SIMULATTING INTERACTION OF TUBERCULOSIS ANTIGEN 85B PROTEIN WITH HYDROPHOBIC SURFACE GRAPHENE

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ABSTRACT

Around two millions people die each year from Tuberculosis. Due to the ability of the pathogen (*Mycobacterium tuberculosis*) to evade host defense system and remains undetected for decades in the host cell, it is very difficult to control and cure the disease. Therefore, it is a challenge to invent an easy, cheap, and fast detection method to control and cure the disease. The maintenance of the highly hydrophobic cell wall of the pathogen is crucial to the survival of this bacterium in the host cell and the antigen 85, a major secretory protein, of the bacterium helps maintain the integrality of the cell wall. Since antigen 85 protein is directly linked to the survival mechanism of the bacteria, the protein is a very good marker candidate for disease detection and drug target. This study was exploration of the possibilities to immobilize the protein on hydrophobic graphene surface for separating the protein from the blood sample at the initial processing stage of the detection process. Predicting the conformation of the protein adopted on the surface should provide a clue about the possibility to immobilize the protein on the surface. Molecular dynamics (MD) simulation was carried out to study adsorbed conformation of antigen 85 at the graphene (hydrophobic) surface. 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 were no significant changes of Ag85B protein conformation. Also the protein preferred to locate middle of water box rather than close to graphene surface. Based on thermodynamic energy findings, the system equilibrated well and the energy of the system is reasonably conserved which is the fundamental requirement for molecular dynamics methodology.

ABSTRAK

Kira-kira dua juta orang meninggal setiap tahun disebabkan oleh jangkitan tibi. Jangkitan patogen *Mycobacterium tuberculosis* sukar dikawal dan dikesan. Oleh itu, adalah satu cabaran untuk mencipta satu kaedah pengesanan yang mudah, murah, dan cepat untuk mengawal dan menyembuhkan penyakit ini. Penyelenggaraan dinding sel yang sangat hidrofobik adalah penting untuk kehidupan bakteria ini di dalam sel tuan rumah dan antigen 85 merupakan protein utama yang dihasilkan oleh bakteria yang membantu mengekalkan keutuhan dinding sel. Memandangkan antigen 85 berkait secara langsung dengan mekanisme penting bakteria, protein ini boleh dipilihpakai sebagai calon petanda yang sangat baik untuk pengesanan penyakit dan sasaran ubat. Kajian ini dijalankan bertujuan mengkaji kemungkinan imobilasi antigen 85 pada permukaan grafen hidrofobik. Ramalan imobilasi antigen 85 dibuat melalui simulasi komputer untuk menentukan perubahan struktur umum protein. Molekul dinamik (MD) simulasi telah dijalankan untuk mengkaji pengesahan terjerap antigen 85 pada permukaan grafen (hidrofobik). Keputusan awal menunjukkan bahawa terdapat beberapa perubahan struktur protein dalam fasa air manakala protein itu tidak terjerap ke permukaan graphen. Sebagai hasilnya, tiada perubahan ketara di atas struktur protein. Protein lebih cenderung untuk duduk di dalam kotak simulasi air berbanding dengan permukaan grafen. Berdasarkan analisa tenaga termodinamik, sistem juga equilibrated dan tenaga sistem ini agak dipelihara dan stabil.

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LIST OF ABBRIVATION

MD	Molecular Dynamic
Ag85B	Antigen 85B
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
Rg	Radius of gyration
TB	Tuberculosis Bacterium
VMD	Virtual Molecular Dynamic
TDM	Trehalose dimycolate
TMM	Trehalose monomycolate
TB	*Tuberculosis* Bacteria
PG	peptidoglycan
AG	arabinogalactan

CHAPTER 1

INTRODUCTION

1.1 Background of study

Due to the cheap availability of computational power, molecular dynamics (MD) technique is of great interest in exploring the many particles structures system at atomic level study. Modeling of these systems can be used for predicting properties of useful materials such as biopolymers, nanomaterials, biological composites and so on (Todorova, 2009).

