MOLECULAR DYNAMIC SIMULATION ALPHA CRYSTALLIN ADSORPTION IN BULK PHASE AND AT WATER VACUUM INTERFACE

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APPRECIATION TO MY BELOVED FAMILY.

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In the name of God the merciful and the compassionate

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ABSTRACT

In both civilized and undeveloped countries Tuberculosis is a fundamental killer infective disease and can be considered as a threat among them. Furthermore, due to an increment of drug resistance and substantial level of TB occurrence in human immunodeficiency, virus-infected individuals. Tuberculosis disease is often the result of the bacteria sequestered inside lung macrophages being activated when the immune system of the infected individual is weakened. Bacterium can spend many years in a dormant state inside lung granulomas. Mycobacterium tuberculosis would have two small heat shock proteins: Acr1 and Acr2. Like all SHSPs, these two heat shock proteins share a domain of 90 amino acids called the α-crystallin domain and have divergent N- and C-terminal extensions. The α crystallin protein (sHSP)2 family is ubiquitous throughout nature and carries out a general cellular protective role in preventing aggregation of denatured proteins and facilitating subsequent refolding by other chaperones. As for the Mycobacterium tuberculosis perspective, this function plays an ultimate role which must be able to survive an inhospitable environment while sequestered within phagosomes of alveolar macrophages. This study was actually considered exploration of the possibilities to immobilize the protein interaction with water vacuum interface. A clue of the possibility of immobilization the protein on the surface would be provided by predicting the conformation of the protein adopted on the surface. Molecular dynamics (MD) simulation was carried out to study adsorbed conformation of α crystallin at the water vacuum interface. The preliminary results showed that there were some conformational changes of protein in water phase while the protein was not preferentially adsorbed on the surface at that particular orientation. As the result, there was no significant change of αcrystallin protein conformation.

ABSTRAK

Tuberculosis adalah pembunuh asas bagi penyakit berjangkit dan boleh dianggap sebagai ancaman di negara-negara yang bertamadun dan mundur. Oleh itu, disebabkan oleh rintangan dadah tinggi dan tahap besar berlakunya TB bagi manusia yang mempunyai immunasi rendah; virus senang dijangkiti individu. Penyakit ini dijangkiti disebabkan oleh bakteria yang terdapat di dalam makrofaj paru-paru diaktifkan apabila sistem imun individu menjadi lemah . Bakteria boleh hidup untuk beberapa tahun dalam keadaan tidak aktif dalam granulomas paru-paru. Mycobacterium tuberculosis mempunyai dua jenis protein kejutan haba: Acr1 dan Acr2 . Seperti semua SHSPs , kedua-dua protein ini berkongsi domain 90 amino asid yang dikenali sebagai α - crystallin domain dan mempunyai sambungan dekat N- dan C- terminal. Protein α crystallin (SHSP)2 keluarga sentiasa terdapat di seluruh alam dan menjalankan peranan perlindungan sel umum dalam mencegah pengumpulan protein denatured dan memudahkan refolding berikutnya oleh chaperones lain. Bagi perspektif Mycobacterium tuberculosis, fungsi ini memainkan peranan penting; yang mesti berupaya untuk terus hidup dalam persekitaran yang ganas manakala diasingkan dalam phagosomes makrofaj alveolar. Kajian ini sebenarnya dijalankan untuk melumpuhkan interaksi protein dengan muka vakum air. Satu penunjuk tentang kemungkinan immobilization protein di permukaan akan dilakukan melalui pengubahan bentuk protein. Simulasi molekul dinamik (MD) telah dijalankan untuk mengkaji bentuk terjerap daripada α crystallin di muka vakum air. Kajian awal menunjukkan bahawa terdapat beberapa perubahan bagi pembentukan protein dalam fasa air manakala protein itu tidak terjerap pada permukaan orientasi yang tertentu. Oleh itu, tidak terdapat sebarang perubahan ketara bagi permukaan α crystalline protein dilakukan..

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LIST OF ABBREVIATIONS

MD	-	Molecular dynamics
BPTI	-	bovine pancreatic trypsin inhibitor
STWV	-	simulated the whole virus
Å	-	Angstrom
α	-	Alpha
XRD	-	X-ray diffraction
CPK	-	Corey-Pauling-Koltun
GEP	-	enetically engineered peptides have been done
TB	-	Tuberculosis
sHSPs	-	small heat shock proteins
NMR	-	Nuclear Magnetic Resonance
TB	-	*tuberculosis* bacteria
MS	-	Mass Spectroscopy
PG	-	peptidoglycan
LPS	-	lipopolysaccharide
LAM	-	Lipoarabinomannan
TDM	-	trehalose dimycolate
AG	-	arabinogalactan
MA	-	mycolic acids
MAGP	-	arabinogalactan-peptidoglycan
NO	-	nitric oxide
HSP	-	Heat shock proteins
HSF	-	heat shock factor
OMPs	-	stress outer membrane proteins
SCF	-	self-consistent-field
RCSB	-	research collaborator for structural bioinformatics

RMSD	-	root-mean-square deviation
RMSF	-	root-mean square fluctuation
D	-	Distance

CHAPTER 1

INTRODUCTION

1.1 Background of Information

In exploring many particles structures system at atomic level study, Molecular dynamics (MD) technique is of great interest because of the cheap availability of computational power. Prediction properties of useful materials can be utilized by modeling of these systems such as biopolymers, nano materials, biological composites and so on (Todorova, 2009).

