



UNIVERSITI PUTRA MALAYSIA

**PREPARATION OF CONJUGATED THERMO-SENSITIVE
POLY (NIPAM) FOR AFFINITY PRECIPITATION OF ENZYME**

SYAUBARI

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**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2007



**PREPARATION OF CONJUGATED THERMO-SENSITIVE
POLY (NIPAM) FOR AFFINITY PRECIPITATION OF ENZYME**

By

SYAUBARI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

January 2007



Dedication

My Father, H. Zainon Hasan

My Wife, Mardalena

And

My Children, Muhammad Furqan Adhi

Nur Irhamni Sabrina

Nurul Muthmainnah Sabrini

Noor Qurrota Aiyun

In the Memory of

My Love Mother, Hajjah Safiah Abdullah

And

My relatives of Tsunami disaster on 26th December, 2004

Especially

My Brother, Muhammad Asnawi and Family

My Parent in law: M. Ali Nago and Mariana

My Mother: Hajjah Mardhiyah Sumarsono



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**PREPARATION OF CONJUGATED THERMO-SENSITIVE
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January 2007

Chairman : Associate Professor Saari Mustapha, PhD

Faculty : Engineering

Affinity precipitation is a recent technique for the purification of enzyme. The precipitation is based upon the interaction between a target enzyme and ligand which binds to the enzyme in a specific manner. Primary-effect affinity precipitation, in which precipitation is a direct result of ligand-enzyme binding, has been the focus of this research.

The n-isopropylacrylamide (NIPAM) monomer was synthesized using two methods, chemical and irradiation grafted process polymerization. The various amount of mercaptopropionic acid (MPA) to monomer preparation used resulted in hydrophobic modification of carboxylated poly (NIPAM). Both methods did not have big impact on the lower critical solution temperature (LCST) of polymer but resulted in conjugation of ligand indicating significant difference in synthesized process.



A ligand-enzyme system used in this study was p-aminobenzamidine-trypsin (PABA-trypsin). Initial studies with the PABA-trypsin system succeed to produce precipitation due to complications with the use of p-aminobenzamidine (PABA) as a ligand. An affinity precipitant for ligand-polymer was then successfully synthesized by conjugation of PABA to thermo-sensitive poly (NIPAM). The effect of molecular weight, reaction time, ligand PABA load, pH condition and addition of NHS on conjugated PABA-poly (NIPAM) were examined including the amount of ligand conjugated and dried weight precipitate of polymer. Trypsin as single enzyme was replaced by enzyme mixture of trypsin and chymotrypsin to evaluate the effect of the specific interaction ligand-enzyme during affinity precipitation. Both methods of preparation polymer chemical and irradiation grafted process successfully were used in the evaluation the reversibility of the precipitation and recovery of the selected enzyme after precipitation.

PABA conjugated with thermo-sensitive poly (NIPAM) was used in the affinity precipitation of trypsin. Polymer and ligand concentrations used in conjugate preparation showed remarkable effect on the trypsin recovery. Trypsin precipitation efficiency amounted to 97% and recovery was 81% using prepared polymer by chemical process, and synthesized conjugated poly (NIPAM) polymers by irradiation grafted process show the trypsin precipitation efficiency amounted to 95% and recovery was 83%.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENYEDIAAN POLI (NIPAM) TERMOSENSITIF TERKONJUGASI
UNTUK AFINITI PEMENDAKAN ENZIM**

Oleh

SYAUBARI

Januari 2007

Pengerusi : Profesor Madya Saari Mustapha, PhD

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Afiniti pemendakan adalah suatu kaedah baru untuk penulenan enzim. Kaedah pemendakan berdasarkan kepada interaksi antara enzim pilihan dengan *ligand* dan konjugat enzim dengan cara khas. Kesan utama daripada afiniti pemendakan ialah ianya boleh menunjukkan hasil langsung diantara konjugat *ligand* dengan enzim merupakan fokus utama penyelidikan ini.

Monomer NIPAM yang telah disintesisikan dengan menggunakan dua kaedah yaitu kimia dan iradiasi kepelbagaian kuantiti mercaptopropionic acid (MPA) ke atas monomer yang menghasilkan perubahan sifat hidrofobik gugus karboksil poly (NIPAM). Kedua kaedah ini tidak memberikan kesan kepada temperatur kritis larutan lebih rendah (LCST) ke atas polimer, tetapi kemampuan konjugat ligan menunjukkan kesan yang berbeza pada proses sintesis.



