



**UNIVERSITI PUTRA MALAYSIA**

**PROTEIN STRUCTURE-BASED DESIGN OF A NOVEL  
SEMISYNTHETIC METALLOTRYPSIN**

**AZIZAH MISRAN**

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**PROTEIN STRUCTURE-BASED DESIGN OF NOVEL SEMISYNTHETIC  
METALLOTRYPSIN**

**By**

**AZIZAH MISRAN**

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**PROTEIN STRUCTURE-BASED DESIGN OF NOVEL SEMISYNTHETIC  
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**October 2006**

**Chairman: Associate Professor Mohd Basyaruddin Abd. Rahman, PhD**

**Faculty: Science**

A detailed study of the surface region trypsin from bovine pancreas was performed to gain insight into its biological functions and interactions that helped to determine the binding specificity. Twenty four pockets were identified in trypsin from Protein Data Bank (PDB) file entry 1AUJ using Computed Atlas of Surface Topography of proteins (CASTp). Nevertheless, only five biggest pocket cavities were selected; pocket 20, 21, 22, 23, and 24 since those pocket cavities would provide insight of location where ligand could bind as well as to identify the cavities that can aid in diffusion of the ligands. It also offered the identification of surface features and functional region of protein. Analyses of volume, surface area, and amino acids that participated in each pocket cavity were also determined. Systematic molecular docking studies using AutoDock 3.0.5 was performed on the five largest pocket cavities in trypsin. A set of ten chemical ligands was docked onto five biggest pocket cavities. The results showed that the biggest rigid ligand 1,10-phenanthroline (PHN) preferred to bind at pocket 24 as indicated by the lowest docked energy value (-8.74



kcal/mol). Systematic analyses on molecular docking for various metal ions such as  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$  to the protein ligand complex showed almost similar docked energy at all pocket cavities. Docking results of trypsin-PHN complex showed that the lowest docked energy of -9.63 kcal/mol for  $\text{Fe}^{2+}$  at pocket cavity 21, followed by  $\text{Mg}^{2+}$  (-7.00 kcal/mol) at pocket 23,  $\text{Ca}^{2+}$  (-5.56 kcal/mol) and the highest docked energy value that was  $\text{Zn}^{2+}$  with -0.02 kcal/mol at the pocket cavity 21. Experimental studies have focused on PHN as an intermediate ligand since PHN was a bidentate ligand that provides more sites for interactions which capable of generating stable complex. It was found that at concentration 100  $\mu\text{M}$  of PHN increased the activity of trypsin by 40 % higher than native trypsin. However, analysis among the metal ions on trypsin-PHN complex indicated that  $\text{Ca}^{2+}$  was the only metal ions capable of enhancing the activity of trypsin about 10 % than native trypsin at the concentration of 5  $\mu\text{M}$ .



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
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**KAJIAN METALLOTRIPSIN SEMISINTETIK BERDASARKAN  
REKABENTUK STRUKTUR PROTEIN**

Oleh

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**Oktober 2006**

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Satu kajian terperinci terhadap kawasan permukaan trypsin telah dilakukan memandangkan ia memberikan maklumat mengenai fungsi biologi dan interaksi yang dapat membantu dalam mengenalpasti spesifikasi ikatan yang terbentuk. Sebanyak 24 protein kaviti telah dikenalpasti secara automatik menggunakan program Pencirian Berkomputer ke atas Topografi Atlas Permukaan Protein (CASTp) daripada Data Bank Protein (PDB) fail 1AUJ. Namun begitu, hanya lima poket kaviti terbesar sahaja yang dipilih memandangkan ia memberikan maklumat mengenai lokasi di mana ligan berkemungkinan tinggi berinteraksi dan untuk mengenalpasti poket kaviti yang dapat membantu penyebaran ligan. Ia juga memberikan informasi sifat permukaan dan kawasan yang berfungsi bagi protein. Analisis mengenai isipadu, luas permukaan dan asid amino yang terlibat turut dilakukan. Kajian secara bersistematik telah dilakukan terhadap percantuman