The molecular dynamics method was first introduced by Alder and Wainwright in the late 1950's (1959) to study the interactions of hard atomic spheres of liquids. Many important concepts of simple liquids action were considered thorough their study. Stillinger and Rahman carried out more realistic simulation of water in 1974. Since 1977, it was the beginning of the protein simulation of the bovine pancreatic trypsin inhibitor (BPTI). Latour and Wei were reported that the whole of virus (STMV) has been simulated by MD method (10^6 atoms, 50 ns) (2009).

Molecular modeling of proteins was initiated by Corey and Pauling (1953) with models originally made of hard wood (1 inch per Å) and plastic (0.5 inch per Å). The wood models were connected with steel rods and clamps, the plastic models with snap fasteners. This model was based on structural data obtained from X-ray diffraction (XRD) and used correct atom proportions based on their van der Waals radii. Todorova has been reported that Koltun who improved the original Corey-Pauling model, resulting in the Corey-Pauling-Koltun (CPK) model which has proven to be very useful in visualizing and making accurate measurements of protein structure (Todorova, 2009).

One of the most important classes of biomolecules is the protein that has numerous applications in various fields such as medicine, disease detection, etc. Proteins are expected have biological functions and it is often very challenging to preserve the functions in application settings due to the surface induced denaturation. In this regard, particular protein adsorption on the surfaces extent has been paid attention at bio nanotechnology. Sarikaya *et al.* (2006) and Serizawa *et al.* (2007) have performed a series of experiments to study the adsorption behavior of genetically engineered peptides (GEP) from phage display on various material substrates. From these studies, they showed that different sequences of the 20 primary amino acids exhibit a unique fingerprint of interaction with different material surfaces. White *et al.* (2005) have used pent peptides models to study the partition free energy of unfolded polypeptides at cell membrane interfaces in 2005, from which they developed an experimentally based algorithm to predict the binding free energy and secondary structure of peptides and proteins that partition into the lipid bilayer interface. Because of existing inaccessible unfolded proteins through interface of cellular membrane and problems of hydrophobicity thermodynamics, the partitioning free energy was created to calculate the virtual free energy of transfer of unfolding chains into the interface. These studies are being used to provide insight into the processes that influence cellular function (Latour and Wei, 2009).

M. tuberculosis is the notorious species of this genus and is a facultative intracellular pathogen that persists within immune phagocytes and is responsible for more than 1.5 million deaths per year. The hydrophobic mycobacterial cell wall consists of several components including the free glycolipids, trehalose monomycolate (TMM), and trehalose dimycolate (TDM). The antigen 85 (Ag85) complex, composed of three proteins (Ag85A, 85B, and 85C), is a major protein component of the mycobacterial cell wall, all of which contribute to cell wall biosynthesis by catalyzing the transfer of mycolic acid from one molecule of TMM to another, resulting in TDM and free trehalose (Belisle *et al.,* 1997). The group of Ag85B proteins has been listed as high confidence drug targets because of their important role in the maintenance of the cell wall (Ronning *et al.,* 2000). The structure of the binding site near the catalytic triad (Ser-126, His-262 and Glu-230) has potential to bind the carbohydrate moiety and the two branches of the mycolic acids. The antigen 85 groups of proteins catalyze the following biochemical reaction (Ronning *et al.,* 2000).

