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Foreword of the Chairmen of the 16th International HLA and Immunogenetics Workshop and Local Organising Committee

On behalf of the organising committee, we are delighted to welcome you to Liverpool for the Joint Conference of the 16th International HLA and Immunogenetics Workshop, 26th Federation for European Immunogenetics Conference and 23rd British Society of Histocompatibility and Immunogenetics Conference.

This is the first time that the three organisations have come together in a single event and we are excited that so many people have registered to extend our knowledge and understanding of immunogenetics and histocompatibility.

Liverpool, the 2008 European Capital of Culture, is a perfect venue for the event and we hope that you will all take time to explore the city and take advantage of the activities arranged for you.

Needless to say coordinating and organising a meeting of this sort involves a great many people, all of whom we most sincerely thank. In particular we would like to express our deep appreciation to the members of the EFI and BSHI Executive Committees, EFI Scientific Committee and EFI Education Committee for their help and support in organising the Scientific Program, the Teaching Sessions and in the evaluation of the abstracts. More than 500 abstracts have been submitted from all over the world, which is probably some kind of record, but more than anything reflects the excellent work being carried out across the world in this field. We would also like to thank all of our sponsors without whom meetings of this kind would simply not be possible.

We are delighted to host you on Liverpool's famous waterfront, a UNESCO world heritage site, and hope that you will be stimulated by both the meeting and your surroundings.

Derek Middleton and Steven Marsh
Chairmen 16th IHIWC

Foreword of the President of EFI

With great pleasure, I welcome the participants of the combined European Federation for Immunogenetics and British Society for Histocompatibility and Immunogenetics Conference in Liverpool. It is a historical masterpiece of vision having not only two societies cooperating for the annual meeting but also harbouring the International HLA and Immunogenetics Workshop Conference. It is work at its best. It mimics the tri-molecular structure of MHC, peptide and receptor. In this case the T-cell receptor. Other members of the community could see different things such as the three important immune cells; the three classes of MHC or even other associations.

The program is excellent and the organising committee have done their utmost to offer to the community all the attributes for an excellent and wonderful meeting. However, a meeting lives with its participants, their input to discussions in the lectures and abstract sessions, it lives with the awardees and their vision and it lives with all the educational activities, and last but not least, with the input and efforts of the sponsors of the societies and the meeting. All of the aforementioned have done their best beforehand. A grateful thank-you to all: participant; sponsor and local organiser and not forgetting, to the committees of EFI and BSHI with all their members. I wish you a fruitful unforgettable Conference, for both the scientific and personal experience.

Ilias Doxiadis
President of EFI

Foreword of the Chairman of BSHI

As Chair of the British Society for Histocompatibility and Immunogenetics (BSHI) and on behalf of the BSHI, I am delighted to welcome you to Liverpool to the joint 16th International HLA and Immunogenetics Workshop Conference, 26th European Federation for Immunogenetics (EFI) and 23rd BSHI Conference. We are grateful to the organisers and the committee for compiling a stimulating and diverse scientific programme and are pleased to welcome eminent speakers for the BSHI lectures, the Hilliard Festenstein lecture, the Terasaki lecture and the BSHI Presidential Address. Thanks, too, to the corporate partners and sponsors of this Conference whose support is vital. This Conference promises to be a great opportunity for exchange of ideas and to develop collaborations with colleagues internationally. I hope that you will find the Conference stimulating and that you are able to take time to visit the fascinating city of Liverpool.

Susan Fuggle,
Chair BSHI

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IL-12B GENE POLYMORPHISMS INFLUENCE PSORIATIC ARTHRITIS CLINICAL SUBTYPES IN ROMANIAN POPULATION

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Psoriatic arthritis (PsA) is a chronic disorder characterized by skin psoriasis and the inflammation of the peripheral joints (oligo or polyarticular) and/or spine (axial). PsA has a genetic predisposition and recent findings indicate that the genes participating in interleukin (IL)-12/23 signaling play an important role in the disease pathogenesis. IL-12B gene encodes the common subunit p40 of both IL-12 and IL-23. The aim of this study was to investigate a possible influence of IL-12B gene single nucleotide polymorphisms (SNPs) on the clinical symptoms of PsA in Romanian patients. PsA patients (n = 94, 42.5% polyarticular disease, 33% axial involvement, 24.5% isolated oligoarticular) and 161 controls of Romanian ethnicity were genotyped for 6 SNPs in the IL-12B gene. Genotyping was performed with the Sequenom MassARRAY platform (Sequenom, San Diego, CA). Association tests for each polymorphism, linkage disequilibrium (LD) and haplotype frequency estimations were performed with the software PLINK v 1.07 and p values $\leq$0.05 were considered significant. The rs6887695 variant was associated with polyarthritis subtype (MAF 16.2% in patients versus 32.6% in controls, p = 0.003, pcorr = 0.01, OR 0.401, 95% CI 0.211–0.759). Two other SNPs showed associations with the same subtype (rs3212227, MAF 16.2% in patients vs 27.1% in controls, p = 0.04 and rs1363670, MAF 22.5% in patients vs 12.9% in controls, p = 0.03). These associations did not remain significant after correction. The combined haplotype CATACC of all studied SNPs (rs3212227/rs2853694/rs3212220/rs1433048/rs1363670/rs6887695) was associated with polyarthritis subtype (7.6% in patients vs 21.8% in controls, p = 0.004). No association of the investigated SNPs was found with other disease characteristics. In conclusion, in the Romanian population, IL-12B gene influences the clinical phenotype of PsA, mainly of the polyarticular subtype. Grant support: IGA PU_LF_2012_007.

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HLA AND TUMOR NECROSIS FACTOR POLYMORPHISMS IN PATIENTS WITH PROSTATE CANCER

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Tumor necrosis factor-alpha (TNF-alpha) is a proinflammatory cytokine that has been implicated in pathogenesis of many forms of cancer, including prostate cancer. The polymorphisms located within the gene for TNF-alpha have shown an influence on the production of this cytokine. The aim of the study was to evaluate the potential association of TNFa, TNFb and TNFd as well as HLA-A, -B and -DRB1 with prostate cancer. Eighty-nine patients, diagnosed and treated for prostate cancer at the University Hospital Centre Zagreb in the period 2009–2011, were included in the study. The HLA typing was performed using sequence specific oligo probes and Luminex methodology. TNF microsatellites were analyzed using sequence specific primers and PCR followed by electrophoresis on a polyacrylamide gel in an automated sequencer (ALFexpress). The control subjects were healthy individuals (n = 150) typed for HLA-A, -B, -DRB1, TNFa, TNFb and TNFd polymorphisms. The analysis of the patients' HLA typing results revealed *HLA-A*02* (33.7%), *-B*51* (14.6%) and *DRB1*11* (16.9%) as the most frequent specificities. This, as well as the distribution of other HLA specificities at tested loci did not show any significant difference in comparison to the controls. The most frequent alleles at TNF loci were TNFa2 (28.1%), TNFb (36.5%) and TNFd (47.8%). The distribution of TNF alleles revealed a significant difference only for TNFa2 and TNFd5 alleles that were present significantly more among patients in comparison to controls (TNFa2: 28.1% vs. 16.7%, p = 0.0043; TNFd5: 32.0% vs. 21.3%, p = 0.0128). When the patients were divided according to the Gleason score, those two TNF alleles showed a significant increase only among patients with a higher Gleason score (GS equal or higher of 7; TNFa2: 28.4% vs. 16.7%, p = 0.0214; TNFd5: 36.4% vs. 21.3%, p = 0.0062). The obtained results indicate an association of TNFa and TNFd polymorphisms with prostate cancer, especially in cases of higher tumor grade.

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LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AS A SCREENING TEST FOR HLA-B*57:01 AND 58:01 IN DRUG HYPERSENSITIVITY PREDICTION

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Association studies between *HLA-B*57:01* and abacavir hypersensitivity, and *HLA-B*58:01* and allopurinol hypersensitivity in many Asian populations including the Thai population have been reported in recent years. A mortality rate of those who suffer with Stevens Johnson Syndrome and toxic epidermal necrolysis (SJS-TEN), severe drug hypersensitivity, is about 30%. Screening for these alleles before initiating the drug drastically prevents the severe adverse reaction. Most HLA typing techniques depend on conventional techniques e.g. sequence specific primer – polymerase chain reaction or sequence specific oligonucleotide probe – polymerase chain reaction that require well equipped laboratory and specialized scientists. Thus it is important to develop an alternative method to allow HLA typing in a limited resources area. In this report, we used LAMP (Loop-mediated isothermal amplification), an isothermal amplification technique, to amplify *HLA-B*57:01* and *B*58:01*. This technique requires 4 primers which specifically target 6 regions allowing both *HLA-B*57:01* and *B*58:01* to be detected. From 99 blind purified DNA samples, in 40 minutes we could successfully achieve 100% sensitivity and 98.99% specificity (4 positive for *HLA-B*57:01*, 12 positive for *B*58:01* and 82 negative for *HLA-B*57:01* and *B*58:01*) with one false positive sample, compared to commercial SSOP-PCR technique. This technique is faster than PCR and also allows instant detection by using SYBR green. In addition, an expensive thermocycler machine is not required. However, there is a potential problem of false positive results. Therefore, this technique still needs further development to make it more specific. Ongoing research includes the use of an oligonucleotide probe labelled with fluorescence to increase detection specificity and further differentiate *HLA-B*57:01* from *B*58:01*.

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BLACK PATIENTS OF AFRICAN DESCENT AND HLA-DRB1*1503 FREQUENCY OVERREPRESENTED IN EPIDERMOLYSIS BULLOSA ACQUISITA

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Epidermolysis bullosa acquisita (EBA) is a rare autoimmune bullous disease (AIBD). A higher EBA incidence and HLA predisposing genetic factor have been suspected in some populations. A retrospective study from the French Referral Center for Autoimmune and Toxic Acquired Bullous Dermatoses showed that 54% EBA in contrast to 3% AIBD patients were black people ($p = 10^{-6}$) and that 54% black patients suffering from AIBD had EBA. Between 1983 and 2009, 19 black EBA patients were seen. Among the 19 black EBA patients, 9 were natives of sub-Saharan Africa, 1 from Reunion Island, 7 from West Indies and 2 were of mixed ancestry. The frequency of *HLA-DRB1*1503* was 50% for African patients, significantly higher than for the control population ($p < 10^{-3}$) and 21% for the West Indians (ns). A high EBA frequency has already been reported in American blacks significantly associated with HLA-DR2. In conclusion, this retrospective study assessed the overrepresentation of black patients with EBA, its link with HLA-*DRB1*1503* allele, and suggests that EBA should be evoked systematically for every AIBD seen in this population.

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IMPACT OF HLA AND CYTOKINE POLYMORPHISMS ON INHIBITOR DEVELOPMENT IN CHILDREN WITH SEVERE HAEMOPHILIA A.

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The development of inhibitors (Inh) against factor VIII in Hemophilia A (HA) patients is the most serious complication and seems to be multifactorial. The type of FVIII gene mutation, HLA genes and cytokine polymorphisms are included among genetic predisposing factors for Inh formation. To investigate any possible correlation of FVIII gene intron-22 inversion, HLA alleles and cytokine polymorphisms with the risk for Inh development in 52 Greek children with severe HA, exclusively treated with recombinant products, we performed Long Range PCR for detection of intron-22 inversion. PCR-SSP and PCR-SSO were applied for HLA Class I and II genotyping and also for polymorphisms of TNF-a, TGF-b1, IL-10, IL-6, IFN-gamma. Statistical analysis was performed by χ^2 test and Fischer's exact test. Twenty-eight children had developed inhibitors (Group I), while 24 had not (Group II). Analysis of HLA frequencies between the two groups showed statistically significant differences in the following genotypes i) positive associations with Inh development: *DRB1*01* ($p = 0.014$, OR = 10.9), *DRB1*01:01* ($p = 0.011$), *DQB1*05:01* ($p = 0.005$, OR = 12.8), and ii) negatively associated with Inh development: *DRB1*11* ($p = 0.011$, OR = 0.2), *DRB1*11:01* ($p = 0.031$, OR = 0.15), *DQB1*03* ($p = 0.004$, OR = 0.15), *DQB1*03:01* ($p = 0.014$, OR = 0.22). The differences between the two groups regarding the polymorphisms of cytokines were not statistically significant. However, the homozygocity of the haplotypes ACC and ATA for IL-10 -1082G>A, -819C>T and -592C>A polymorphisms showed a trend for Inh development. This study shows that *HLA-DRB1*01*, *DRB1*01:01*

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BLACKBERRY-LIKE PARTICLES ASSEMBLED FROM FLUORESCENT-LABELED QUATERNIZED AMPHIPHILIC CHITOSAN: PREPARATION AND THEIR POTENTIAL FOR BIOIMAGING

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Introduction

Self-assembly is well recognized as a versatile technique for inducing particle formation from amphiphilic polymer having both hydrophobic and hydrophilic entities. In particular, the method has been successfully employed to generate particles with controllable size range and great potential as drug carriers from amphiphilic chitosan, a natural, non-toxic, and biodegradable biopolymer.[1-2] Fluorescent labeling has been known as an effective tool to monitor substance uptake by biological systems. In the case of particle, the fluorescent dye can be either chemically tagged to or physically encapsulated in the particles. Taking advantage of pyrene being a hydrophobic fluorescent dye, fluorescent labeled quaternized chitosan particles can be conveniently prepared by self-assembly of amphiphilic chitosan having pyrene as a hydrophobic entity and *N*-[(2-hydroxyl-3-trimethylammonium)]propyl (HTAP) as a hydrophilic entity. It is anticipated that the quaternary ammonium groups from HTAP should enhance electrostatic attraction between the particles and negatively charged phospholipid cell membranes and subsequently promote cellular uptake so that the particles may be applicable for bioimaging applications.

Experimental

Stepwise procedure for the preparation of fluorescent-labeled amphiphilic chitosan is schematically outlined in Figure 1. *N*-phthaloylchitosan (PhCS) was first synthesized in order to protect amino groups of chitosan. The *C-6*-azido-*N*-phthaloylchitosan (N_3-PhCS) was then obtained after bromination of *C6* hydroxyl group of chitosan followed by azidation.[3] Click reaction between the N_3-PhCS and 1-ethynylpyrene was conducted using Cu(I) as catalyst and *N,N*-diisopropylethylamine as base to yield pyrene-functionalized PhCS (Pyr-PhCS). Deprotection of phthaloyl groups was carried out via hydrolysis using hydrazine solution. The positively charged HTAP groups were then introduced by a reaction between Pyr-PhCS and glycidyltrimethylammonium chloride (GTMAC) in DMF. Finally, fluorescent-labeled quaternized chitosan particles were obtained by self-assembly of the resulting amphiphilic chitosan (Pyr-CS-HTAP) after dialysis against deionized water for 4 days and lyophilization.

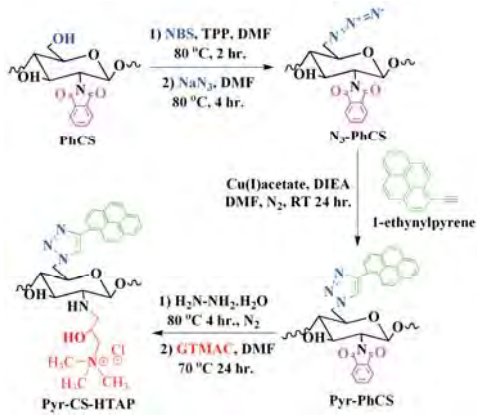

Figure 1. Schematic representation of stepwise procedure used for the preparation of fluorescent-labeled amphiphilic chitosan.