berkomputer menggunakan AutoDock 3.0.5 terhadap lima poket kaviti terbesar pada trypsin. Satu set yang mengandungi sepuluh ligan telah dicantumkan secara berkomputer pada poket kaviti yang telah dipilih. Keputusan menunjukkan ligan yang paling besar dan tegar iaitu 1,10-fenantrolin dipilih untuk berinteraksi pada poket kaviti 24 dengan tenaga percantuman paling rendah (-8.74 kcal/mol). Kajian terhadap percantuman ion logam pada kompleks trypsin-ligan menunjukkan sedikit perbezaan. Keputusan percantuman berkomputer mempamerkan tenaga percantuman yang paling rendah iaitu -9.63 kcal/mol bagi  $\text{Fe}^{2+}$  pada poket kaviti 21, diikuti dengan  $\text{Mg}^{2+}$  (-7.00 kcal/mol) pada poket kaviti 23,  $\text{Ca}^{2+}$  (-5.56 kcal/mol) dan tenaga percantuman yang tertinggi  $\text{Zn}^{2+}$  dengan -0.02 kcal/mol pada poket kaviti 21. Eksperimen makmal dilakukan dengan menumpukan PHN sebagai ligan perantara kerana PHN adalah ligan bidentat yang menyediakan tapak untuk berinteraksi dan dapat membentuk kompleks yang lebih stabil. Kepekatan PHN pada 100 $\mu\text{M}$  telah meningkatkan kadar tindak balas trypsin sebanyak 40%. Manakala analisis mengenai kompleks trypsin-PHN-ion logam menunjukkan  $\text{Ca}^{2+}$  satu-satunya ion logam yang mampu meningkatkan kadar tindakbalas trypsin sebanyak 10% pada kepekatan 5  $\mu\text{M}$ .



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I certify that an Examination Committee met on .....to conduct the final examination of Azizah Misran on her degree thesis entitled ‘Protein Structure-Based Design of Novel Semisynthetic Metallotrypsin’ in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher degree) Regulations 1981. The Committee recommends that candidate awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby 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 UPM or other institutions.

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Date : 19 MARCH 2007



## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL</b>	viii
<b>DECLARATION</b>	x
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF CHART</b>	xvii
<b>LIST OF ABBREVIATIONS</b>	xviii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>5</b>
Semisynthetic system	5
Introduction	
Importance of engineering protein	5
Recent development of semisynthetic enzyme	7
Semisynthetic enzyme	11
Protein pocket	11
Protein Conjugates	13
Ligands	13
Metal ions	16
Role metal ions in biological system	17
Metalloenzyme and metal-activated enzyme	19
The presence metal ions in enzyme	19
Possible function of metal ions in enzymatic reaction	20
Correlation between enzyme catalysis and metal catalysis	22
Computational Docking	23
Molecular docking	23
Molecular docking software	24
AutoDock 3.0	25
Major component of AutoDock	26
Overview search method	27
Hybrid search technique	27
Structure-based scoring function	27
Application of docking	29
Proteases	31
Protein and Structure of Trypsin from bovine pancreas	31
Serine protease	32



	Pancreatic bovine trypsin	33
	Chemistry of trypsinogen and trypsin	35
	Mechanisms of catalysis and active site	36
<b>3</b>	<b>MATERIALS AND METHOD</b>	<b>38</b>
	Computer Hardwares and Softwares	39
	Chemicals	40
	Computer Simulation	41
	Preparation of the ligands	42
	Identifying pocket cavities	42
	Docking procedure	43
	Analysis	44
	Experimental procedure	45
	Purification of trypsin	45
	Synthesis of the trypsin-PHN	47
	Synthesis of trypsin-PHN-metal ion	48
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	
	Molecular docking studies	49
	Characterization of pockets / cavities	49
	Detailed analysis of five biggest cavities	51
	Screening of the ligands	56
	Identifying rotatable bond in ligands	59
	Molecular docking of multiple ligands onto	60
	five biggest pockets/cavities in trypsin	
	Docking PHN onto five biggest pocket	
	cavities in trypsin	63
	Molecular docking of metal ion onto each complex	
	pocket-ligands	71
	Experimental Studies	78
	Synthesis of complex semisynthetic metalloenzyme	78
	Purification of trypsin	78
	Synthesis of trypsin-PHN	81
	Synthesis of trypsin-1,10-phenanthroline-metal ion	85
<b>5</b>	<b>CONCLUSION</b>	<b>91</b>
	<b>REFERENCES</b>	<b>93</b>
	<b>APPENDICES</b>	<b>101</b>
	<b>BIODATA OF THE AUTHOR</b>	<b>122</b>