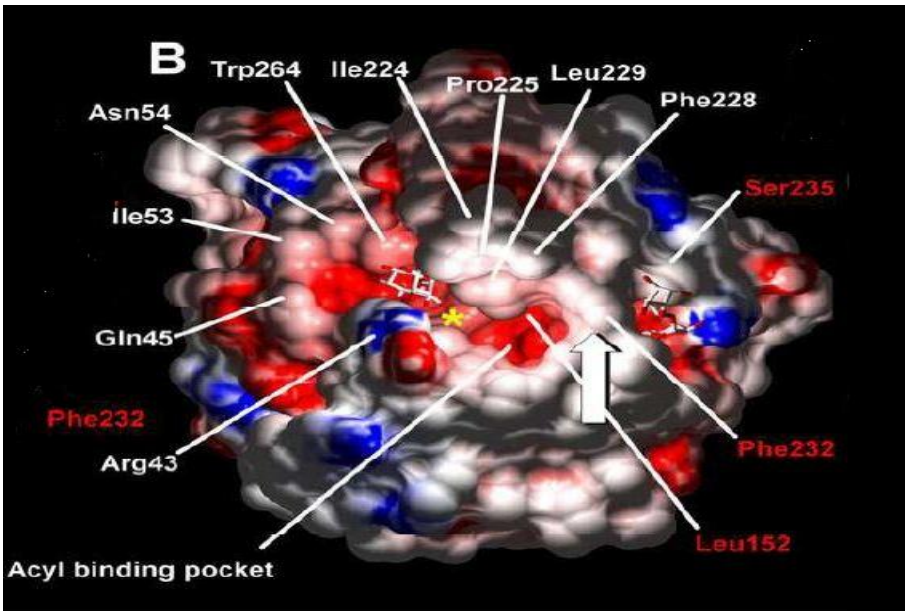

Figure 1.1 Illustration of antigen 85B.The ball and stick model of trehalose molecules (Elamin *et al.,* 2012)

The position of the catalytic serine is indicated by a yellow asterisk in Figure 1.1. The carbohydrate binding pocket is conserved completely (Arg43, Gln45 Ile53, Asn54, Trp264). The separation of the second carbohydrate binding pocket from the acyl binding pocket by the Phe232 in Ag85B and the corresponding smaller Leu230 in Ag85C is highlighted by an arrow. The surface is colored by electrostatic potential: The red and blue coloring represent negative and positive electrostatic potential, respectively (Elamin *et al.,* 2012).

The geometries of the catalytic residues among the antigen 85 proteins (A, B, C) are highly similar (Ronning *et al.,* 2004). This raises the possibility of identifying a single inhibitor for all three antigen 85 proteins. The importance of TDM to mycobacterial cell wall stability provides an excellent basis for the development of novel antitubercular drugs (Gahoi *et al.,* 2013).

Molecular dynamics simulation (MD) computes series of atomic coordinates as a function of time. Hence, the details of protein conformational fluctuations or 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 structures from experiments (Salsbury, 2010).

Graphene is a monolayer of carbon atoms that is arranged in a honeycomb structure and the carbon atoms are joined together by strongest C-C bond. Graphene possess remarkable mechanical, optical, and electronic properties. It is easy and cheap to produce high quality graphene in the laboratory (Geim and Novoselov, 2007). There are various potential bioapplication of graphene. Due to its unique mechanical properties, graphene can be used in tissue engineering, support material for imaging biomolecules in transmission electron microscopy, etc. It has also been proposed that the functionalized graphene has potential application in drug delivery and in fast and ultrasensitive measurement devices, capable of detecting a range of

biological molecules including glucose, cholesterol, haemoglobin and DNA. Graphene is also lipophilic and due to its lipophilic nature and availability, graphene surface was considered in the current study (Zhang *et al.*, 2013).

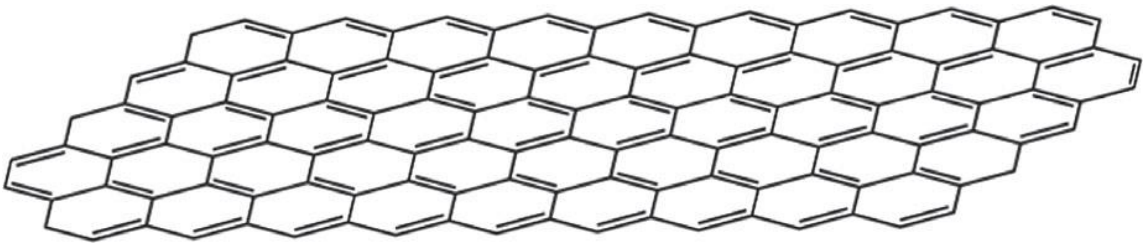

Figure 1.2 Schematic models of Graphene (Zhang *et al.*, 2013)

