UNIVERSITI PUTRA MALAYSIA

# PROTEIN STRUCTURE-BASED DESIGN OF A NOVEL SEMISYNTHETIC METALLOTRYPSIN

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**MASTER OF SCIENCE**
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# PROTEIN STRUCTURE-BASED DESIGN OF NOVEL SEMISYNTHETIC METALLOTRYPSIN

**By**

**AZIZAH MISRAN**

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**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 $Fe^{2+}$, $Mg^{2+}$, $Ca^{2+}$, and $Zn^{2+}$ 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 $Fe^{2+}$ at pocket cavity 21, followed by $Mg^{2+}$ (-7.00 kcal/mol) at pocket 23, $Ca^{2+}$ (-5.56 kcal/mol) and the highest docked energy value that was $Zn^{2+}$ 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 μM 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 $Ca^{2+}$ was the only metal ions capable of enhancing the activity of trypsin about 10 % than native trypsin at the concentration of 5 μM.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KAJIAN METALLOTRIPSIN SEMISINTETIK BERDASARKAN REKABENTUK STRUKTUR PROTEIN**

Oleh

**AZIZAH MISRAN**

**Oktober 2006**

**Pengerusi : Profesor Madya Dr. Mohd Basyaruddin Abd. Rahman, Ph.D**

**Fakulti: Sains**

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 ligand 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 $Fe^{2+}$pada poket kaviti 21, diikuti dengan $Mg^{2+}$ (-7.00 kcal/mol) pada poket kaviti 23, $Ca^{2+}$ (-5.56 kcal/mol) dan tenaga percantuman yang tertinggi $Zn^{2+}$ 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 100μM telah meningkatkan kadar tindak balas trypsin sebanyak 40%. Manakala analisis mengenai kompleks trypsin-PHN-ion logam menunjukkan $Ca^{2+}$ satu-satunya ion logam yang mampu meningkatkan kadar tindakbalas trypsin sebanyak 10% pada kepekatan 5 μM.

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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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**AZIZAH MISRAN**

Date : 19 MARCH 2007

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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 charaterize 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