Results and Discussion

FT-IR technique was used to monitor the stepwise functionalization of chitosan. As shown in Figure 2, a characteristic stretching of azide group in N_3-PhCS emerges at 2100 cm^{-1} and disappears after the click reaction with ethynylpyrene (See Pyr-CS-HTAP spectrum). The presence of C-H stretching peak of -$N(CH_3)_3$ at 1480 cm^{-1} and the absence of C=O stretching at 1700 cm^{-1} (found in PhCS) in the Pyr-CS-HTAP spectrum verified the success of HTAP incorporation and the complete removal of phthaloyl entities, respectively.

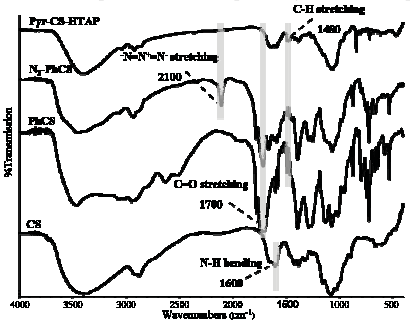

Figure 2. FT-IR spectra of chitosan (CS), PhCS, N_3-PhCS and Pyr-CS-HTAP.

According to SEM analysis (Figure 3a), the self-assembly of the amphiphilic Pyr-CS-HTAP interestingly yielded particles with blackberry-like morphology and a size range of 1-2 µm. As expected, the particles exhibited positive charges with a zeta-potential of +40 mV as determined by photon correlation spectroscopy. The bright green fluorescence of the particles appeared in Figure 3b indicated the existence of pyrene in the structure of Pyr-CS-HTAP particles.

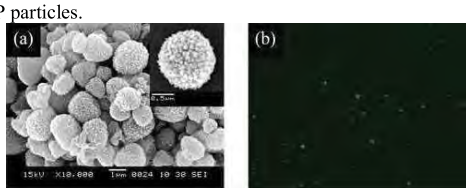

Figure 3. SEM (a) and fluorescence (b) micrographs of Pyr-CS-HTAP particles.

The self-assembled Pyr-CS-HTAP particles are relatively stable considering that there was no detectable morphological change under extreme variation of temperature (0-100°C) and pH (1-12). The de-aggregation only occurred upon the treatment with non-polar hexane. Nonetheless, the blackberry-like particles can be recovered after hexane removal and water replacement indicating that the self-assembly process is reversible. Interactions of the Pyr-CS-HTAP particles with representative biological systems such as bacteria (*S.aureus* and *E.coli)* and cells (macrophage, cancer cells) are being investigated to demonstrate their potential for bioimaging applications.

Conclusions

Fluorescent-labeled quaternized chitosan particles can be successfully prepared by self-assembly of amphiphilic chitosan. These positively charged particles have an interesting blackberry-like morphology and are relatively stable. With these desirable characteristics, the particles should potentially be used for bioimaging applications.

Acknowledgement. The financial support for this project was provided by The Thailand Research Fund (RTA 5280002).

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Postharvest Non-destructive Determination of Fruits: a Model on Fruit Maturity Assay via Biosensor Based on Colorimetric Change of Gold Nanoparticles

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Keywords: pineapple, *Ananus comosus*, *AcSUT*1

Abstract

New approach for non-destructive fruit maturity determination using pineapple as a model had been established. The assay was based on expression patterns of the putative sucrose transporter (*AcSUT*1) in crown leaves during the course of physiological maturity that could be employed as an index marker for fruit maturity. Determination of *AcSUT*1 cDNA was initially designed using isothermal cDNA amplification and the DNA signal detection via biosensor based on colorimetric platform with gold colloid nanoparticles, 20 nm in diameter. In the immature fruit, the *AcSUT*1 gene was expressed allowing target DNA amplification to occur. DNA signals detection in later step was based on plasmon phenomena of gold nanoparticles. Presence of target DNA affected gold colloid nanoparticles in term of non aggregation with no colour change (still ruby red). However, in the mature fruit, the expression of the *AcSUT*1 gene was terminated resulting in no target cDNA product amplification. When this was tested with gold colloid nanoparticles, it induced aggregation of particles which in turn resulted in a plasmonic change of colloid solution from ruby red to dark purple, visible by naked eye. All processes from RNA extraction to cDNA signal detection could be completed within 80 min without the need for a thermocycler as normally employed during RT-PCR. The developed assay demonstrated the integration of the novel DNA biosensor approach for a non-destructive determination of fruit quality.

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INTRODUCTION

Maturity of fruit at harvest is an important factor determining fruit quality. Especially, in several fruits, immature fruit may leads to inferior flavor quality and undesirable physiological changes, which in turn shortening their shelf-life(Kader, 1999). In general, fruits reach their best quality when they were left maturity ripen on plant. Nevertheless, most practical harvesting can not wait until reaching that stage. Measuring

the maturity through some characters or markers was then required. When considering maturity measurement, maturity of fruits for customer was basically determined by their appearance quality including freshness firmness and color, however, for farmer maturity indices were further employed as important factors for determination of time for fruit harvesting. So far several indices including total solid, soluble solid concentration, titratable acidity concentration and ratio between soluble solid to titratable acidity, and flesh firmness were used for determining maturity and quality of fruit (Pattee, 1985). Nevertheless, those appearance factors and chemical indices alone could not perfectly predict real maturity of fruits. Moreover, measurement through most of those indices was performed with risk of destructive manner.

Maturation is a stage of development leading to the attainment of physiological maturity. In most fruits, even fruits were detached from plant the processes of development were still going on. We had interested in investigation of pineapple fruit development as a model because it was the most agronomical important to Thailand with annual gross export value more than 690 million US$.

Pineapple is classified as a non-climacteric fruit whose processes of ripening can not markedly be continued once removed from the plant. It produces small quantities amount of ethylene and has almost no response to it (Kader, 1999). Thus any abundance of transcripts relating to fruit quality along the period of development may not be influenced by ethylene as found in the other of climacteric one.

So far several genes of pineapple had been investigated for their expression profiles. Taira *et al*., (2005) had studied a group of chitinase genes and found the different among their stage of expression and physiological roles. Type A chitinase expressed at all stages while type B and C was terminate at early stage of fruit development. These early termination were not related directly to fruit maturation. Moyle *et al*., (2005) had surveyed a range of expressed sequences during fruit development from green unripe to yellow ripe fruit and found that some genes, for example, fruit bromelain gene and their inhibitors, were abundant at unripe fruit stage, while MADS box and others associated with protein synthesis (ribosomal L10 and some translation factors) were expressed at yellow ripe fruit. Although the expressions were covering stages of fruit maturation, these un unique expression profiles were not able to employ as marker for maturity detection.

During fruit maturation, sugar storage and assimilate partitioning in fruit play an important role in fruit carbohydrate metabolism. The genes involving to these storage and assimilate partitioning phenomena were then attacked interests since sugar accumulation was one of the importance phenomena influencing fruit maturity and quality. Zhang *et al*., (2010) had reported the important of sucrose phosphate synthase (SPS) as a key enzyme over invertase and sucrose synthase, and had cloned gene SPS of pineapple (*Ac-SPS1*). Expression studies of this *Ac-SPS1* gene revealed low expression at early stage of fruit growth, increasing from 20 days after anthesis and gradually decreasing again on 40 days after anthesis. The results although implied *Ac-SPS1* and an activator of this gene might be important regulatory events of sugar during pineapple fruit maturation, its uninterrupted transcription pattern could not be employed for fruit maturation detection.

Antony *et al*., (2008) had investigated the sugar storage in pineapple (*Ananus comosus*) and had identified a putative hexose transporter gene (*AcMST1*: *A comosus* monosaccharide transporter), a putative inositol transporter gene (*AcINT1*), a putative sucrose transporter (*AcSUT1*) and tonoplast hexose transporter like (*AcMST2*). In pineapple, *AcMST1* was more highly expressed in fruits compared to leaves, whilst transcripts of *AcINT1, AcSUT1, and AcMST2* were more abundant in leaves (Antony *et*

al., 2008). The expression patterns of *AcMST1* and *AcSUT1* implied the incidence of the synthesis and accumulation periods of monosaccharide in fruit, and also the transportation of monosaccharide from leaf during maturation, which might be employed in fruit maturation detection.

In this paper, we had further studied the abundance of transcripts of both *AcMST1* and *AcSUT1* comparing with that of 18S rRNA in fruits and in crown leaf of during the course of fruit development. Results revealed pattern of gene expression of *AcSUT1* in crown leaf was finally terminated when fruit was in mature stage. Based on this, we had demonstrated the feasibility of using the *AcSUT1* expression phenomenon as an indicator for fruit maturity determination. It was based on biosensor technique, combining a rapid cDNA amplification of *AcSUT1* transcripts using reverse transcription loop mediated isothermal platform and a novel rapid nucleic acid hybridization using colorimetric change of gold nanoparticles for a simple non destructive determination of fruit maturity.

MATERIALS AND METHODS

Pineapple fruits used in the experiment were Smooth Cayenne type *Ananus comosus* (L.) Merr., cv. Sriraja from local orchards in Cholburi province, Thailand. Fruits were picked up covering all 5 stages of fruit developments; stage1 anthesis (2 months after flowering treatment), stage2 late anthesis stage (2.5 months after stage 1), stage3 small fruit stage (3.5 months after flowering treatment), stage 4 medium fruit stage (4-4.5 months after treatment), and stage 5 mature fruit (5 months after flowering treatment)(Fig. 1.). Transportation of fruits to laboratory was finished within two hours post detachment.

The transcripts abundance of each genes were investigated using RT-PCR (Antony *et al*., 2008). Amplification of primers set for the *AcMST1, AcSUT1* gene and for the 18S rRNA gene, were based on criteria of specific DNA amplification to both genes without leaving non-specific products. In detail, first, total RNA was extracted from 500 mg of fruit and the second crown leaf (scale) from basal closed to fruit of each treatment with TRIZOL solution according to the manufacturer's protocol (Invitrogen, USA). Two-step RT-PCR of each samples was performed using reverse M-MuLV transcriptase (New England Biolabs Inc., USA), and *Taq* DNA polymerase (Promega, USA) with incubation condition for reverse transcription at 42°C 60 min and 90°C for 10 min inactivation of enzyme, and for PCR using denaturation at 93°C 40 sec, annealing at 53°C 1 min, and extension at 72°C 1min with 40 cycles of amplification as described (Sambrook *et al*., 1989). RT-PCR products were visualized after electrophoresis.

For detection of cDNA of *AcSUT1* gene using reverse transcription loop mediated isothermal amplification (RT-LAMP), set of primers was designed to recognize 6 distinct areas of *AcSUT1* gene of pineapple (accession number EF460878) as described (Chaumpluk and Chaiprasart, 2010) (Figure 3.) and reaction preparations were carried out as described (Notomi *et al*., 2000). The mixture was incubated at 63°C for 30 min. No post heat incubation at 80°C was applied. The detection limit of the reaction was determined using equivalent copy numbers of cloned *AcSUT1* at 10-fold dilutions as described (Anonymous, 2003, Kuribara *et al.*, 2002). The sensitivity of the assay was also confirmed and compared with that of RT-PCR by the gel electrophoresis (Antony *et al*., 2008).

Confirmation of the *AcSUT1* cDNA was by novel colorimetric nucleic acid hybridization in gold colloid solution. This was based on plasmonic change of gold nanoparticles upon hybridized with probe (Kanjanawarut and Su, 2009). Gold nanoparticles 20 nm in size in water solution were prepared and selected under increasing alkaline condition using soluble starch as reducing agent (Pienpinijtham *et al.*, 2010). Analog nucleic acid probe (5'Flu-O-aatcagtaatta-LysNH_2) was synthesized according to the previously reported Fmoc solid-phase peptide synthesis protocol (Suparpprom *et al*., 2005, Vilaivan and Srisuwannaket, 2006). The crude oligo probe was cleaved from the solid support by treatment with trifluoroacetic acid (TFA) and purified using reversed-phase HPLC with UV detection at 260 nm. A Varian Polaris C18 (3 μm particle size 4.6x50 mm) analytical HPLC column was used and eluted with a gradient of 0.1% TFA in acetonitrile and 0.1% TFA in deionized water. Binding of probe to surface of gold nano particles was performed by adding probe to a final concentration of 100 pM to 20μL gold solution (1000 ppm) at room temperature. Nucleic acid hybridization was performed after adding 1 μL of RT-LAMP products and 1mM PBS. Changing of gold color was observed with naked eye and measured through UV-Visible Spectroscopy (Ocean Optics ® USB2000, USA) with λ=380-800nm.

RESULTS AND DISCUSSION

Sugar accumulation was found in fruit during maturation. Close correlation of the abundance of *AcMST1* in fruits was demonstrated (Antony *et al*., 2008). However in fruit, there were limited expressions of *AcSUT1* gene especially during fruit maturation due to the processes of sugar transportation was nearly ceased during that period. Although abundance of both gene were studied in fruit(Antony *et al*., 2008), there were no investigation of both gene following the course of fruit development as well as the fate of the gene in crown leaf at each stages. Thus, in order to find the relationships between transcripts abundance of both gene during the course of fruit development in both fruit and crown leaf, cDNA corresponding to both genes covering all 5 stages ranking from stage1 anthesis (2 months after flowering treatment), stage2 late anthesis stage (2.5 months after stage1), stage3 small fruit stage (3.5 months after flowering treatment), stage 4 medium fruit stage (4-4.5months after flowering treatment), and stage 5 mature fruit (5 months after flowering treatment)(Fig. 1) were carried out. The mature fruit at stage 5 was selected based on 80% of yellow ripen which represent stage of physiological maturity. The investigation of *AcMST1 and AcSUT1* gene expression of in crown leaf during fruit development were shown in Fig. 2.

Results revealed that, in fruit, the expression of *AcMST1* increased from stage 2 and steadily expressed with a level more than that of 18S, house keeping rRNA. Also the expression of *AcSUT1* started to express at similar stage with that of *AcMST1*. The expression of *AcSUT1* though continued to the last, its level of expression decreased after stage 3. Interestingly, level of expression of both genes, in crown leaf, were much lower than that found in fruit. Also level of expression of both genes continued to decrease after stage 4 and the expression of *AcSUT1* was totally discontinued at stage 5.

AcSUT1 protein is a sucrose transporter localized to pre-vacuoles. Since in Arabidopsis a similar protein called SUT2, localized at plasma membrane of sieve element, was found to highly express in sink tissues, similar to *AcSUT1*, a role of

AcSUT1 as efflux carriers, implicated in phloem unloading had been proposed (Antony *et al*., 2008).

The halting of *AcSUT1* expression in crown leaf at stage of fruit maturity was attracted interest because this phenomenon could be employed for maturity prediction. Based on this, a method for a simple non destructive determination of fruit maturity using the *AcSUT1* expression phenomenon in crown leaf as an indicator was developed. It was based on specific cDNA amplification via reverse transcription loop mediated isothermal amplification platform in combination with nucleic acid hybridization based on colorimetric assay of gold nanoparticles (Kanjanawarut and Su, 2009).

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) provides an alternative simple yet rapid testing option (Notomi *et al*., 2000, Mori *et al*., 2001). The mechanisms of RT-LAMP and its accesses had been described elsewhere (Notomi *et al*., 2000, Parida *et al*., 2008). RT-LAMP has several advantages comparing with RT-PCR and real-time RT-PCR especially in reaction simplicity, its rapidness, its highly specific to target RNA due to the simultaneously primed of multi primers on RNA, and its higher amplification efficiency, all attributes that clearly distinguish RT-LAMP from the existing detection methods (Parida *et al*., 2008).