1.2 Problem statement

As the statistical figures of World Health Organization (WHO), one-third of the world's population, around two billion people, infected with *tuberculosis* bacteria (TB) and two millions of those die annually (World Health Organization website, 2012) . This epidemic phenomenon has been known as seventh most common reason of mortality in the world (Mathers *et al.*, 2009). TB is an airborne disease and transmitted through the contaminated air, not by surface contact. *M. tuberculosis* can attack any part of human body, such as lung, spine, kidney and brain. Symptoms include a long-lasting cough, which can produce blood or phlegm, fever, fatigue, weight loss, and chest or breathing pain. Air is normally contaminated by the infected person through his or her coughs, sneezes or speaks (Cramer *et al.*, 2006). 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), so it is very difficult to control and cure the disease.

There are more than twenty drugs available for TB treatment, and they are used in differing combinations in different circumstances, such as for the treatment of new patients where there is no suggestion of any drug resistance whereas, others are only used for the treatment of drug resistant patients (Todar, 2012). Since it is very difficult to detect the bacteria, infected patients are often treated with various combinations of antibiotic on the basis of assumption. As a result, the bacteria may become resistant to the antibiotics in the patient's body. Therefore because of antibiotics interference, difficulty along treatment, drugs resistance and rapid spreading of the disease the lack of better detection way is seen (Gahoi *et al.*, 2013). It is a challenge to devise an easy, cheap, and fast detection method to control and cure the disease. The antigen 85B, a major secretory protein of the *Mycobacterium tuberculosis*, helps maintain the integrality of the highly hydrophobic cell wall in the host cells. So it can be appropriate candidate for the disease detection.

1.3 Significant of the study

Several strategies for discovering new drugs for TB are being followed worldwide. A major focus has been toward attacking the unique cell wall composition of *M.tuberculosis* (Gahoi *et al.,* 2013) .Since antigen 85 proteins is directly linked to the survival mechanism of the bacteria, the protein is a very good marker candidate for disease detection and drug target. It was explored the possibilities to immobilize the protein on hydrophobic graphene surface for separating the protein from the blood sample at the initial processing stage of the detection process.

In this regard, the protein 85B-graphene interaction and adsorption happening experiments and modeling has great importance. The measurement, prediction and understanding the protein conformation, surface interaction, shift structures and kinetic details of protein-surface contact provide significances on this study. Moreover, proteins are one of the major structures in all living cells. The deliberation

of interaction proteins with surfaces can help to progress and finding out well of macromolecules. Moreover, molecular dynamic simulation method provides the ability to surmount on limitation of experimental working.

1.4 Statement objectives of the study

The overall aims of the current work is to understand the mode of interaction of antigen-85B protein with a hydrophobic solid surface such as graphene, and to explore the possibilities of using antigen 85B in devising tuberculosis detection method. Following objectives are intended to achieve:

1) To construct the model graphene surface
2) To run molecular dynamics simulations
3) To calculate mechanical (e.g. radius of gyration) and thermal properties (e.g. entropy) of adsorbed protein from simulation data.

1.5 Scope of the study

The current study is exclusively computational in nature and the computational facilities in the Faculty of Biosciences and Medical Engineering (FBME) was used to perform the computer simulations. In this preliminary study, the basic molecular dynamics methodology was followed to observe the conformational changes of antigen 85 protein at or towards the hydrophobic graphene surface. The extent of conformational changes was measured in terms of root means square deviation (RMSD), radius of gyration, and root means square fluctuations (RMSF).

Based on these measurements and 3D of the conformations, the results are assessed and discussed.

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