## LIST OF TABLES

Table		Page
1	List of some metal-binding properties and general roles of metal ions in biological processes.	17
2	The centres coordinate in five biggest pockets cavities performed in molecular docking.	43
3	Docking parameters.	44
4	Analysis of volume, surface area and mouth opening of five biggest pockets cavities in pancreatic bovine trypsin computed by CASTp.	52
5	List of five largest pocket cavities in trypsin that described its residues constituent.	54
6	Structure of chemical ligands involved in the molecular docking to five selected trypsin pocket cavities. The ligand selections were based on small molecules which consist of amine group, hydroxyl group and both amine and hydroxyl group.	57
7	AutoDock calculated docked energy for each ligand to the protein pocket.	61
8	Results obtained for docking of PHN to five biggest pockets in trypsin.	63
9	The most favourable docked energy of metal ions involved at pocket cavity 21, 23 and 24.	72
10	Purification table of trypsin from bovine pancreas.	78



## LIST OF FIGURES

Figure		Page
1	A stereo representation of the X-ray crystal structure of the semisynthetic transaminase adipocyte lipid-binding protein (ALBP)-pyridoxamine.	9
2	The concave regions and surface packing of proteins. There are three types of concave regions on protein surfaces: Fully enclosed voids with no outlet, pockets accessible from the outside but with constriction at mouths, and shallow depressions.	12
3	Molecular surface models (a) van der Waals (b) solvent accessible surface (c) molecular surface.	13
4	Schematic diagram showing how electrostatic interactions can influence the binding of a ligand.	16
5	Possible function of metal ion in enzyme system.	21
6	A three dimensional lattice of grid maps surrounding and centered on some region of interest.	26
7	3D structure of trypsin.	34
8	Catalytic residues in trypsin. The residues rendered as sticks represent the catalytic triads.	37
9	Structure of the pockets cavities in trypsin from bovine pancreas (1AUJ). The five biggest pockets are shown as ball and stick representations (pockets 24, 23, 22, 21 and 20).	51
10	Each pocket was separated in their own physical structure rendered by secondary structure with stick display style is shown in (a) pocket 20 (b) pocket 21 (c) pocket 22 (d) pocket 23 (the biggest pocket) and (e) pocket 24.	56
11	Orientation of PHN at its lowest docked energy to each pocket cavity. Result of docking simulation pocket 24 (-8.74 kcal/mol). The presentation of interaction trypsin-PHN complex using (a) scaled ball and stick and (b) tube.	66
12	Interactions of PHN at pocket 24 of pancreatic bovine trypsin. Hydrophobic interactions of the lowest docked energy of PHN with the pocket 24 of pancreatic bovine	66



trypsin represented by LigPlot. The comb-like arrangements indicate the hydrophobic contacts.

- 13 Orientation of PHN at its lowest docked energy to each pocket cavity. Result of docking simulation pocket 23 (-5.96 kcal/mol). The presentation of interaction trypsin-PHN complex using (a) scaled ball and stick and (b) tube 68
- 14 Interactions of PHN at pocket 23 of pancreatic bovine trypsin. Hydrophobic interactions of the lowest docked energy of PHN with the pocket 23 of pancreatic bovine trypsin represented by LigPlot. The comb-like arrangements indicate the hydrophobic contacts. 69
- 15 Orientation of PHN at its lowest docked energy to each pocket cavity. Result of docking simulation pocket 21 (-6.36 kcal/mol). The presentation of interaction trypsin-PHN complex using (a) scaled ball and stick and (b) tube. 69
- 16 Interactions of PHN at pocket 21 of pancreatic bovine trypsin. Hydrophobic interactions of the lowest docked energy of PHN with the pocket 21 of pancreatic bovine trypsin represented by LigPlot. The comb-like arrangements indicate the hydrophobic contacts. 69
- 17 Orientation of PHN at its lowest docked energy to each pocket cavity. Result of docking simulation pocket 22 (-4.76 kcal/mol). The presentation of interaction trypsin-PHN complex using (a) scaled ball and stick and (b) tube. 70
- 18 Orientation of PHN at its lowest docked energy to each pocket cavity. Result of docking simulation pocket 20 (-5.11 kcal/mol). The presentation of interaction trypsin-PHN complex using (a) scaled ball and stick and (b) tube. 70
- 19 Orientation of complex 21-PHN-metal ions at its lowest docked energy to each pocket cavity. Result of docking simulation of metal ions at pocket 21. The docked metal ions were rendered by CPK structure. The blue ball represents  $\text{Ca}^{2+}$  (-5.54 kcal/mol), green ball represents  $\text{Mg}^{2+}$  (-7.00 kcal/mol), red ball represents  $\text{Fe}^{2+}$  (-9.33 kcal/mol) and  $\text{Zn}^{2+}$  represented by yellow ball (-0.02 kcal/mol). 73
- 20 Orientation of complex 23-PHN-metal ions at its lowest docked energy to each pocket cavity. Result of docking simulation of metal ions at pocket 23. The docked metal ions were rendered by CPK structure. The blue ball represents  $\text{Ca}^{2+}$  (-5.54 kcal/mol), green ball represents  $\text{Mg}^{2+}$  (-6.96 74