Likewise, the novel nucleic acid hybridization, based on colorimetric assay of gold nanoparticles, was employed for further identify cDNA signals. In the colorimetric detection, the β-pyrrolidinyl peptide nucleic acid (PNA) was used as probe. β-pyrrolidinyl PNA is a derivative of oligonucleotide analogues, in which the sugar phosphate backbone had been replaced by a pseudopeptide chain of *N*-aminoethylglycine monomers, the recognition of target DNA via PNA/DNA hybridization could be performed at exceptionally high affinity and specificity (Suparpprom et al., 2005, Vilaivan and Srisuwannaket, 2006). The merits of using PNA here were not only property of PNA that allowed complementary DNAs to be hybridized according to the Watson–Crick hydrogen-bonding rules, it also offered advantages over classical DNA hybridization since its neutral-charged backbone increases the binding strength (Vilaivan and Srisuwannaket, 2006). This provided a rapid sequence-specific detection in solution without the need of membrane in short period of time. Moreover, this PNA probe typically showed superior selectivity against mismatches compared to DNA probes and would not hybridize with RNA molecules in the system, the latter was not found in that of Nielsen (Nielsen and Haaima 1997). Thus these properties improve discrimination of target DNA to a perfect level.

PNA also acts as a coagulant to alter the intrinsic stability of gold nanoparticles when mixing together allowing nucleic acid hybridization to be simply and rapidly carried out. In principle, the binding of PNA probe on surface of gold nanoparticles is by both the nitrogen-/oxygen-containing and the hydrophobic neutral structure of peptide backbone leading to a higher affinity to gold than that of DNA. During hybridization, PNA oligomers can induce immediate particle aggregation, if they are not bind with their target DNA, however, when target DNAs are available, successful PNA-DNA hybridizations result in forming of hybridization complexes, although having a stable double helix structure similar to dsDNA, that can effectively protect gold nanoparticles from salt induced aggregation (Kanjanawarut and Su, 2009). The result of gold nanoparticles aggregation provides a colorimetric change of colloid particles from ruby red to purple that can be seen with naked eye.

In the first step of experiment, a primer set for *AcSUT1* cDNA amplification, comprising two outer (F3 and B3) and two inner primers (FIP and BIP), was chosen and

depicted in Fig. 3. It was specific to 6 area of *AcSUT1* (Accession No. 460878) between position 1574 to 1741(see Fig. 3 for detail).

Amplification using the designed primer set at 63 °C delivered ladder DNA products of multiplied size of ~170 nucleotides (Fig. 4) within 30 min. Only sample having *AcSUT1* RNA had been amplified. The primer set was priori tested by computer program Blastn for specific amplification of only target RNA but not others (Altschul *et al.*, 1990) and tested for non-reacting with RNA of plant viruses (TMV, CMV, PRSV) (data not shown). When compared with RT-PCR of Antony *et al.*, (2008), RT-LAMP provided similar results (Fig. 4., lower).

Sensitivity in *AcSUT1* detection using known copy number of clone cDNA of *AcSUT1* as templates was also performed. Results, in Fig. 5, revealed the LAMP unique DNA pattern observed even when the template molecules were diluted to a level of 10 copies per reaction. Results of cDNA amplifications were also well corresponding with that of previously reported RT-PCR (Antony *et al.*, 2008). All these results supported the reliability of amplification system for *AcSUT1* signals amplification.

Further step confirmation of the nucleotide sequence of target products was by novel nucleic acid hybridization based on colorimetric assay of gold nanoparticles. Successful detection of *AcSUT1*'s cDNA each time when fruit were still immature came with ladder like DNA products that hybridized well with PNA at surface of gold nanoparticles. This resulted in effective protection of gold nanoparticles from salt induced aggregation, thus, remaining of ruby red color for gold solution. Whereas in mature fruit, where the expression of *AcSUT1* in crown leaf had terminated, no DNA amplification were observed. The PNA oligomers, not binding with their target DNA, were remained on the gold surface and could induce immediate particle aggregation changing the color of gold solution from ruby red to purple one. Ruby red color of gold solution after hybridization could be detected in samples having *AcSUT1* expression within 2 min(Fig. 4 and Fig 5, bottom). They provided further evidences of nucleic acid specific detection following *AcSUT1* amplification system. These specificity and sensitivity indicated a satisfactory performance of the combined RT-LAMP and rapid nucleic acid hybridization method for maturity detection. The result of clear color of ruby red signals came from the yield of DNA products, delivered more by reverse transcription isothermal DNA amplification than that of PCR, especially at near limit of detection. The RT-LAMP/nucleic acid hybridization also provided several merits of RT-LAMP in cDNA amplification and signals detection in terms of the incubation at constant temperature which eliminates the need for a thermocycler device, making possible to perform DNA amplification outside laboratory (Mori *et al.*, 2001, Nagamine *et al.*, 2001) and detection rapidly at room temperature. Furthermore, time for amplification reaction is reduced to 30 min which enables the whole processes of the detection to be completed within 1 hour. Additionally, this detection can be observed visually with naked eye

Further demonstrations of fruit maturity determination using the above method, an application for field testing had been carried out to using 25 samples randomly selected from fields (15 mature and 10 immature fruits, based on color and soluble solid criteria). Results revealed the complete detection of all blind 15 samples of mature fruits among 25 samples tested but one false negative result was found from both tests. The mature fruit results predicted by RT-LAMP were agreed with the results previously judged by the physiological criteria. Based on the data tested (n=25), sensitivity of the test was

calculated from number of positive results divided by sum of number of positive plus number of false negative, which equally to 15/16 or 93.75%. Similarly, the specificity of the test was calculated from number of negative results divided by sum of number of negative plus number of false positive, which equally to 10/10 or 100% (Fig.6.) . It should be noted that the detection of gene expression here was a qualitative test, suitable to detect on and off gene expression, for quantitative assay further modification of the method was needed.

Detection of target *AcSUT1* gene expression in crown leaf was a sample model for non destructive determination of fruit maturity. In the rest of fruits, either climacteric or non climacteric one, the incidences of the synthesis and accumulation periods of monosaccharide in fruit, and also the transportation of monosaccharide from leaf during maturation, were still key factors in fruit development, fruit quality and fruit maturity, awaited elucidation. These exploring could be employed in fruit maturation detection.

Based on the above results, we had developed the method for simple fruit maturity detection and demonstrated the use of this method for fruit maturity determination. By detecting of *AcSUT1* gene expression in crown leaf, one can predict state of fruit maturity without damage the fruit. This was the first time that isothermal nucleic amplification platform together with nucleic acid hybridization on the surface of gold nanoparticles were applied for fruit maturity detection. The detection via RT-LAMP and nanoparticles here costs approximately US$8 per test, at least 8 times cheaper than the cost of a standard laboratory test. Also reaction can be adjusted to an easy-to-operate reagent kit which better provides an opportunity to monitor the expression of *AcSUT1* gene wherever needed.

CONCLUSION

A technique for the pineapple fruit maturity detection based on RT-LAMP of *AcSUT1* and nucleic acid hybridization on the surface of gold nanoparticles was developed. Direct amplification of *AcSUT1* by reverse transcription isothermal DNA amplification using a set of primers allowed target gene to be ready for nucleic acid hybridization in 30 min. Target gene products in ladder DNA starting from size of 170 nt. could be observed. Determination of technique's sensitivity revealed the limit of detection at 10 copies of *AcSUT1* gene. Further detection of *AcSUT1* signals was based on colorimetric nucleic acid hybridization platform with gold colloid nanoparticles, 20 nm in diameter. In the immature fruit, the *AcSUT*1 gene was expressed allowing target DNA amplification to occur. Presence of target DNA affected gold colloid nanoparticles in term of non aggregation with no color change (still ruby red). However, in the mature fruit, the expression of the *AcSUT*1 gene was terminated resulting in no target cDNA product amplification. When this was tested with gold colloid nanoparticles, it induced aggregation of particles which in turn resulted in a plasmonic change of colloid solution from ruby red to dark purple, visible by naked eye. The technique has several merits on its rapidity and simplicity in performing the test, on the characteristic of colorimetric change of gold nanoparticles which required no sophisticated devices, and on its ability to perform the test wherever needed.

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Figures

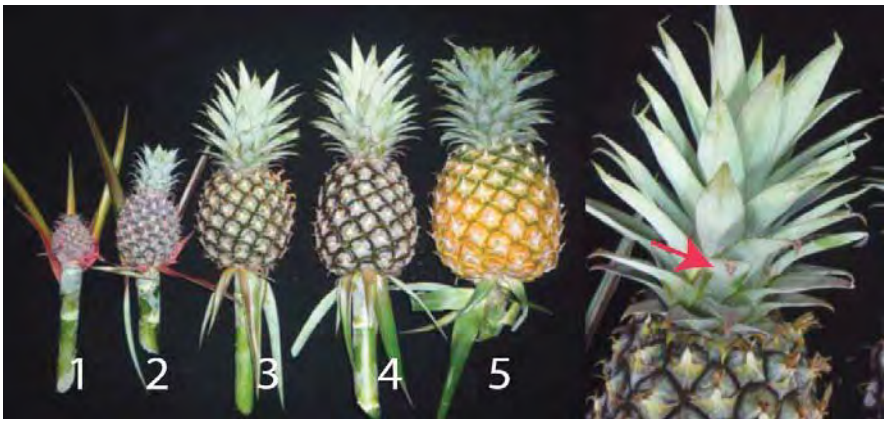


Figure 1. Stage of fruits development of Sriraja, a Cayenne type pineapple. A) From left to right, Stage1 anthesis (2 months after flowering treatment), Stage2 late anthesis stage (2.5 months after stage1), Stage3 small fruit stage (3.5 months after flowering treatment), Stage 4 medium fruit stage (4-4.5months after flowering treatment), and Stage 5 mature fruit (5 months after flowering treatment). B) Position of crown leaf used for maturity detection (arrow).

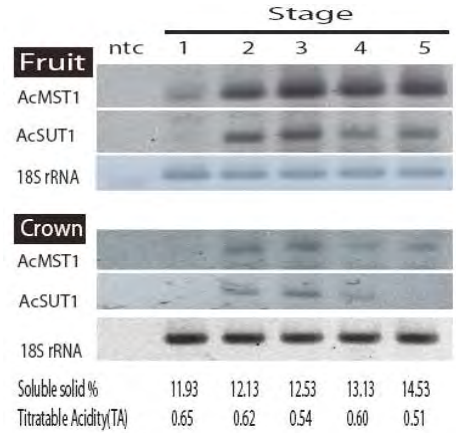
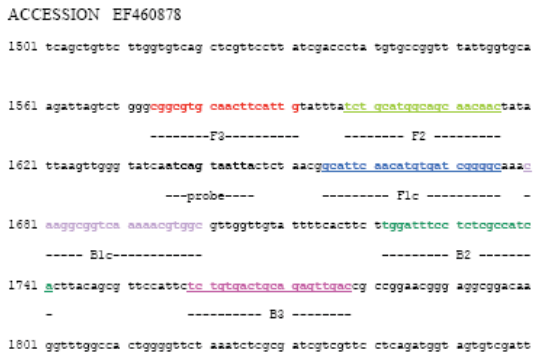


Figure 2. Abundance of transcripts of AcMST1 and AcSUT1 in fruits and in crown leave during the course of fruit development (Stage 1 anthesis to stage 5 mature fruit) compared with the 18S rRNA house keeping gene. Soluble solid and titratable acidity (TA) of each stage were also shown. The ntc represents non template control.



Primer	Sequence	Location
F3	CGGCGTGCAACTTCATTG	1574-1591
B3	GTCAACTCTGCAGTCACAGA	1759-1778
FIP	GCCCCGATCACATGTTGAATGC TTTTTCTGCATGGCAGCAACAAC	1597-1616 and 1655-1676
BIP	CAAGGCGGTCAAAAACGTGGC TTTTTGATGGCGAGAGGAAATCCA	1680-1700 and 1722-1741

Figure 3. Schematic illustration of related sequence of *AcSUT1* where 6 locations of primers were assigned on the sequence including F3, F2, F1, B1, B2 and B3 respectively. The detail of primers was demonstrated on the right.

Figure 4. Amplification of target *AcSUT1* using RT- LAMP, their corresponding RT-PCR detection (lower) and the confirmation by nucleic acid hybridization using gold nanoparticles. Lane M, 100 nucleotide ladder, Lane NTC, non template control, Lane –ve, negative amplification of *AcSUT1*, and +ve, positive amplification of *AcSUT1*.

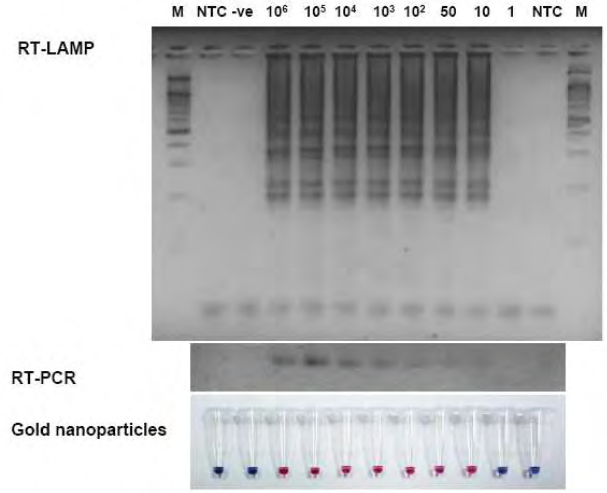
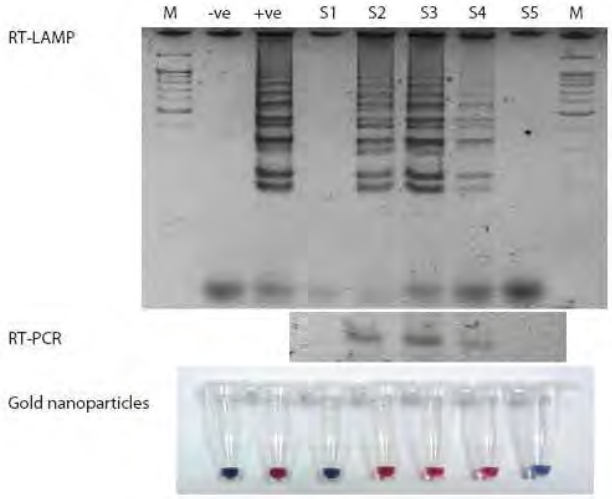


Figure. 5. Sensitivity of primers for amplification of target cDNA of known copies using cloned *AcSUT1* gene via LAMP (upper) and its nucleic acid hybridization using gold nanoparticles (lower). The confirmation by PCR based on Antony, (2008) was shown in the middle. Lane M, 100 nt ladder marker. Lane –ve , negative amplification of DNA using *AcMST1* gene as a template, Lanes with numbers represent the number of DNA copies involved in detection.



	Sample*	RT-PCR	RT-LAMP+ Goldnano
Mature (true positive)	15	15	15
False positive	-	0	0
Immature(true negative)	10	9	9
False negative	-	1	1

n = 25

$$\text{Sensitivity} = \frac{\text{true positive}}{\text{true positive+false negative}} = \frac{15}{15+1} = 93.75\%$$

$$\text{Specificity} = \frac{\text{true negative}}{\text{true negative+false positive}} = \frac{10}{10+0} = 100\%$$

Figure 6. A) Representative detection of *AcSUT1* in pineapple fruits collected from fields. Results based on RT-LAMP (upper) and RT-PCR as previously reported (Antony, *et al*., 2008) (middle) and nucleic acid hybridization using gold nanoparticles (lower) were illustrated. Lane M, 100 nt ladder marker, Lane –ve , negative amplification of *AcMST1*, Lane +ve , positive *AcSUT1* DNA, Lanes S with numbers represent the commercial samples from source 1 to 5. B) Overall fruit maturity detection using 15 mature fruits and 10 immature fruits revealed a precise testing results of mature fruits with one of false negative in both methods. The sensitivity and specificity were demonstrated.