In order to study the interactions of hard atomic spheres of liquids, at first, Alder and Wainwright in the late 1950's introduced the molecular dynamics method. Thorough their study, many important concepts of simple liquids were put into consideration. In 1974, more realistic simulation of water has been carried out by Stillinger and Rahman. The actual protein simulation's beginning bovine pancreatic trypsin inhibitor (BPTI) was started since 1977. The whole virus (STMV) has been simulated by MD method (106 atoms, 50 ns) as (Wei and Latour, 2009) have stated.

Molecular modeling of proteins with models made of hard wood originally (1 inch per Å) and plastic (0.5 inch per Å) was initiated by Corey and Pauling, (1953).

Moreover, the plastic models were connected with snap fasteners and the wood models with steel rods and clamps. X-ray diffraction (XRD) is the source where this model and structural data was obtained from and used correct atom proportions based on their van der Waals radii. Koltun who improved the original Corey-Pauling model, resulting in the Corey-Pauling-Koltun (CPK) model has proven to be very useful in visualizing and making accurate measurements of protein structure (Todorova, 2009).

Molecular dynamics simulation (MD) computer series of atomic coordinates as a function of time. Hence, the details of protein conformational fluctuations its changes can be accessed through MD simulation. It is an efficient method to study of the construction, dynamics and thermodynamics of proteins and their complexes even, also refinement of X-ray crystallography and NMR structure from experiment (Salsbury, 2010).

Protein in various fields such as medicine, disease detection and etc., it can be known as one of the most important classes of biomolecules. As the surface induced denaturation, preservation of the functions in application settings is very challenging and in addition of it, proteins are expected have biological functions. Furthermore, bio nanotechnology is the concept which has been concentrated by particular protein adsorption on the surfaces extent. An experiments series to study the adsorption behavior of genetically engineered peptides have been done (GEP) by Sarikaya *et al*., (2006) and Serizawa *et al*., (*2007*) from phage display on various material substrates.

A unique fingerprint of interaction with different material surfaces can be illustrated by different sequences of the 20 primary amino acids based on these studies (White *et al*., 2005). In order to study the partition free energy of unfolded polypeptides at cell membrane interfaces in 2005 they have used pent peptides models from which they developed an algorithm which was based on experiment to predict proteins that partition into the lipid bilayer interface, the binding free energy and secondary structure of peptides.

In order to calculate the virtual free energy transfer of unfolding chains into the interface, the partitioning free energy was created, due to problems of hydrophobicity thermodynamics and existing inaccessible unfolded proteins through interface of cellular membrane. Last but not least, in order to provide insight into the processes that influence cellular function, these studies have been utilized accordingly (Wei and Latour, 2009).

The actual cause of more than 1.5 million deaths per year would be *M. tuberculosis* which is the notorious species of this genus and a facultative intracellular pathogen that persists within immune phagocytes. The hydrophobic mycobacterial cell, roughly around two billion people have been infected with tuberculosis (TB) which constitutes one out of three of the world's population which two millions of those pass away each year (Organization, 2013). This disaster has also been known as the seventh most common cause of mortality in the world (Alday, 2010). This disease would not be transmittable through surface contact, but by the contaminated air. Different part of body such as lung, spine, kidney and brain can be attacked by *M. tuberculosis*. Moreover, it would have some symptoms including a long-lasting cough, which can produce blood or phlegm, fever, fatigue, weight loss, and chest or breathing pain (Organization, 2013). Coughs, sneezes or speaks are the ways that air can be contaminated by the infected person (Cramer *et al*., 2006). Host defenses can be rescued by Tuberculosis and remain undetected within the body for decades, plus most people infected with TB are symptom-free. It would be very difficult to control the disease within in a population, as this symptom-free nature of the disease exists. It goes without saying that, as for the undetected patients, treatments would be challenging. Roughly twenty drugs are available for TB treatment which would be utilized differently in various combinations and situations. For instance, as for the treatment of new patients, there is no suggestion of any drug resistance while, others are only used for the treatment of drug resistant patients (Todar and Kanabus, 2012). Mostly, various combinations of antibiotic on the basis of assumption would be needed and used for infected patients, as it is so hard to detect the bacteria. Consequently, the resistance of bacteria may occur to those antibiotics in the patients.