Dalam suatu sistem konjugat *ligand* dengan enzim yang digunakan sebagai model dalam penyelidikan ini adalah p-aminobenzamidine (PABA) dengan tripsin. Penyelidikan awal dengan menggunakan sistem PABA-trypsin telah berjaya membentuk pemendakan yang disebabkan oleh komplikasi PABA sebagai *ligand*. Afiniti pemendakan antara *ligand* dan polimer telah berjaya dilakukan dengan sintesis konjugat PABA ke atas termosensitif poli (NIPAM). Kesan berat molekul, masa tindak balas, beban *ligand* PABA, kondisi pH dan penambahan NHS terhadap konjugat PABA-poli (NIPAM) telah dikaji termasuk kuantiti konjugat *ligand* dan berat kering daripada pemendakan polimer. Enzim tunggal yang dikaji adalah tripsin kemudian digantikan dengan enzim campuran berupa tripsin dan chymotripsin untuk penilaian keberkesanan interaksi antara *ligand* dan enzim sepanjang proses ini. Kedua-dua kaedah penyediaan polimer dengan cara kimia dan iradiasi telah berjaya digunakan untuk penilaian pemendakan balik dan penulenan enzim secara proses pemendakan.

PABA konjugat dengan termosensitif poli (NIPAM) digunakan untuk afiniti pemendakan tripsin. Nisbah kepekatan polimer dan *ligand* meninggalkan kesan yang besar terhadap perolehan enzim. Kecekapan pemendakan tripsin adalah 97 peratus dan perolehan enzim ialah 81 peratus dengan menggunakan polimer yang disediakan dengan kaedah kimia. Manakala, dengan menggunakan kaedah iradiasi didapati bahawa kecekapan pemendakan tripsin adalah 95 peratus dan perolehan enzim ialah 83 peratus.



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I convey my thanks to all of my colleagues, friends, housemate and UPM support staff.



I certify that an Examination Committee has met on 17th January 2007 to conduct the final examination of Syaubari on his Doctor Of Philosophy thesis entitled “Preparation of Conjugated Thermo-sensitive Poly (NIPAM) for Affinity Precipitation of Enzyme” In accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

SYAUBARI

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LIST OF ABBREVIATIONS/NOMENCLATURE

ABBREVIATIONS

ADH	Alcohol dehydrogenase
AIBN	2,2'-Azobis- (isobutyronitrile)
ATPS	Aqueous two-phase systems
ATRP	Atom transfer radical polymerization
AIBN	Azoisobutyronitrile
AML	Affinity Macroligand
BAPNA	N-Benzoyl-DL-arginine-p-nitroanilide
BTPNA	N-Benzoyl-L-tyrosine-p- nitroanilide
Con A	Concanavalin A
DCC	Dicyclohexylcarbodiimide
DIFP	Diisopropylfluorophosphate
DMA	Dynamic mechanical analysis
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
DMTA	Dynamic mechanical thermal analysis
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EGTA	Ethylene glycol bis(β -aminoethyl ether)-N,N'-tetraacetic acid
FTIR	Fourier transform infrared
GDH	Glutamate dehydrogenase
GMA	Glycidyl methacrylate
GPC	Gel permeation chromatography



IDA	Iminodiacetic acid
IgG	Immunoglobulin G
LCST	Lower critical solution temperature
LDH	Lactate dehydrogenase
LET	Linear energy transfer
Mab	Monoclonal antibody
MALLS	Multy angle laser light scattering
MPA	Mercaptopropionic acid
Mw	Molecular weight
MWCO	Molecular weight cut-off
NaCl	Natrium chlorida
NASI	N-Acryloxysuccinimide
NHS	N-Hydroxysuccinimide
NIPAM	N-Isopropylacrylamide
OD	Optical density
PABA	p-Aminobenzamidine
PEG	Poly (ethylene glycol)
PMDETA	N,N,N',N',N''-Pentamethyldiethylenetriamine
PVA	Polyvinylalcohol
PVCL	Poly (N-vinyl caprolactam)
STI	Soybean trypsin inhibitor
THF	Tetrahydrofuran
TMEDA	Ttetramethylenediamine
WGA	Wheat germ agglutinin



NOMENCLATURE

E	Concentration of active enzyme (mol L^{-1})
EI	Inhibitor enzyme complex (mol L^{-1})
ES	Enzyme-substrate complex (mol L^{-1})
E_T	Total complexed of enzyme
I	Inhibitor (mol L^{-1})
K_d	Dissociation constant (mol L^{-1})
K_i	Inhibitor constant (mol L^{-1})
K_m	Michaelis constant (mol L^{-1})
S	Substrate (mol L^{-1})
V	Velocity ($\text{mol L}^{-1}\text{min}^{-1}$)
V_m	Maximum velocity ($\text{mol L}^{-1}\text{min}^{-1}$)
V_{\max}	Maximum velocity ($\text{mol L}^{-1}\text{min}^{-1}$)