SYN-O-004

CYCLODEXTRINS DISCRIMINATION BY TRICATIONIC PHENYLENE-ETHYLYNYLENE FLUOROPHORE

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ABSTRACT

The cyclodextrins (CyDs) containing six (α), seven (β) and eight (γ) glucosyl-membered cyclic structure are widely used in controlled release system, separation and molecular recognition due to their complexation ability and low cytotoxicity. Size selective inclusion complexation with CyDs and detection of this phenomenon are thus important for development toward these applications. In this contribution, the complexation of trimethylphenyl quaternary ammonium groups with γ–CyD is readily observed by fluorescence enhancement and ^{1}H NMR signals shifts of tricationic phenylene-ethynylene fluorophore. Benesi-Hilderbrand plot and the NMR data revealed a 1:1 complexation between the fluorophore and γ-CyD. The data also indicate no inclusion interaction between the fluorophore and other CyDs (α and β). This tricationic fluorophore should be useful for identify γ–CyD from the other sizes CyDs and probing its inclusion interaction.

Keywords Fluorescence; Sensor; Inclusion complex

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Cytocompatibility and Cellular Uptake of Blackberry-like Particles Assembled from Fluorescent-labeled Quaternized Chitosan

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Abstract

Upon self-assembly of amphiphilic chitosan having *N*-[(2-hydroxyl-3-trimethylammonium)] propyl (HTAP) as hydrophilic entities and ethynylpyrene (fluorescent dye) as hydrophobic entities, fluorescent-labeled quaternized chitosan (Pyr-CS-HTAP) particles can be formed. The functionality of the quaternized chitosan particles were verified by FT-IR and NMR spectroscopy. According to SEM analysis, the particles had a size range of 1-2 μm with blackberry-like morphology. And the particles exhibited positive charges with a zeta-potential of +40 mV as determined by PCS. The particles had bright green fluorescence and were stable in a broad pH and temperature range. The cytotoxicity test revealed that the particles were biocompatible with Macrophage cells (RAW 264.7 cells). Moreover, cellular uptake of the particles by the cells can be determined by confocal laser scanning microscopy (CLSM) and flow cytometry techniques. The results showed that approximately 42% of cellular uptake was observed after 1 h incubation. It has also been found that the aggregation of the particles has a strong impact on the extent of cellular uptake. These fluorescent-labeled quaternized chitosan particles possess a number of desirable features that may be used for bioimaging application.

Keywords: blackberry-like particles; fluorescent-labeled particles; self-assembly; chitosan particles; cytocompatible particles

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Functional Polymer-stabilized Gold Nanoparticles: Preparation and Peptide Nucleic Acid Immobilization

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Abstract

Poly(methacrylic acid)-*ran*-(2-methacryloyloxyethyl phosphorylcholine) (PMAMPC) which can provide binding site and protein-repellent property was prepared via Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization and grafted onto citrate-stabilized gold nanoparticles (citrate-AuNPs) to obtain functionalizable AuNPs having anti-fouling characteristic. Optical property, size, and morphology of PMAMPC-stabilized AuNPs (PMAMPC-AuNPs) were evaluated by UV-vis spectroscopy, dynamic light scattering (DLS) technique and transmission electron microscopy (TEM). The presence of PMAMPC around the AuNPs was also confirmed by Fourier transform infrared spectroscopy (FTIR). PMAMPC-AuNPs were highly stable because of both electrostatic and steric stabilizations of the polymeric shell. Then, fluorescein-labeled peptide nucleic acid (PNA-flu) was immobilized to carboxyl groups of PMAMPC on PMAMPC-AuNPs via EDC/NHS coupling chemistry. Effects of PMAMPC molecular weight, surfactant (Tween-80) concentration on PNA-flu immobilization was determined. The amount of immobilized PNA-flu as determined by fluorescence spectroscopy after KCN digestion was found to be 2.72×10^{13} probe/cm^2. This value is two times higher than that (1.28×10^{13} probe/cm^2) obtained via a direct PNA immobilization by self-assembly monolayer (SAM) formation of PNA-terminated thiol compound. This discovery strongly suggests that the 3-D structure of the polymeric shell can provide greater probe binding capacity than the 2-D SAM system. It is anticipated that this newly developed polymer-stabilized AuNPs can potentially be developed into effective PNA-based DNA biosensor.

Keywords: Gold nanoparticles; Zwitterionic polymer; Peptide nucleic acid; Protein repellent surface

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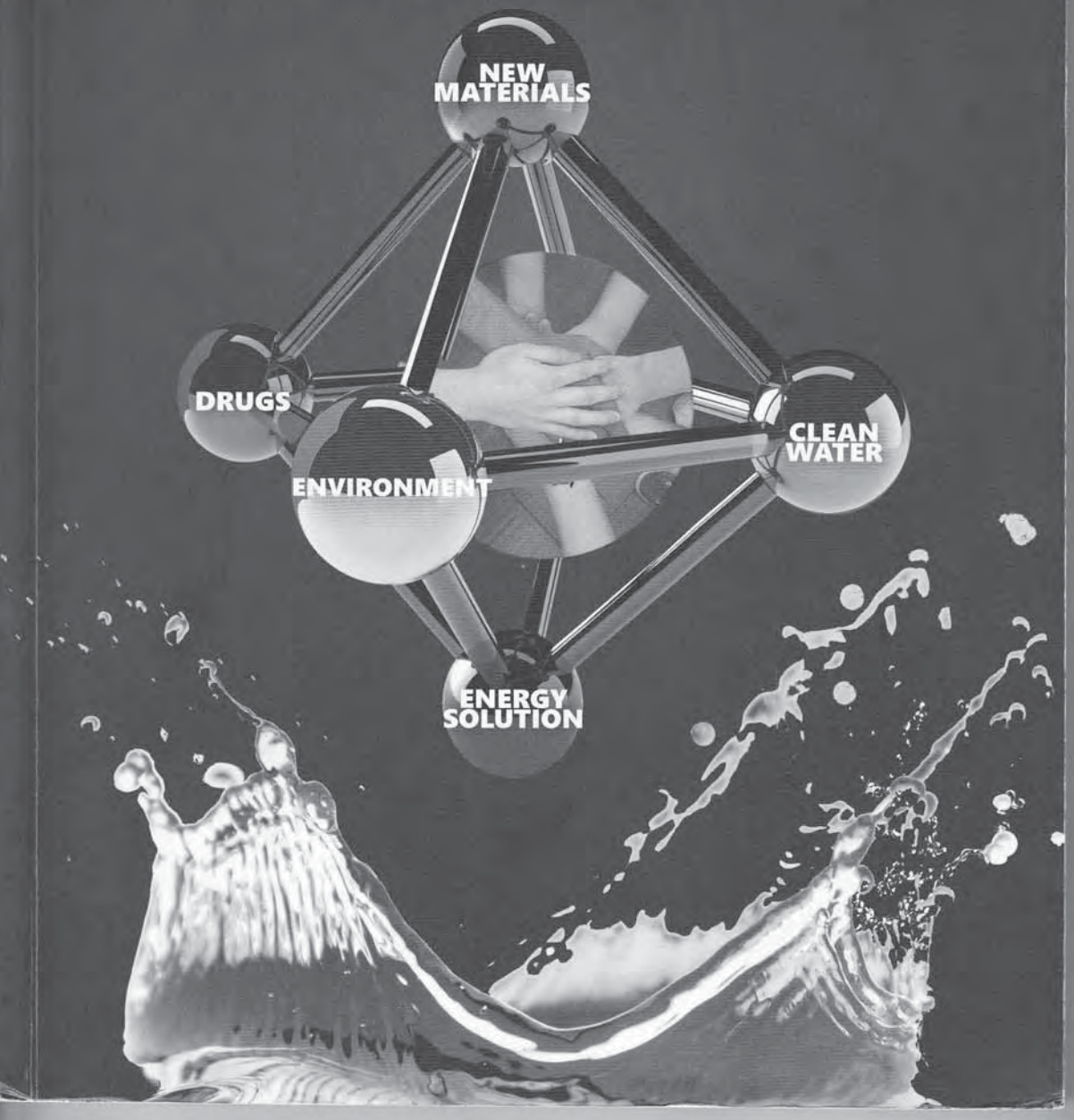
11 - 14 Dec 2012, Singapore
Nanyang Technological University



17:30 OP12 p.37
Interfacial Self-Assembly and Theranostic Applications of Organic-Inorganic Hybrid Nanomaterials
Ken C.-F. Leung, *Hong Kong Baptist University / The Chinese University of Hong Kong*

18:00 OP13 p.38
Biomimetic Total Synthesis of Complex Sesquiterpenoid
Xiaoguang Lei, *National Institute of Biological Sciences*

THU - 13 DEC 2012

Oral Presentation | Classroom 1

08:30 - 10:00 | Session Chair: Sunggak Kim

08:30 OP14 p.39
Formation of Ligand-Assisted Complex of Two RNA Hairpin Loops
Kazuhiko Nakatani, *Osaka University,The Institute of Scientific and Industrial Research*

09:00 OP15 p.40
Protein Total Synthesis
Xuechen Li, *The University of Hong Kong*

09:30 OP16 p.41
Bioactive Compounds from Endophytic and Marine-Derived Fungi
Prasat Kittakoop, *Laboratory of Natural Products, Chulabhorn Research Institute/Chulabhorn Graduate Institute*

11:00 - 12:30 | Session Chair: Somsak Ruchirawat

11:00 OP17 p.42
Highly Efficient Methodologies via Nitrogenation
Ning Jiao, *Peking University, School of Pharmaceutical Sciences*

11:30 OP18 p.43
Efficient synthesis of biologically active chromenone derivatives via Pd-catalyzed direct cross-coupling
Sungwoo Hong, *KAIST*

12:00 OP19 p.44
D-D-π-A Type Organic Dyes for Dye-Sensitized Solar Cells: Structure-Property Relationships
Vinich Promarak, *Department of Chemistry, Faculty of Science, Ubon Ratchathani University*

13:30 - 15:30 | Session Chair: Yixin Lu

13:30 OP20 p.45
Organocatalyst in Total Synthesis
Yujiro Hayashi, *Tohoku University*

14:00 OP21 p.46
Asymmetric Diels-Alder Reactions via HOMO-activation
Ying-Chun Chen, *Department of Medicinal Chemistry, West China School of Pharmacy, Sichuan University*

14:30 OP22 p.47
N-Heterocyclic Carbene Catalysis: Towards Developing New Activation Modes
Robin Chi, *NTU-SPMS-CBC*

15:00 OP23 p.48
Transition Metal-Catalyzed Fluorination
Guosheng Liu, *Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences*

16:30 - 18:00 | Session Chair: Atsushi Nishida

16:30 OP24 *p.49*
Functionalized Nanoparticle for Cell Targeting and Protein Enrichment
Chun-Cheng Lin, *National Tsing Hua University*

17:00 OP25 *p.50*
Internally-Labeled Pyrrolidinyl Peptide Nucleic Acid as Hybridization-Responsive Fluorescence Probes for DNA Sequence Detection
Tirayut Vilaivan, *Department of Chemistry, Faculty of Science, Chulalongkorn University*

17:30 OP26 *p.51*
Catalytic Asymmetric Construction of Stereochemically Diversified Pyrrolidines
Takayoshi Arai, *Chiba University*

18:00 OP27 *p.52*
Structural Revisions/Assignments of Marine Natural Products by Total Synthesis
Tao Ye, *Dept. of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University*

FRI - 14 DEC 2012

Oral Presentation | Classroom 1

08:30 - 10:00 | Session Chair: Minoru Isobe

08:30 OP28 *p.53*
Modulation of oxacarbenium ions: a Simple Approach for Stereoselective Glycosidic bond formations
Kwok Kong Mong, *National Chao Tung University*

09:00 OP29 *p.54*
Ni-Catalyzed Multi-component Coupling Reaction of Organozinc Reagents, Unsaturated Hydrocarbons, and Carbonyl Compounds
Masanari Kimura, *Nagasaki University*

09:30 OP30 *p.55*
Aromatic C-H Bond Functionalization: Transition Metal-Free SET C-H Arylation, Pd-Catalyzed Cross-Dehydrogenative-Coupling (CDC) and Ketone-Directed Hydroxylation Reaction
Fuk Yee Kwong, *The Hong Kong Polytechnic University*

10:30 - 12:00 | Session Chair: Pauline Chiu

10:30 OP31 *p.56*
Weak Hydrogen Bonding as Stereocontrolling Element in Asymmetric Palladium Catalysis
Steve Zhou, *NTU-SPMS-CBC*

11:00 OP32 *p.57*
Synthesis of Azabicycles via Lewis- and Brφnsted-Acid-Promoted Cyclization of N-Containing Cyclic Enynols
Ming-Chang Yeh, *National Taiwan Normal University*

11:30 OP33 *p.58*
Site Selective Borylation of Unactivated Internal C(sp3)–H Bonds Catalyzed by Rh or Ir Complexes with Silica-Supported Monophoshine Ligands
Masaya Sawamura, *Hokkaido University*

WED - 12 DEC 2012

Poster Session A | NTU -

10:30 - 11:30, 15:30 - 16:30

PA01
Design, Synthesis, and Electr Phthalocyanine Analogues
Atsuya Muranaka, *Advanced Elem*

PA02
Collective Syntheses of Tetro
Toshio Nishikawa, *Nagoya Univ*

PA03
The Use of Functionalized Ra Resolution
Kwunmin Chen, *National Taiwan N*

PA04
Structure-based Optimizatio G-quadruplex DNA Stabilize
Edmond Dik Lung Ma, *Hong Kong*

PA05
Selective Synthesis of Hetero
Boshun Wan, *Dalian Institute of Ch*

PA06
Palladium-Catalyzed Arylati Malonate Esters with Aryl H
Hideki Yorimitsu, *Kyoto Universit*

PA07
Pd(II)-Catalyzed C-H Activat Hydrogen Phosphates
Sunggak Kim, *NTU-SPMS-CBC*

PA08
Hydrogen Bonded Aryl Ami
Zhan-Ting Li, *Department of Chemis*

PA09
π-Extended, Expanded Arom Protonation-Coupled Redox
Chang Hee Lee, *Kangwon National U*

PA10
ortho-Methyl Substituted W the DNA strand displacemer
Shigeki Sasaki, *Kyushu University*

PA11
Small Molecule Regulator o
Zhang Yan, *Department of Chemistry*

PA12
Enantioselective Conjugate Catalyzed by Ni(acac)2 and
Biing-Jiun Uang, *National Tsing Hua*

PA13
Deacylation of Unactivated Transamidation
Takashi Ohshima, *Kyushu Universi*

PA14
Muraymycins: A Guide to a
Satoshi Ichikawa, *Hokkaido Universi*

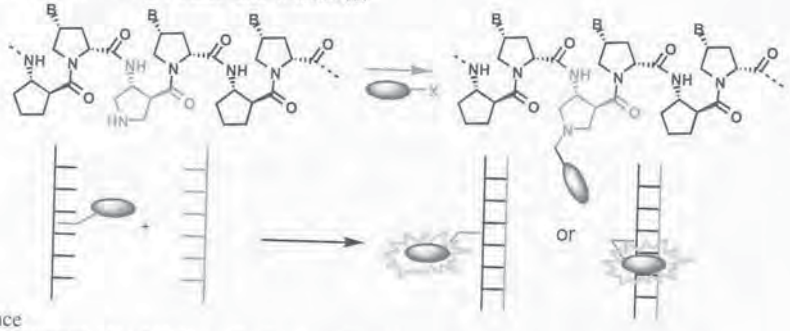
OP25

Internally-Labeled Pyrrolidinyl Peptide Nucleic Acid as Hybridization-Responsive Fluorescence Probes for DNA Sequence Detection

Tirayut Vilaivan,* Boonsong Ditmangklo, Chalothorn Boonlua
Organic Synthesis Research Unit, Department of Chemsitry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand
E-mail: vtirayut@chula.ac.th

Classical molecular beacons require two different labels, usually a fluorophore and a quencher attached to different end of the same DNA strand. The sequence of DNA beacons are designed so that the fluorophore and hte quencher are in close proximity in the normal state, resulting in suppression of the fluorescence. Upon hybridization with the correct DNA target, the fluorophore and quencher are separated and this is accompanied by fluorescence increase. There are attempts to simplify the design of molecular beacons by using just one fluorophore that can change its fluorescence in response to the different microenvironment in the single- and double-stranded DNA. Little work in this area had been applied to peptide nucleic acid (PNA) – a DNA analogue with the deoxyribose phosphate backbone is replaced by a peptide-like backbone. PNA recognizes DNA/RNA with higher affinity and specificity than DNA, and offered a number of other advantages such as complete resistant to proteases and nucleases.