Through inhaling minute aerosol droplets carrying a small number of bacteria, humans may become infected with the bacteria. The lung mycobacterial bacilli are phagocytized by alveolar macro phases at the site of infection. In order to combat microbial intruders and to entirely eliminate them, these macrophages are executed promptly. Nevertheless, escaping of mycobacteria eradication by macrophages and survive within these cells might happen as well. Within macrophages the bacteria would replicate themselves instead, inducing the release of pro-inflammatory cytokines, which would be leading to formation of granulomas, tissue destruction, liquefaction and cavity formation.

By employing multiple receptors, like mannose receptors, the unique composition of the mycobacterial cell wall and envelope mostly enables the tubercle bacillus to enter macro phases (Gengenbacher and Kaufmann, 2012). Bacterium would need to survive in these two different environments as, phagocytic compartment of macrophages and the potentially hypoxic environment of granulomas during this lifelong infection (Stewart *et al*., 2006). The bacteria would have the possibility to persist in human tissues for long periods in a clinically latent or dormant state if it could survive under these environments. Persist in human tissues for long periods in a clinically latent or dormant state.

1.2 Problem Statement

Around two billion people across the world have suffered the Infections with tuberculosis bacteria (TB). This would indicate that around one out of three population of world have been infected as indicated by World Health Organization (WHO) in its statistical figure (world health organization website, 2012). As an air bone disease TB is a transmittable disease that would be not transmitted through the surface contact but in contaminated air. Different part of body as lung, spine, kidney and brain can be attacked by *M. tuberculosis*. Blood, phlegm, fever, fatigue, weight loss, and chest or breathing pain can be produced by symptoms include a long-lasting

cough. People can get infected by contamination Air through different ways such as, coughs, sneezes and speaking (Cramer *et al*., 2006).

It would be very much difficult to control and cure the disease due to the ability of the pathogen (*Mycobacterium tuberculosis*) to evade host defense system and remains undetected for decades in the host cell (symptoms free). As for TB treatment, there are around twenty drugs available which would be used in different combination and situation/circumstances. As an instance, there is no suggestion of any drug resistance in the treatment of new patients while, others are only used for the treatment of drug resistant patients (Todar, 2012). Various combinations of antibiotic would be utilized to cure the infected patients on the basis of assumption as the bacteria are very much hard to be detected. At last, in the patient's body, the bacteria may become resistant to the antibiotics. Lack of better detection way is seen as the antibiotics interference, difficulty along treatment, drugs resistance and rapid spreading of the disease (Gahoi *et al*., 2013). In order to control and cure the disease, it would be a considerable challenge to devise an easy, cheap, and fast method for detection. An inhospitable environment would have the possibility to survive by getting hand of α crystallin as a major secretory protein of the *Mycobacterium tuberculosis* while sequestered within phagosomes of alveolar macrophages (Kennaway *et al*., 2005). Therefore, as for disease detection perspective, it can be an appropriate candidate. I have utilized alpha crystallin in bulk phase and water vacuum interface to show the interaction and change conformational of alpha crystallin for devising method.

1.3 Objectives of the Research

Understanding the mode of interaction of α crystallin protein with a water-vacuum interface is the actual aim of this project along with exploring the possibilities of using α crystallin in devising tuberculosis detection method. The objectives tended to be accomplished are as follows:

1) To construct the water model and water-vacuum interface
2) To run molecular dynamics simulations
3) To calculate physical propertied of adsorpted protein from simulation data

1.4 Scope of the Study

This would go without saying that this study is computational specifically in nature, plus the computational facilities of the faculty of biosciences and medical engineering (FBME) was utilized to perform the simulation.

A single monomer of α-crystallin will be used in the simulation along with consideration of the computational expenses and facilities available. Besides, in the current study, Standard molecular dynamics was used as well. The software GROMACS will be utilized to execute the molecular dynamics simulations. Gromos 96 force field parameters would provide the potential energy for the simulations. Root means square deviation (RMSD) radius of gyration and root means square fluctuations (RMSF) is the criteria and measurement of the extent of conformational changes. The final results would be assessed and discussed according to these measurements and 3D of the conformations.

1.5 Significance of the Study

Discovering new drugs for TB along with many different strategies are being followed worldwide. Attacking the unique cell wall composition of *Tuberculosis* is the major concentration point (Gahoi *et al*., 2013). Protein can be considered as a very good candidate for disease detection and drug target as α crystallin is straightly connected to the survival mechanism of the bacteria.

There would be a great importance in the protein α crystallin water-vacuum interaction, adsorption happening experiments and modeling. The significances of this study would be provided through the measurement, prediction and understanding the protein conformation, interface interaction, shift structures and kinetic details of protein-interface. Furthermore, in all living cells, the major and actual structures are proteins. The progress and finding out the macromolecules well would be helped through deliberation of interaction proteins with interface. Moreover, surmount ability on limitation of experimental would be provided through working molecular dynamic simulation method.

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