kcal/mol), red ball represents  $\text{Fe}^{2+}$  (-9.62 kcal/mol).

- |    |  |    |
|----|--|----|
| 21 | Orientation of complex 24-PHN-metal ions at its lowest docked energy to each pocket cavity. Result of docking simulation of metal ions at pocket 24. The docked metal ions were rendered by CPK structure. The blue ball represents $\text{Ca}^{2+}$ (-5.56 kcal/mol), green ball represents $\text{Mg}^{2+}$ (-6.99 kcal/mol), red ball represents $\text{Fe}^{2+}$ (-9.33 kcal/mol). | 75 |
| 22 | A protein elution profile for affinity chromatography 10ml of 1mg/ml of trypsin which prepared and applied to Hi Trap Benzamidine 1 ml column equilibrated with 20 mM Tris-HCl pH 7.6, 10 mM NaCl.   | 79 |
| 23 | Analysis of commercial trypsin by SDS-PAGE after using Hi-Trap Benzamidine Lane 1 protein marker ; Lane 2 Hi-Trap Benzamidine.   | 80 |
| 24 | Absorption of spectrum of trypsin and 1,10-phenanthroline using UV/vis spectra.  | 81 |
| 25 | Temperature profile of purified trypsin and trypsin-PHN complex.   | 83 |
| 26 | pH profile of purified trypsin and trypsin-PHN complex.  | 84 |
| 27 | Effect of four metal ions (in different concentration) in trypsin-PHN complex  | 86 |



## LIST OF CHART

1	The frame work of the project research	38
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## LIST OF ABBREVIATIONS

Å	Angstrom
kcal/mol	kilocalorie per mol
M	Molar
mg	milligram
ml	milliliter
mM	milliMolar
µM	micromolar
µl	microliter
v/v	volume per volume
w/v	weight per volume
BEN	Benzamidine
PBZ	P-aminobenzamidine
PHN	1,10-phenanthroline
EOH	Ethanol
POL	1- Propanol
PAC	2- Phenylacetic acid
PDO	1,3 -Propandiol
ETA	Ethanolamine
PSE	o- Phosphoethanolamine
PHE	Phenylalanine
Ala (A)	Alanine
Arg (R)	Arginine



Asn (N)	Asparagine
Asp (D)	Aspartic acid
Cys (C)	Cysteine
Glu (Q)	Glutamic acid
Gly (G)	Glycine
His (H)	Histidine
Ile (I)	Isoleucine
Leu (L)	Leucine
Lys (K)	Lysine
Met (M)	Methionine
Phe (F)	Phenylalanine
Pro (P)	Proline
Ser (S)	Serine
Thr (T)	Threonine
Trp (W)	Tryptophan
Tyr (Y)	Tyrosine
Val (V)	Valine



# CHAPTER 1

## INTRODUCTION

Enzymes catalyze chemical reactions with great specificity and rate enhancement. These reactions are the basis of the metabolism of all living organisms and provide tremendous opportunities for industry to carry out elegant, efficient, and economical biocatalytic conversions. The function of enzymes as catalyst remarkably increase the velocity of a reaction by  $10^8$  to  $10^{12}$  fold compared with catalysts employed by organic chemists which are only able to accelerate reactions a  $10^2$  to  $10^3$  times over the non-catalyzed rate (Karp, 1999).