In this work, we summarize progress in the design of hybridization responsive fluorescence probe for DNA sequence determination based on our recently-developed pyrrolidinyl peptide nucleic acid consisting of D-prolyl-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid backbone (acpcPNA). As an example, we had developed reliable reductive alkylation and Click chemistry-based protocols for site-specific labeling of acpcPNA backbone by pyrene or thiazole orange. The pyrene-labeled acpcPNA showed a fluorescence increase (4–14 folds) in the presence of the correct DNA target. The thiazole orange-labeled acpcPNA showed even higher fluorescence increase (up to 100 folds) upon binding to DNA. The specificity can be further improved with the help of S1 nuclease digestion, which allows perfect discrimination between complementary and single mismatched DNA at ambient conditions under a wide sequence context.



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Tirayut Vilaivan (ธีรยุทธ วิไลวัลย์). Chulalongkorn University (B.Sc., 1992), Oxford University (D.Phil., 1996). Associate Professor, Department of Chemistry, Faculty of Science, Chulalongkorn University (since 2004). Research field: peptide nucleic acids, asymmetric catalysis, medicinal chemistry.

OP26

Catalytic Asymme

Department of Ch
1-3

Precise control of stereoche
is a highly desired goal
asymmetric synthesis
functionalized molecules.
exploring efficient asymm
we prepared a library
imidazoline-aminophenol
catalysts, and developed
throughput screening metho
reaction mixtures carried
solid-phase catalysts were di
by circular dichroism (CD).
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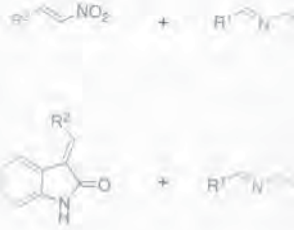
explored to give the tetrasub
pyrrolidines. The chiral



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Chemical Science for Green Community

January 23-25, 2013

Bangsaen Beach

THAILAND

PEPTIDE NUCLEIC ACID PROBE FOR ELECTROCHEMICAL DETECTION OF HUMAN PAPILLOMA VIRUS DNA TYPE 16

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**Corresponding Author: corawon@chula.ac.th*

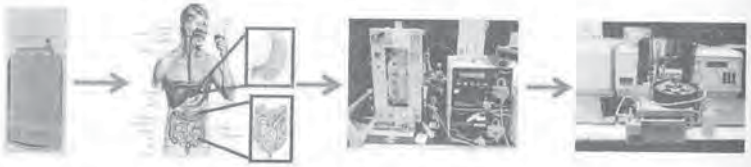
ABSTRACT

A novel electrochemical biosensor for the detection of high-risk human papilloma virus (HPV) DNA type 16 based on pyrrolidinyl peptide nucleic acid (acpcPNA) as a sensor probe was proposed. A 14-mer PNA probe was designed to specifically target the DNA sequence of HPV type 16. The probe was modified at the N-terminus via N-acylation with anthraquinone (AQ) as a redox-active label. The success of the synthesis of the AQ labeled PNA (PNA-AQ) was confirmed by MALDI-TOF mass spectrometry and by thermal denaturation study with a complementary synthetic DNA target with a sequence corresponding to the HPV DNA (T_m= 69.9 ^oC). An inexpensive screen print carbon electrode (SPCE) was used in this study. Ink compositions containing graphite powder, carbon ink and binder solution were mixed together with the ratio of 0.2 g: 1 g: 1 mL, respectively. The PNA-AQ probe (20 μM) was covalently immobilized onto the electrode surface using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) as a coupling agent after the electrochemical pretreatment of electrode. Hybridization between the PNA-AQ probes and the synthetic DNA target was studied by measuring the peak current of AQ using a square-wave voltammetric (SWV) method. The results showed that the redox signal response decreased by three folds after addition of the DNA. This is explained by the rigidity of PNA-DNA duplexes, which affected the accessibility and electron transfer between the AQ label and the electrode surface. It is hoped that this method will be applicable in screening for the HPV-DNA type 16 in the primary stage of cervical cancer in the developing country.

Keywords Human Papilloma Virus (HPV); Peptide Nucleic Acid (PNA); Anthraquinone (AQ); screen print carbon electrode (SPCE); Redox-active label

ANC O 001 SIZE CHARACTERIZATION OF SILVER NANOPARTICLES (AgNPs) PREPARED UNDER VARIOUS CONDITIONS AND UPON INCUBATION IN *IN VITRO* GASTROINTESTINAL DIGESTION

Jakrawan Yostawonkul, Juwadee Shiowatana, Atitaya Siripinyanond
Faculty of Science, Mahidol University, Thailand



Keywords Silver nanoparticles; Tannic acid; Flow field-flow fractionation

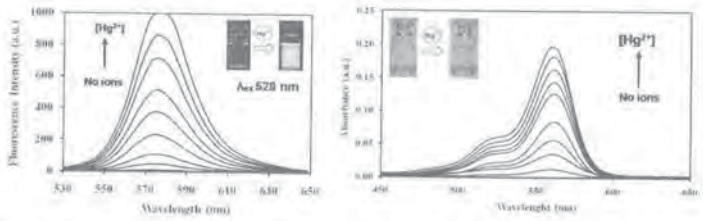
ANC O 002 METHOD DEVELOPMENT FOR ESTIMATION OF *IN VITRO* BIOACCESSIBILITY OF PHYTOESTROGENS IN SOYBEAN MILK USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AFTER DIALYSIS METHOD

N. Butboon, J. Shiowatana, T. Tiensing, A. Siripinyanond
Faculty of Science, Mahidol University, Thailand

Keywords Phytoestrogens; Daidzein; Genistein; Solid-phase extraction; Bioaccessibility

ANC O 003 DUAL OPTICAL DETECTION OF A HIGHLY SENSITIVE MERCURY SENSOR THROUGH FRET PROCESS

O. Hanmeng, T. Puangsamlee, C. Wainiphithapong, K. Suksat, K. Grudpan, N.Wanichacheva
Faculty of Science, Silpakorn University, Thailand



Keywords Mercury sensor; Fluorescence sensor; Hg2+-selectivity

ANC O 004 SIMULTANEOUS DETERMINATION OF ISOPROTURON AND CARBENDAZIM IN WATER SAMPLES BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY

Peeyanun Noyrod, Suchada Chuanuwatanakul, Orawon Chailapakul, Wanida Wonsawat
Faculty of Science, Chulalongkorn University, Thailand

An electrochemical method for simultaneous determination of two pesticides, namely isoproturon and carbendazim, in water samples are presented. The determinations are established by square wave anodic stripping voltammetry (SWASV) using graphene-based electrochemical sensor fabricated in our laboratory. Linear calibration graphs were established in the range of 0.02 - 10.0 mg L^{-1} for isoproturon and 0.50 - 10.0 mg L^{-1} for carbendazim. The limits of detection were 0.02 mg L^{-1} for isoproturon and 0.11 mg L^{-1} for carbendazim. The relative standard deviations were 9.17 and 10.0 % for isoproturon and carbendazim, respectively. The satisfactory recoveries of 106.9% for isoproturon and 85.46% for carbendazim in rice-field water and 92.06% for isoproturon and 84.41% for carbendazim in Chao Phraya river water, were obtained.

Keywords Pesticides; Isoproturon; Carbendazim; Modified-electrodes; Graphene

ANC O 005 MULTILAYER ELECTROCHEMICAL PAPER-BASED ANALYTICAL DEVICE WITH SODIUM DODECYL SULPHATE FOR THE SELECTIVE DETERMINATION OF DOPAMINE IN HUMAN SERUM

Poomrat Rattanarat, Wijitar Dungchai, Weena Siangproh, Orawon Chailapakul, Charles S. Henry
Faculty of Science, Chulalongkorn University, Thailand

A modern platform of paper-based analytical device for selective detection of dopamine over uric acid and ascorbic acid in serum is introduced. Multilayer paper-based analytical device was constructed for preconcentration and selectively electrochemical detection of catecholamines in complex matrix. DA determination in the presence of AA and UA was performed using square-wave voltammetry between −0.2 and 0.8 V vs. Ag/AgCl, and linear range and detection limit (S/N = 3)were found to be 1–100 μM and 0.37 μM, respectively. Nonetheless, novel preconcentration mechanism based on electrostatic interactions between dopamine and sodium dodecyl sulfate will be discussed.

Keywords Paper-based analytical device; Dopamine; Ascorbic acid; Uric acid

ANC O 006 PEPTIDE NUCLEIC ACID PROBE FOR ELECTROCHEMICAL DETECTION OF HUMAN PAPILLOMA VIRUS DNA TYPE 16

Sakda Jampasa, Wanida Wonsawat, Tirayut Vilaivan, Orawon Chailapakul
Faculty of Science, Chulalongkorn University,Thailand

A novel electrochemical biosensor for the detection of high-risk human papilloma virus (HPV) DNA type 16 based on pyrrolidinyl peptide nucleic acid (acpcPNA) as a sensor probe was proposed. Anthraquinone (AQ) was used as a redox-active label. The success of the synthesis of the AQ labeledPNA (PNA-AQ) was confirmed by MALDI-TOF mass spectrometry and by thermal denaturation study. An inexpensive screen print carbon electrode (SPCE) was used in this study. The PNA-AQ probe was covalently immobilized onto the electrode surface. The result showed that, the redox signal response decreased by three folds after the hybridization between the PNA-AQ probes and the synthetic DNA target.

Keywords Human Papilloma Virus (HPV); Peptide Nucleic Acid (PNA)

ORAL PRESENTATION PROGRAM

Thursday, 24 Jan 2013

Presidential Symposium : Thai-Korean Nanotechnology (SYM-II)			
24 Jan 2013		15.30 - 17.20 Pacific 2	Room:
17.00-17.30	**SYM-I-008**	Kajornsak Fuangnawakij	Nanostructured Spinel Oxides and Their Applications in Heterogeneous Catalysis
Analytical Chemistry (ANC-I)			
24 Jan 2013		10.40 - 12.10 Pacific 1	Room:
Chairperson : Assoc.Prof.Dr.Orawan Chailapakul Co-Chair : Dr.Apinya Navakun			
10.40-11.10	**ANC-I-001**	Takashi Kaneta	Selective Detection of Glycoproteins by Two-Color Laser-Induced Fluorescence in Capillary Electrophoresis
11.10-11.30	ANC-O-001	Jakrawan Yostawonkul	Size Characterization of Silver Nanoparticles(AgNPs) Prepared Under Various Conditions and Upon Incubation in In Vitro Gastrointestinal
11.30-11.50	ANC-O-002	Nathanika Butboon	Method Development for Estimation of In Vitro Bioaccessibility of Phytoestrogens in Soybean Milk Using High Performance Liquid Chromatography (HPLC) after Dialysis Method
11.50-12.10	ANC-O-003	Thamon Puangsamlee	Dual Optical Detection of a Highly Sensitive Mercury Sensor Through Fret Process
Analytical Chemistry (ANC-II)			
24 Jan 2013		13.10 - 15.10	Room: Pacific1
Chairperson : Dr.Yupaporn Sameenoi ; Co-chair : Dr.Nudnuda Rodthongkhum			
13.10-13.40	**ANC-I-002**	Charles Sherman Henry	Personal Exposure Assessment Using a Paper-Based Analytical Devices

ORAL PRESENTATION PROGRAM

Thursday, 24 Jan 2013

Analytical Chemistry (ANC-II)			
24 Jan 2013		13.10 - 15.10	Room: Pacific1
Chairperson : Dr.Yupaporn Sameenoi ; Co-chair : Dr.Nudnuda Rodthongkhum			
13.40-14.00	ANC-O-004	Peeyanun Noyrod	Simultaneous Determination of Isoproturon and Carbendazim in Water Samples by Square Wave Anodic Stripping Voltammetry
14.00-14.20	ANC-O-005	Poomrat Rattanarat	Multilayer Electrochemical Paper-Based Analytical Device with Sodium Dodecyl Sulphate for the Selective Determination of Dopamine in Human Serum
14.20-14.40	ANC-O-006	Sakda Jampasa	Peptide Nucleic Acid Probe For Electrochemical Detection of Human Papilloma Virus DNA Type 16
14.40-15.00	ANC-O-007	Thanakorn Pluangklang	Improvements of Membraneless Vaporization Device and Its Application with Sequential Injection Analysis
Analytical Chemistry (ANC-III)			
24 Jan 2013		15.30 - 17.20	Room: Pacific 1
Chairperson : Prof.Dr. Charles Sherman Henry ; Co-Chair : Dr.Yupaporn Sameenoi			
15.30-16.00	**ANC-I-003**	Nadnudda Rodthongkum	Selective Enrichment and Sensitive Detection of Cancer Biomarkers Using Amphiphilic Nanoassemblies along with MALDI-MS
16.00-16.20	ANC-O-008	Nupattaranee Thammasoontaree	Ultra Fast Liquid Chromatography Coupled With Graphene Modified Electrode for Simultaneous Determination of Sulfonamides
16.20-16.40	ANC-O-009	Nipapan Ruecha	Nanodroplets Graphene-Polymer as a Novel Electrodes for Cholesterol Detection
16.40-17.00	ANC-O-010	Jirapom Kitikul	Development of Glutamate Biosensor Based on Immobilized Glutamate Oxidase on the Chitosan Cross Linked with Carbon Nanotube Modified Gold Nanowire
17.00-17.20	ANC-O-011	Dakhil.N.Taha	Simple and Highly Sensitive FIA Method for Determination of Vanadium (V) Ion by Using Merging Z zone Technique

การสังเคราะห์พิร์โรลิดินิลเพปไทด์นิวคลีอิกแอซิด (พีเอ็นเอ) ที่มียูนิเวอร์ซัลเบส

SYNTHESIS OF PYRROLIDINYL PEPTIDE NUCLEIC ACID (PNA) CARRYING UNIVERSAL BASE

วิมลมาศ ศรีนาราง, ชโลธร บุญเหลือ, ณัฐวุฒิ โยธาพันธ์, โชติมา วิไลวัลย์, ธีรยุทธ วิไลวัลย์*

Wimonmas Srinarang, Chalothorn Boonlua, Nuttawut Yothapan, Chotima Vilaivan, Tirayut Vilaivan*

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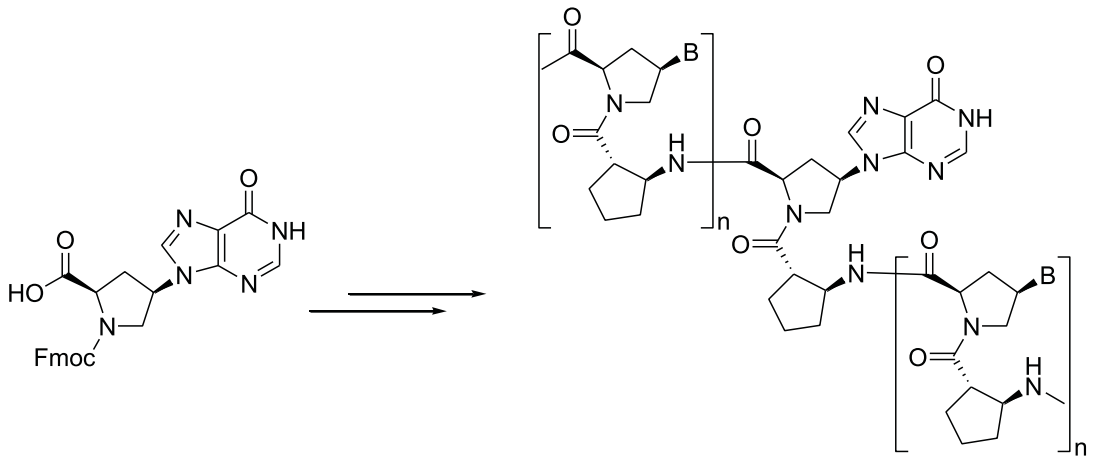
บทคัดย่อ: การจดจำระหว่างดีเอ็นเอสองสายอาศัยการเข้าคู่กันอย่างจำเพาะเจาะจงของเบสไทมีน- อะดีนีน และไซโตซีน- กัวนีน แต่ความจำเพาะเจาะจงดังกล่าวเป็นสิ่งที่ไม่พึงประสงค์ในบางวัตถุประสงค์ ตัวอย่างเช่น การตรวจสอบลำดับเบสของดีเอ็นเอที่มีลำดับเบสคล้ายคลึงกัน แต่ไม่เหมือนกันเสียทีเดียว หากส ามารถเตรียมยูนิเวอร์ซัลโพรบที่สามารถจับยึดกับดีเอ็นเอที่มีความแตกต่างกันเพียงเล็กน้อยเหล่านี้ได้ดีพอๆ กันก็จะช่วยลดจำนวนโพรบที่ต้องสังเคราะห์ลงไปได้มาก โดยยูนิเวอร์ซัลโพรบจะต้องมียูนิเวอร์ซัลเบสซึ่งแสดงสมบัติการจับยึดกับเบสธรรมชาติทั้งสี่ชนิดได้ใกล้เคียงกัน ในงานวิจัยที่ผ่านมามีผู้สังเคราะห์และศึกษาการจับยึดของดีเอ็นเอที่มียูนิเวอร์ซัลเบสอยู่บ้าง แต่มีการศึกษาพีเอ็นเอที่มียูนิเวอร์ซัลเบสมีน้อยมาก ดังนั้นในงานวิจัยนี้จึงมีแนวคิดที่จะสังเคราะห์พีเอ็นเอชนิดพิร์โรลิดินิลที่มีอิโนซีนเป็นยูนิเวอร์ซัลเบสและศึกษาสม บัติการจับยึดของ พีเอ็นเอดังกล่าวกับดีเอ็นเอที่มีเบสธรรมชาติโดยเริ่มจากการสังเคราะห์อิโนซีนยูนิเวอร์ซัลเบสโมโนเมอร์ ที่มีหมู่ปกป้องเป็น Fmoc โดยพบว่าสามารถสังเคราะห์มอนอเมอร์ดังกล่าวได้ และสามารถสังเคราะห์พิร์โรลิดินิลพีเอ็นเอที่มีมอนอเมอร์ดังกล่าวได้จริง และจะได้ศึกษาความสามารถในการเป็นยูนิเวอร์ซัลโพรบของพีเอ็นเอที่สังเคราะห์ได้ต่อไป

Abstract: Recognition between two strands of DNA relies on highly specific base pairing between thymine-adenine and cytosine-guanine. Such a high specificity is undesirable in certain purposes, for example, in determination of DNA sequences that are closely related, but not identical. A universal probe that can bind to these similar DNA targets with equal affinities will reduce the number of probes to be synthesized. The universal probe must contain one or more universal base which can bind to all four natural nucleobases with equal strength. A considerable progress in this area has been made with DNA probe, but not PNA. The purpose of this work is to synthesize and determine the DNA binding properties of a new pyrrolidinyl PNA carrying inosine as a universal base. The Fmoc-protected inosine universal base monomer has been successfully synthesized and incorporated into the pyrrolidinyl PNA. The ability of the synthesized PNA to behave as universal probe is being investigated.


HO
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การสังเคราะห์เพปไทด์นิวคลีอิกแอซิดชนิดใหม่ที่มีสะพานเชื่อมเป็นบีตา-2-อะมิโนไซโคลโพรเพนคาร์บอกซิลิกแอซิด

SYNTHESIS OF NOVEL PEPTIDE NUCLEIC ACIDS BEARING *β*-2-AMINOCYCLOPROPANE CARBOXYLIC ACID SPACER

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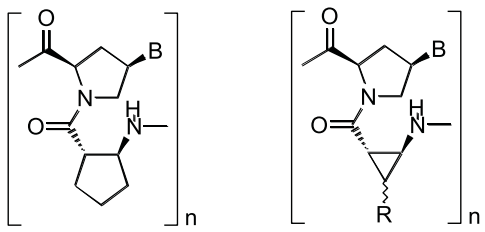
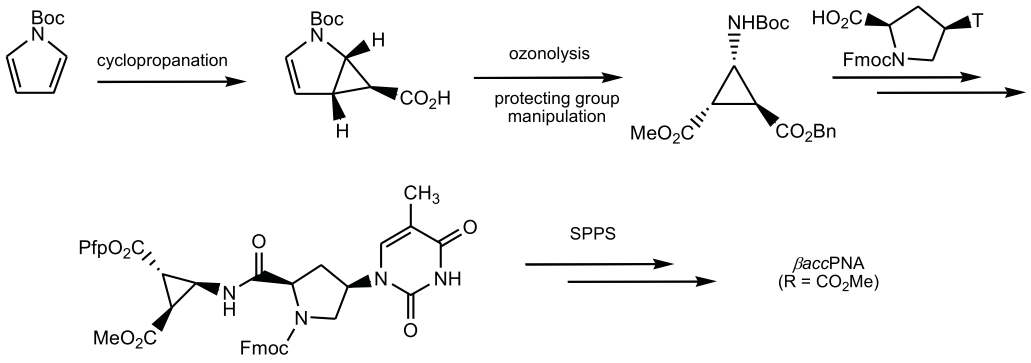
บทคัดย่อ: การสังเคราะห์และสมบัติการจับยึดดีเอ็นเอของเพปไทด์นิวคลีอิกแอซิด (พีเอ็นเอ) ที่มีคอนฟอร์เมชันจำกัดเป็นหัวข้อที่มีผู้ให้ความสนใจมากในช่วงหลายปีที่ผ่านมา กลุ่มวิจัยของเราได้เคยรายงานพิโรลิดินิลพีเอ็นเอชนิดใหม่ที่ประกอบไปด้วยโปรลีนและกรดเบตาอะมิโนที่เป็นวงห้าเหลี่ยมที่ชื่อ (1*S*,2*S*)-2-อะมิโนไซโคลเพนเทนคาร์บอกซิลิกแอซิดในโครงสร้างหลัก (*acpc*PNA) พีเอ็นเอชนิดนี้แสดงสมบัติการจับยึดกับดีเอ็นเอที่มีลำดับเบสคู่สมได้อย่างแข็งแรงและจำเพาะเจาะจง แต่แสดงความแข็งแรงและจำเพาะเจาะจงต่ออาร์เอ็นเอน้อยกว่า แม้ว่าพีเอ็นเอที่มีกรดบี ตาอะมิโนแบบวงห้าเหลี่ยมเป็นตัวเชื่อมจะให้ผลการทดลองที่ดี แต่วงห้าเหลี่ยมยังคงมีความยืดหยุ่นในโครงสร้างค่อนข้างสูง ซึ่งอาจมีผลกระทบในทางลบต่อการจับยึดกับดีเอ็นเอ ดังนั้นในงานวิจัยนี้จึงมุ่งเ น้นถึงการสังเคราะห์พีเอ็นเอชนิดใหม่ (*β-acc*PNA) ที่มีตัวเชื่อมเป็นกรดบีตาอะมิโนที่เป็นวงสามเหลี่ยมซึ่งเป็นโครงสร้างที่มีความยืดหยุ่นน้อยกว่าเดิม การสังเคราะห์เริ่มจากการเตรียมมอนอเมอร์ของ *β-acc*PNA ที่มีไทมีนเป็นเบสและมีหมู่ปกป้องเป็น Fmoc ในรูปแบบที่เป็นอิแนนชิโอเมอร์เดี่ยวโดยเริ่มต้นจากเอ็น-บอค-พิร์โรล จากนั้นจึงนำมอนอเมอร์ดังกล่าวไปใช้ในการสังเคราะห์พีเอ็นเอที่เป็นไทมีนโอลิโกเมอร์โดยใช้วิธีการสังเคราะห์บนวัฏภาคของแข็ง และจะได้ศึกษาสมบัติการจับยึดกับดีเอ็นเอเทียบกับพีเอ็นเอที่มีตัวเชื่อมเป็นวงแหวนห้าเหลี่ยมต่อไป

Abstract: Synthesis and DNA binding properties of conformationally restricted peptide nucleic acid (PNA) have been the subject of intense research in the past few years. Our group previously reported a pyrrolidinyl peptide nucleic acids consisting of proline and a five-membered cyclic β-amino acid namely (1*S*,2*S*)-2-aminocyclopentanecarboxylic acid in the backbone (*acpc*PNA). This PNA system showed a strong binding affinity and high sequence specificity with DNA, but less so with RNA targets. Although, the 5-membered cyclic β-amino acid spacer has been successfully employed as spacer in PNA, the 5-membered ring system appeared to be flexible which could compromise the

DNA binding properties. In this work, a new PNA system bearing potentially more rigid 3-membered cyclic β-amino acid (β-*acc*PNA) was synthesized. The *N*-Fmoc protected *β-acc*PNA monomers containing thymine base have been successfully synthesized in enantiomerically pure form starting from pyrrole. These monomers were used to synthesize homothymine PNA by solid phase peptide synthesis. DNA binding properties of the PNA with DNA will be explored and compared with the 5-membered ring analogue.



*acpc*PNA *βacc*PNA



Abstracts

THE 36th CONGRESS on SCIENCE and TECHNOLOGY of THAILAND (STT 36)

การประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 36 (วทท 36)

Towards a Better Society through Science and Technology

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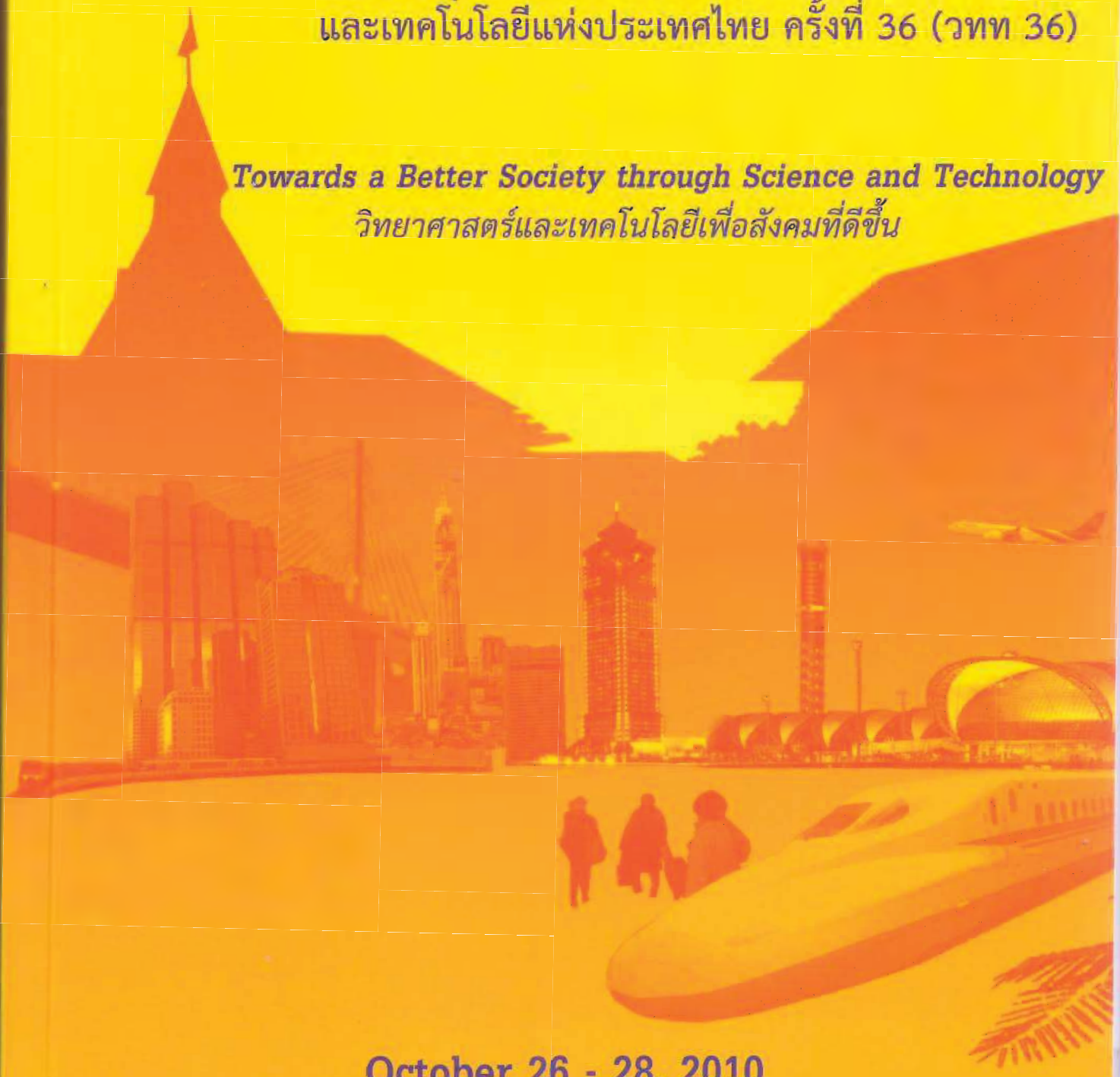

October 26 - 28, 2010

Venue: Bangkok International Trade & Exhibition Centre (BITEC). Bangkok, Thailand.

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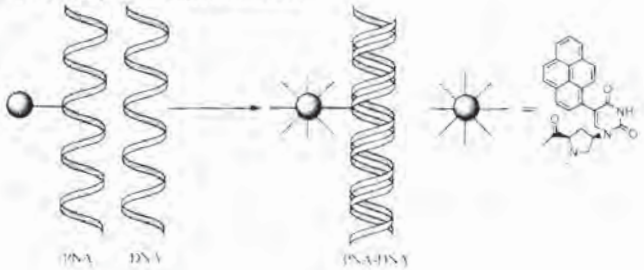
Abstract: Traditional fluorescent labeled probes for nucleic acid hybridization carrying a fluorop either end of the molecule are not responsive to the hybridization state since the position of the fluor is remote from the base pair. In this work a pyrene-labeled uridine pyrrolidinyl peptide nucleic acid monomer was synthesized and incorporated into a PNA molecule. The modified PNA retains the ab specifically recognize its DNA target as determined by melting temperature (T_m) experiments. Further the fluorescence of the modified PNA was enhanced significantly when the PNA was hybridized complementary target. As a result, the pyrene-modified is potentially useful as a hybridization resp fluorescence probe for DNA sequence determination.