Even more interesting, enzymes can accomplish the reactions at mild pHs, temperatures and pressures, thereby consuming less energy. As enzymes are non-toxic that minimizes problems of downstream waste and by-product disposal in ecologically acceptable processes. However, the use of native enzyme is often limited by their inherent specificity. To circumvent this limitation, the development of such artificial enzyme has received high consideration.

Recently, protein engineering and chemical modification has become a successful valuable tool for creating or improving protein function for practical uses. Therefore introducing cofactors or other reactive moieties into proteins provides enormous flexibility for the design of semisynthetic catalysts that could be employed for a variety of purposes (Davies and Distefeno, 1997).

Examination of the protein pocket or cavity as suggested by Ory *et al.*, (1998) would be a good site for the creation of an artificial catalyst. Despite of protein structure being completely organized, a protein has regions on its surface where small molecules or ion can bind. It is possible that there are many binding sites on the surface of enzymes and each binding site has only limited range of the ligands. The design for development of semisynthetic enzyme was based on the use of protein pockets that can accommodate ligand as an intermediate between the pockets at the surface of the enzyme with metal.

In this regards, trypsin from bovine pancreas was selected as the backbone of designing a novel semisynthetic metalloenzyme. Trypsin was chosen due to its high precision information regarding its experimental kinetic data on the enzyme reaction (Pozsgay *et al.*, 1981). Futhermore, it has well-defined mechanisms that are consistent with many structural and kinetic studies (Kraut, 1977). It has also been extensively used in research in developing methods for stabilizing its structures and improving its catalytic properties that increase attention in enzyme engineering (Villalonga *et al.*, 2000).

A number of metals are important in biological systems; these include the alkaline metals; sodium and potassium, the alkaline earths; magnesium, and transition metals; manganese, iron, copper and zinc. Approximately one-third of known enzymes has metals as part of their structure, requires metals to be added for activity or is further activated by metals (Conn, 1987).

The enzyme is in essence single, large, multidentate ligands that bind to metal which occupy three or more coordination sites (Gates, 1992). The activity of metal centers is uniquely determined by the combination of ligands provided by the enzyme and the geometry that is determined by the 3D structure of the enzyme.

In order to understand the interaction of enzyme with other molecules such as ligand, molecular docking may provide the insights on how it is able to mimic and predict the behavior of molecules involved. Molecular docking is important in understanding possible interactions between a protein and a ligand in the formation of a biologically important protein-ligand complex.

Computational approach in this area is always about employing the information in the three-dimensional structure that exploit the structural information in order to understand specific molecular recognitions event and to elucidate the function of target molecules (Joseph-McCarthy, 1999). In parallel of using computational chemistry in predicting the behaviour of the system, conducting experimental work is always the key to understanding on how the system works.

The aim of this research is to prepare a novel semisynthetic metalloenzyme that consist of protein-ligand-metal complex. The objectives of this thesis is therefore

- To screen for the plausible site for designing semisynthetic system
- To design a semisynthetic system that positions coordinated ligands and metal ions at the protein pocket

- To study the structural interaction within the complex of semisynthetic metalloenzyme through computational-aided molecular modeling.
- To characterize the semisynthetic metalloenzyme.



## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **Semisynthetic system**

##### **Introduction**

Broadly defined, semisynthetic system in enzyme is deal with the producing of artificial enzyme by preparing it with the new or useful conjugates in order to create novel properties of the enzyme. The protein can be modified by either using genetic engineering or chemical modification, which involves the creation and modification of an existing protein to change its properties and function in desired and predetermined way (Acquaah, 2004).

Structures are designed based on fundamental rules of design and function. Enzyme is attractive scaffold for the design of new catalyst because their size allows the formation of a large number of interactions between substrate and catalyst (Germanas and Kaiser, 1990). The outcomes of such structuring may include altered substrate specificity of an enzyme or increased stability of a protein for specific application.

##### **Importance of engineering protein**

Protein engineering has become a valuable tool for creating new or improved proteins for practical use using either genetic engineering or chemical modification and has provided new insights into protein structure and functions. A protein engineer has to know a variety of interdisciplinary skills including knowledge of