C3_C0054: Carbazole alkaloids from *Murraya koenigii* stems

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Natural Products Laboratory Research, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand
*e-mail: solica_b@hotmail.com

Abstract: Chemical investigations of *Murraya koenigii* stems led to the isolation and identification of se carbazole alkaloids, murrayazoline (**1**), mahanimbine (**2**), *O*-methylmahanine (**3**), girinimbine (**4**), muko (**5**), murrayazolidine (**6**) and murrayanine (**7**). Their structures were elucidated by spectroscopic meth Moreover, structure of **1** was also comfirmed by X-ray diffraction data.

C3_C0055: Flavanones and dihydrochalcones from *Etlingera littoralis* rhizomes

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[2] Natural products Research Laboratory, School of Science of Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand
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Abstract: The studies of chemical constituents from rhizomes of *Etlingera littoralis* led to the isolation two flavanones, pinocembrin (1) and pinostrobin (2) and four dihydrochalcones, 2', 6'-dihydroxy-4 methoxydihydrochalcone (3), 2',4',6'-trihydroxydihydrochalcone (4) , 2',6'-dihydroxy-4',4-dimethoxydihy drochalcone (5) and 2',6',4-trihydroxy-4'-methoxydihydrochalcone (6), respectively. All structures wer characterized by extensive 1D and 2D NMR spectroscopic methods except compound 3 was characterize by using ^{1}H NMR and X-ray diffraction data.

C3_C0056: Coumarins from *Feronia limonia* roots

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Abstract: Phytochemical investigation of the roots of *Feronia limonia* led to the isolation of four coumarins: isopimpinellin (**1**), osthol (**2**), auraptene (**3**) and 6-methoxy-7-geranyloxycoumarin (**4**). Their structures were elucidated by extensive 1D and 2D NMR spectroscopic methods.

C3_C0058: Chemical interactions between β-carotene and chitosan by coagulation and flocculation: A study on light scattering intensity and infrared spectrum

6th NCCC
The Sixth National Chitin Chitosan Conference

Proceedings

The Sixth National Chitin–Chitosan Conference

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Miracle Grand Convention Hotel,
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Quaternized Chitosan Particles Prepared by Self Assembly of Amphiphilic Chitosan

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[2]Center for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University
[3]Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
Tel: 0-2218-7627 ext 102, Fax: 0-2218-7598, E-Mail: vipavee.p@chula.ac.th

บทคัดย่อ

การตรึงหมู่ทาโลอิลซึ่งเป็นหมู่ที่ไม่ชอบน้ำและตรึงหมู่พอลิ(เอทิลีน ไกลคอล) หรือ เอ็น-[(2-ไฮดรอกซิล-3-ไตรเมทิลแอมโมเนียม)]โพรพิลซึ่งเป็นหมู่ที่ชอบน้ำทำได้โดยการทำปฏิกิริยาทางเคมีของหมู่อะมิโนและหมู่ไฮดรอกซิลบนโครงสร้างไคโทซาน ตามลำดับ สามารถวิเคราะห์เปอร์เซ็นต์การแทนที่ของแต่ละหมู่ที่ตรึงได้โดยโปรตอนเอ็นเอ็มอาร์ สามารถเหนี่ยวนำให้เกิดอนุภาคโดยการประกอบตัวเองของอนุพันธ์แอมฟิฟิลิกไคโทซานที่ได้ผ่านไดอะไลซิสในน้ำ การเพิ่มความเป็นประจุบวกของหมู่ควอเทอร์นารีแอมโมเนียมบนผิวของอนุภาคไคโทซานชนิดที่ตรึงด้วยหมู่พอลิ(เอทิลีน ไกลคอล) จำเป็นต้องอาศัยการทำปฏิกิริยาเมทิลเลชันแบบต่างวัฏภาค ซึ่งแตกต่างจากอนุภาคที่มีหมู่เอ็น-[(2-ไฮดรอกซิล-3-ไตรเมทิลแอมโมเนียม)]โพรพิล ในงานวิจัยนี้สนใจที่ศึกษาผลของน้ำหนักโมเลกุลไคโทซาน ชนิดของหมู่ที่ชอบน้ำ ตลอดจนเปอร์เซ็นต์การแทนที่ของหมู่ที่ชอบน้ำและไม่ชอบน้ำต่อขนาด/สัณฐานวิทยา

Abstract

Phthaloyl groups (Ph) as hydrophobic entities and poly(ethylene glycol) (PEG) or *N*-[(2-hydroxyl-3-trimethylammonium)]propyl (HTAP) as hydrophilic entities were immobilized on chitosan backbone via chemical reactions of amino and hydroxyl groups, respectively. Degree of substitution (%DS) of each functional group was determined by ^{1}H NMR. Formation of particles was then induced by self assembly of the resulting amphiphilic chitosan derivatives upon dialysis in water. Unlike the chitosan particles having HTAP moieties, a subsequent heterogeneous methylation was necessary to introduce positively charged quaternary ammonium groups to the chitosan particles having PEG moieties. In this research, effects of molecular weight of chitosan, type of hydrophilic groups as well as relative %DS of hydrophobic/hydrophilic groups on the particle size/morphology will be determined.

Introduction

Chitosan is a partially deacetylated form of chitin, the second most abundant polysaccharide next to cellulose, which was found in the exoskeleton of insect, shell of crustaceans and fungal cell walls. Chitosan has a number of unique characteristics such as biocompatibity, biodegradability, low toxicity and antibacterial [1]. Chitosan shows its antibacterial activity only in acidic medium above its pKa (pH 6.5). Introducing quaternary ammonium groups to chitosan via quaternization of amino groups has been recognized as a potential way to enhance the antibacterial activity of chitosan in a broader pH range. In addition, it has been recently demonstrated that chitosan as well as quaternized chitosan in the form of nanoparticles exerted higher antibacterial activity than chitosan solution because the greater surface area of the particles provides intimate contact with the surface of bacterial cells. In this research, we have proposed to generate chitosan particles having positive charges of quaternary ammonium groups by self assembly of amphiphilic chitosan. It is believed that this approach should be an efficient way to fabricate the sub-micron particles whose size can be tuned by varying hydrophobic and hydrophilic proportions and types and eligible for a large scale production.

Materials and Methods

Materials:

Chitosan flakes (degree of deacetylation 85%, molecular weight 45,000 Da and degree of deacetylation 95%, molecular weight 100,000 Da) were obtained from Seafresh Chitosan (Lab) Co., Ltd. (Thailand). Phthalic anhydride, poly(ethylene glycol)methyl ether (mPEG) (Mn = 5000), succinic anhydride (SA), *N*-hydroxysuccinamide (NHS) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDC) were purchased from Aldrich. Methyl iodide (MeI), sodium hydroxide (NaOH), sodium iodide (NaI), and glycidyltrimethylammonium chloride (GTMAC) were purchased from Fluka (Switzerland). Methanol (MeOH) was purchased from Merck (Germany). Dimethyl formamide (DMF) was purchased from Carlo. All reagents and materials are analytical grade and used without further purification.

Methods:

Synthesis of phthaloylchitosan (PhCS) was done by a reaction of 3.0 g chitosan with phthalic anhydride (3 mole equiv.) in 20 mL DMF at 110 °C under N_2 atmosphere for 6 h then the temperature was reduced to 60°C and left overnight under N_2 atmosphere. Light yellow powder was obtained as a product after precipitation in cool water and dried *in vacuo*.

Synthesis of poly(ethylene glycol) methyl ether terminated with carboxyl groups (mPEG-COOH) [2]: was done by reacting mPEG (3g) with SA (1 mole equiv. to mPEG) in DMF 10 mL using pyridine as a catalyst at 60°C overnight. White powder was obtained after precipitation in diethyl ether and dried *in vacuo*.

Preparation of amphiphilic chitosan particles

- *Phthaloylchitosan-graft-mPEG (PhCS-g-mPEG) particles* [3]: PhCS(0.2g) was reacted with mPEG-COOH (1, 3, 5 mole equiv.) in 4 mL DMF. NHS (1 mole equiv.) was added and stirred until the solution became clear. Then EDC (1 mole equiv.) was added and stirred for 24 h. Light yellow powder was obtained after dialysis against deionized water for 4 days and lyophilization.
- *Quaternized PhCS-graft-mPEG (QPhCS-g-mPEG) particles:* An anhydrous methanol solution (10 mL) was added into a flask containing 0.05 g PhCS-g-mPEG particles. NaOH (0.13 g, 3.3 mmol) and NaI (0.28 g, 1.9 mmol) were added to the flask. Subsequently, MeI (0.4 mL, 6.4 mmol) was added every 4 h to the mixture. The reaction mixture was stirred at 50°C for 8 h. The QPhCS-*g*-mPEG particles were isolated by centrifugation at 10,000 rpm for 30 min. Supernatant was discarded and the particles were extensively rinsed with methanol and dried *in vacuo*.
- *Phthaloylchitosa-grafted-HTAP (PhCS-g-HTAP) particles:* PhCS (0.2g) was dissolved in 4 mL DMF at ambient temperature. Then GTMAC (1, 3, 5 mole equiv.) was added into the solution. The reaction was stirred at 70°C for 24 h. Light yellow powder was obtained after dialysis against deionized water for 4 days and lyophilization.

Results and Discussion

^{1}H NMR technique was used to confirm the success of amphiphilic chitosan particle formation. Signals at 3.1 ppm appearing in Fig.1C and 1D can be assigned to $-N^+(CH_3)_3$ groups of QPhCS-*g*-mPEG and PhCS-*g*-HTAP, respectively. The signal at 2.1 ppm was assigned to the methoxy group of mPEG (Fig.1B) while the signal at 7.2-8.0 ppm assigned to aromatic groups of phthaloyl entities shown in all spectra.

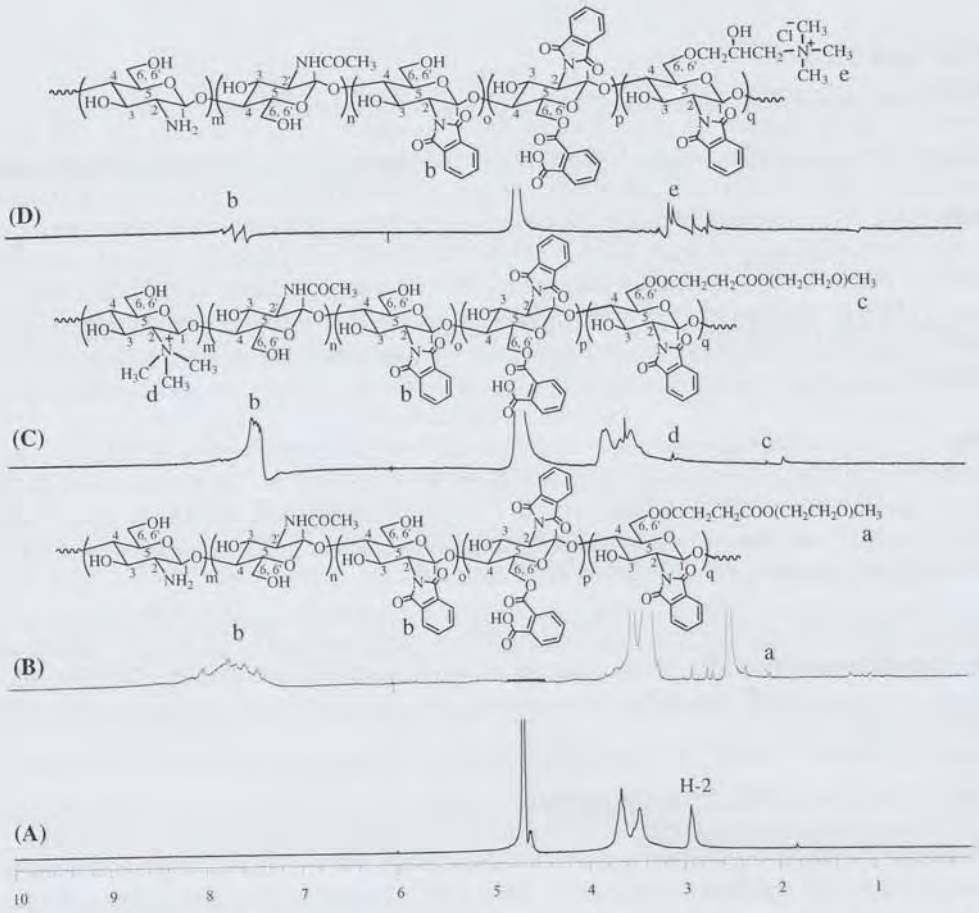

Fig.1: ^{1}H NMR spectra of (A) chitosan, (B) PhCS-*g*-mPEG, (C) QPhCS-*g*-mPEG, and (d) PhCS-*g*-HTAP.

Degree of substitution (%DS) of phthaloyl groups were determined from the relative ratio between the integral of H signal from phthaloyl groups and the signal of H-2 from glucosamine units. As shown in Table 1, %DS of phthaloyl groups in PhCS was 72 and 52% for the chitosan having molecular weight of 45,000 Da and 100,000 Da, respectively. %DS of mPEG was determined from the relative ratio between the integral of H signal from -OCH_3 in mPEG and the signal of H-2 from glucosamine units. Degree of quaternization (%DQ) of QPhCS-*g*-mPEG and PhCS-*g*-HTAP were evaluated from the relative ratio between the integral of H signal from $(CH_3)_3$ and the signal of H-2 from glucosamine units. As demonstrated in Table 1 and 2, the relative ratio between hydrophobic entities (%DS of Ph) and the hydrophilic entities (%DS of mPEG or HTAP) can be varied as a function of mole ratio of PhCS:mPEG and PhCS:HTAP in the case of PhCS-*g*-mPEG, and PhCS-*g*-HTAP, respectively. %DQ was ranged from 6 to 17%.

Table 1. %DS and %DQ of QPhCS-*g*-mPEG

Sample	Mole of PhCS:mPEG	%DS of PhCS	%DS of mPEG	%DQ
$PhCS_{45,000}$-*g*-mPEG	5:1	72	2.67	8.60
	5:3		27.3	9.33
	5:5		16.0	16.1
$PhCS_{100,000}$-*g*-mPEG	5:1	52	5.67	4.00
	5:3		17.3	10.7
	5:5		11.6	10.5

Table 2. %DS of PhCS-*g*-HTAP

Sample	Mole of PhCS:HTAP	%DS of PhCS	%DS of HTAP
$PhCS_{45,000}$-*g*-HTAP	5:1	72	6.56
	5:3		15.1
$PhCS_{100,000}$-*g*-HTAP	5:1	52	5.89
	5:3		16.8

Morphological appearances of PhCS-*g*-mPEG particles determined by SEM are shown in Fig.2. The particles are spherical in shape and have an average size in a range of 100-150 nm. The morphologies of other particles and zeta potential of all particles are yet to be determined to verify whether the sub-micron particles having positive charges can be formed.

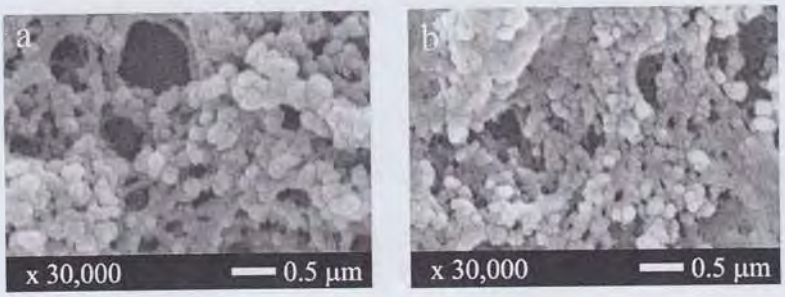

Fig.2: SEM micrographs of (a) $PhCS_{100,000}$-*g*-mPEG (5:1); (b) $PhCS_{45,000}$-*g*-mPEG (5:1)

Conclusion

Amphiphilic chitosan can be successfully synthesized. Self-assembly of the resulting derivatives upon dialysis gave stable colloidal solution in water at room temperature. According to ^{1}H NMR analysis, the relative %DS of hydrophobic/hydrophilic groups can be varied as a function of reagent mole ratio. SEM analysis of representative particles suggested that the sub-micron particles with well-defined spherical morphology can be formed. The investigation on the effects of molecular weight of chitosan, type of hydrophilic groups as well as relative %DS of hydrophobic/hydrophilic groups on the particle size/morphology and zeta potential is on-going and will be presented at the meeting.

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รวมบทคัดย่อ

การประชุมวิชาการเสนอผลงานวิจัยระดับบัณฑิตศึกษา ครั้งที่ 11

บัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น
GRADUATE SCHOOL KHON KAEN UNIVERSITY

Detection of *HLA-B*5801* by In-House PCR-SSP

วิธีการตรวจหาเอชแอลเอบี 5801 ด้วยวิธีพีซีอาร์เอสเอสพี

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ABSTRACT

Pharmacogenetics in HLA allele is very useful for risk assessment in life-threatening drug hypersensitivity. In 2004, *HLA-B*1502* first showed strong association with carbamazepine hypersensitivity in Asian ethnicity. Another association, *HLA-B*5801* involves with allopurinol hypersensitivity with 26% mortality rate. Since allele frequency of *HLA-B*5801* is quite common in some Asian ethnicity, it is important to develop a rapid and cost effective test for Asian, including our Thai population. To get HLA typing result, commercially available PCR-SSP is too expensive. As a result, in-house PCR-SSP would be the most interesting candidates comparing to other molecular techniques. The results showed that only one set of primer can differentiate *HLA-B*5801* from other alleles specifically in 200 DNA samples with 100% sensitivity and >99.9% specificity. The benefit of these tests would help patients to avoid any life-threatening adverse consequences from allopurinol.

บทคัดย่อ

ในปัจจุบันสามารถทำนายการเกิดการแพ้ยาอย่างรุนแรงได้โดยการหาอัลลีลของเอชแอลเอยีน โดยเอชแอลเอแรกที่มีการรายงานคือ เอชแอลเอบี 1502 ซึ่งแสดงความสัมพันธ์ในการเกิดการแพ้ยารุนแรงกับยากันชักชื่อคาร์บามาซีพีน อีกยีนหนึ่งคือเอชแอลเอบี 5801 กับยาโรคเกาต์ชื่ออัลโลพูรินอล ยีนเหล่านี้มีความสำคัญมากเนื่องจากเป็นยีนที่พบมากในกลุ่มประชากรเอเชีย ร่วมกับอัตราการตายอยู่ประมาณ 26 เปอร์เซ็นต์ ดังนั้นจึงมีความสำคัญในการหาวิธีหายีนที่ได้ผลถูกต้องราคาเหมาะสม และรวดเร็ว วิธีหนึ่งก็คือ พีซีอาร์เอสเอสพี ในปัจจุบันมีชุดตรวจสำเร็จแต่ราคายังคงแพงเกินไป จึงควรมีการพัฒนาพีซีอาร์เอสเอสพีอย่างง่าย และราคาถูกเพื่อใช้เอง จากผลการทดสอบพบว่าสามารถทำนายเอชแอลเอบี 5801 ในตัวอย่างดีเอนเอจำนวน 200 รายได้ความไว 100 เปอร์เซ็นต์ และแม่นยำ >99.9% ประโยชน์ของผลการทดลองครั้งนี้ทำให้สามรถช่วยคนไข้ไม่ให้เกิดอาการแพ้ที่รุนแรงถึงชีวิตได้ตั้งแต่ก่อนการให้ยา

Key Words: *HLA-B*5801*, PCR-SSP
คำสำคัญ: เอชแอลเอบี 5801 พีซีอาร์เอสเอสพี

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1. Introduction

Drug response in each patient is different. Up to date, there are many evidence on genetic factors which play an important role in these responses, such as cytochrome P450, thiopurine methyltransferase and human leukocyte antigen (HLA) (Ingelman-Sundberg, 2008). Study in inherited predisposing factors which determine drug response is widely known as pharmacogenetics (Wolf et al., 2000). These diverse reactions include not only level of effectiveness in treatment, but also life-threatening severe symptom. For severe drug-induced reaction aspect, although the incident rate is quite low, identification of these genetic markers helps reduce cause of death dramatically. The strongest and the most specific marker for risk assessment so far is HLA gene (Ingelman-Sundberg, 2008).

The poorest prognosis among severe drug-induced skin reactions are Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (Mockenhaupt, 2009). They are a life-threatening syndrome with high mortality rate. The appearances on mouth, lips, conjunctiva and genital area vary from erythema to blistering second degree burn-like.

In 2004, Chung, *et al.*, first reported HLA gene as a genetic marker for SJS by carbamazepine (Chung et al., 2004). Year after, many other studies in pharmacogenetics of HLA alleles and drug hypersensitivity have also been reported. In addition to HLA-drug specific induced SJS, reports on ethnicity shows important role as well (Chung et al., 2007). Among Asian ethnicity, *HLA-B*5801* involved with severe side effect from antihyperuricemic agents, allopurinol. Its mortality rate is around 26% (Hung et al., 2005). Since allele frequency of this HLA antigens are quite common in some Asian ethnicities, as listed in table 1 (http://www.allelefrequencies.net/default.asp), it is important to develop a rapid test for Asian including our population.

Ethnicity	*HLA-B*5801* (%)
China North Han	2.9
China South Han	8.9
Taiwan	9.8
Japan	0.5
Korea	6.5
Singapore	5.8
Thailand	7.7
Vietnam	6.5

Table1. *HLA–B*5801* frequencies in Asian ethnicity.

For HLA typing, standard serological tests give inconclusive results which were caused by cross reactivity of monoclonal antibodies. As a result, HLA subtypes cannot be identified accurately. Years after, molecular techniques become commercially available and are applied to many routine laboratories. It's still

expensive and also gives lots of unnecessary information, as all alleles are interpreted (Martin et al., 2005). Testing HLA gene from genomic DNA, most laboratories may apply either of four different techniques. First, polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSOP). This technique use sets of probes to detect HLA types of multiple samples at the same time. This is suitable for high throughput HLA typing, preferable used in donor center. Second, reverse SSOP is quite expensive due to the development of high efficiency probes that were designed to bind target allele at the same temperature. The third technique is PCR-SSOP-luminex® system which applies flow cytometry to detect different alleles with up to 100 DNA probe attached colorimetric beads (Itoh et al., 2005). Its limitations are cost and requirement of special instrument. Finally, sequence-specific primer polymerase chain reaction (PCR-SSP) technique works by amplification HLA gene with many sets of primers to specifically differentiate closely related alleles. This technique is available in many forms of commercial sets of primers, which cost is still quite expensive. In order to get the most specific, rapid and cost effective test for *HLA-B*5801*, it's challenging to develop an in-house PCR-SSP to predict drug allergy for each patients before prescription.

2. Objective

To develop an in-house PCR-SSP for *HLA-B*5801* and validate with PCR-SSP commercial kit and direct sequencing.

3. Materials and methods

Sample

In a setup step, known *HLA-B*5801*, *B*1517* and *B*5701*, which have the closest sequences to *HLA-B*5801* and are present in Thai population, along with other alleles (*HLA-B*0705, 1301, 1501, 1511/12/13, 1525, 1801, 3501, 3801, 3901, 4001/02, 4402, 4601, 4801, 5101/02, 5201* and *5701*) that have high frequency in Thai population (http://www.allelefrequencies.net/default.asp), were used. In a validation step, two hundred genomic DNA samples of blood or bone marrow donors at the Thai Red Cross were analyzed by gold standard methods using PCR-SSP commercial kit (Micro SSP™ HLA DNA Typing Trays, One Lambda, Inc.) and direct sequencing. These genomic DNA samples were sent to us blindly to test for *HLA-B*5801*, with 9.5% of *HLA-B*5801*.

Sequence-specific Primer design

*HLA*B* is the most polymorphic gene in human genome. Around 1,249 variants are reported in 2009 from the IMGT/HLA database (http://www.ebi.ac.uk/imgt/hla). In order to design specific primer, HLA-B sequence alignment was

taken from the database with *HLA-B*070201* as consensus sequence (Figure 1). It's noted that polymorphism among *HLA-B*58* group is scattered around the whole sequence, so rare *HLA-B*5705* and other rare subtypes of *HLA-B*58* cannot be differentiated. Reaction mix at 20 µl contains final concentration of 1x Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1.25 µM primer (F1: 5'-ACGGAACATGAAGGCCTCC-3' and R1: 5'-CAGCCATACATCCTCTGGATGA-3'), 0.25 µM internal control primer (IF: 5'-CCTCACATGATATGACTTTGACAT-3' and IR: 5'-AACATCAGAAGCATTGACCTTG-3') and 0.25 U Platinum® Taq DNA polymerase (Invitrogen). Touchdown PCR cycles are used to increase specificity, set up by gradient of temperature and cycles that results in both positive band for *HLA-B*5801* and housekeeping gene. They are composed of 95°C 2 min for 1 cycle; 95°C 30s, 70°C 30s and 72°C 30s for 5 cycles; 95°C 30s, 68°C 30s and 72°C 30s for 5 cycles; 95°C 30s, 67.1°C 30s and 72°C 30s for 5 cycles; 95°C 30s, 65°C 30s and 72°C 30s for 10 cycles; 95°C 30s, 55°C 30s and 72°C 30s for 20 cycles; 72°C 5 min for 1 cycle and holding at 4°C. Total genomic DNA is 100 ng. Electrophoresis uses 2% agarose gel to determine PCR product size by running with 100bp DNA Ladder at 100 V for 40 min.

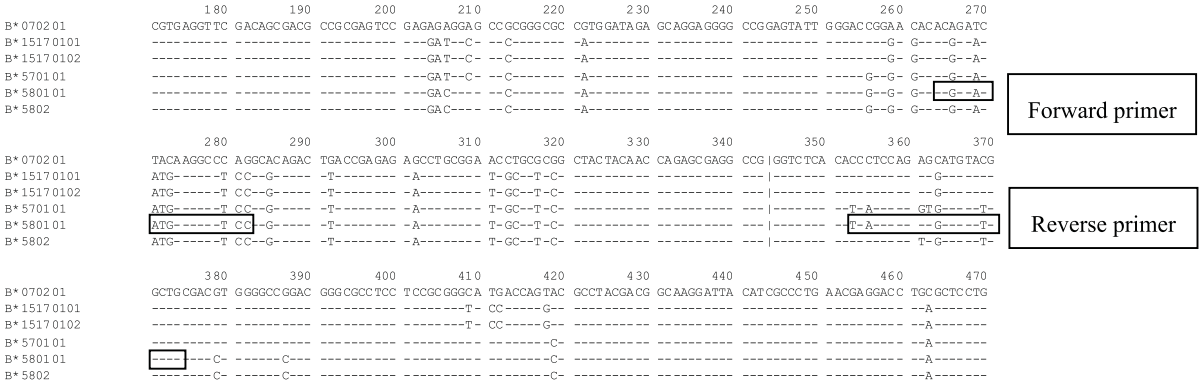

Figure 1 Related *HLA-B* alleles sequence alignment with primer regions. *HLA-B*5801* can be differentiated specifically within those closely related alleles, *HLA-B*1517*, *5701* and *5802*.

Assay Analysis

To compare the efficiency between the new developed method with traditional one, sensitivity and specificity of the assay is determined (Altman and Bland, 1994).

Sensitivity = $\frac{\text{number of true positive}}{\text{number of true positive + number of false negative}}$

Specificity = $\frac{\text{number of true negative}}{}$

number of true negative + number of false positive

4. Results and discussion

Previous report on *HLA-B*5801* detection composed of two sets of primer (Bunce et al., 1995). From the reference, first primer set amplifies *HLA-B*5801, 5104, 5301 and 1513*, while another set amplifies *HLA-B*5801-3*. A successful typing for *HLA-B*5801* will give two positive bands from both sets of primer in separated reactions. The limitation of this method can occur with the combination of non *HLA-B*5801* heterozygote, for example, patient with *HLA-B*1513* and *HLA-B*5802* will result as two positive bands and misinterpret as *HLA-B*5801*. Here, we used only one set of primer to reduce assay number to one reaction and to also exclude false positive from heterozygote. Of 200 samples, we can detect 19 *B*5801* samples correctly. Moreover, this current test could reduce the HLA typing cost from 3,000 baht/test by commercial PCR-SSP to 1,000 baht/test.

In addition, within these 200 samples, all HLA-B alleles presented more than 1% in Thai population including *HLA-B*0705, 1301, 1501, 1511/12/13, 1525, 1801, 3501, 3801, 3901, 4001/02, 4402, 4601, 4801, 5101/02, 5201 and 5701* gave negative result with our primer. A representative gel picture is shown in Fig 2.

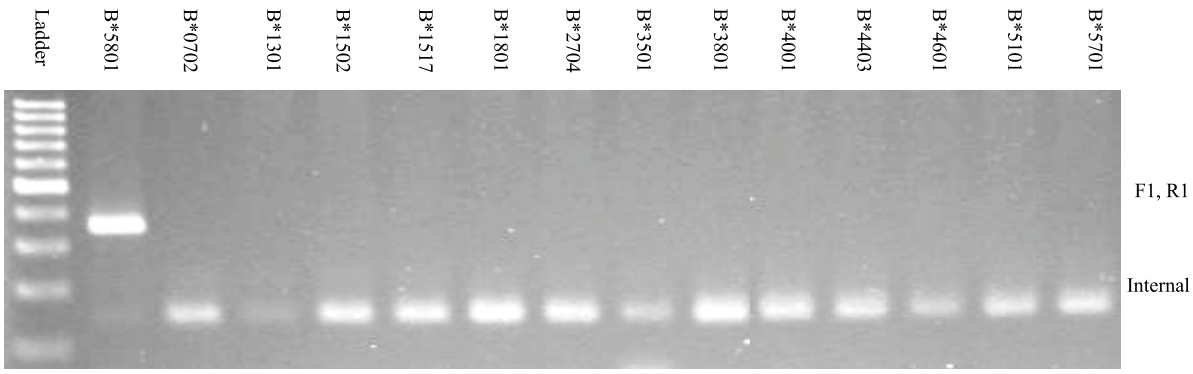

Figure 2 Migration patterns from both *HLA-B*5801* (F1, R1) and housekeeping primer.

As mentioned earlier, polymorphisms among other related alleles, both in intron and exon, are scattered around the sequences, so *HLA-B*5705* and other subtype of *HLA-B*58* cannot be differentiated. Predicted amplified alleles are *HLA-B*5705, 5801, 5804-5, 5809, 5810N, 5811-13, 5815, 5817N, 5819* and *5821-24*. However, from the allele frequencies database (http://www.allelefrequencies.net/default.asp) of world populations, those cross reactive alleles are very

rare, close to 0%. From 200 blind samples, this assay gives 100% sensitivity and >99.9% specificity, as extremely rare alleles mentioned above can still be amplified. According to our samples, we didn't found those extremely rare alleles, positive predictive value and negative predictive value are 100%. Therefore, this SSP assay with one set of primer pairs can be used with high specificity in our Thai population.

5. Conclusion

This PCR-SSP system can be used to define *HLA-B*5801*. The benefit of these tests would help patients to avoid any life-threatening adverse consequences from allopurinol with less cost.

6. Acknowledgement

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