CHAPTER I

INTRODUCTION

Mycoplasma pneumoniae is a pathogenic bacterium that causes respiratory infection in both children and young adults. Most cases of infection are in children aged from 5 to 14 years, and adults aged 30 to 39 years (Bitnun *et al.*, 2001; Coelho *et al.*, 2004). These reports suggest that children acquire *M. pneumoniae* from other children at school and transmit infection to other children and to family members. Outbreaks of infection attributable to *M. pneumoniae* commonly occur in closed or semi-closed communities (Rastawicki *et al.*, 1998; Wattanathum *et al.*, 2003). Epidemics tend to occur at intervals of every 4-8 years (Rastawicki *et al.*, 1998). These outbreaks are difficult to be controlled because of a delaying in outbreak detection, the long incubation period of the bacterium, and an incomplete understanding of the effectiveness of infection control strategies.

Serious infections requiring hospitalization occur in both young adults and children. This pathogen infects not only pulmonary but also multiple organ systems. Extrapulmonary complications can occur in association with *M. pneumoniae* infection as a result of direct invasion especially central nervous system as encephalitis. Encephalitis manifestations are greater severity and more clinical importance than the primary respiratory infection. However, the molecular mechanisms whereby *M. pneumoniae* adheres to and is internalized into host cells still remains unclear. Additionally, virulence factors of *M. pneumoniae* involving human blood brain barrier (hBBB) invasion that cause neurological disorder, has not yet been determined. Therefore understanding of mechanism as to how *M. pneumoniae* enters host cells is a significant subject for pathogenesis of this bacterium. This might lead to the clue on how to inhibit pathogen invasion, which would be beneficial for medication of the infectious disease caused by this organism. The aims of this present study are firstly to identify *M. pneumoniae* virulence factor, secondly to model 3-dimensional (3D) structure of the selected *M. pneumoniae* virulence factor, and thirdly to investigate potential binding between a selected *M. pneumoniae* virulence factor model and the key zymogen within blood brain circulation by using molecular docking approach. Finally all models will be refined by molecular dynamics simulation. In summary, the molecular interaction of this complex would be described in detail. The summary

of this information would lead to a better understanding of the pathogen and its interactions with the human host.

Rationale of the study

It has been reported that many microorganisms including *M. pneumoniae* are able to adhere to host tissues for colonization and internalization, which leads to host infection (Seto *et al.*, 2003; Shin *et al.*, 2005). In addition, the several factors contributing to mistreatment included: misdiagnosing or providing the wrong prescription, patient compliance, and misusing the antimicrobial agents. The major factor causing a peak like in brain infection has been *M. pneumoniae* infection, in that the victims are immuno-compromised by antigenic variation. In addition, the problem is that patients who endure from persistent *M. pneumoniae* are habitually being a continuous transmission source for the rest of their community (Wattanathum *et al.*, 2003). It is noticeable that some recent therapies are no longer effective (Suzuki, 2006). Accordingly, the determination of *M. pneumoniae* virulence factor and its counterpart protein need to be investigated for novel antimicrobial development.

One of the main objectives of this study is to address *M. pneumoniae* virulence factor which might facilitate this bacterium entering hBBB. Although, molecular biology techniques for *M. pneumoniae* research are available, the bacterium is quite difficult to grow and maintain the culture of this pathogen in laboratory. Moreover, *in vitro* model of hBBB has not been established perfectly yet. These are considered to be an important drawback for conducting this research. To help counteract these problems, this study uses computer-aided screening technique to scan putative virulence factor which can potentially be novel targets. In particular, the study was carried out using bioinformatics and bacterial genomic data to find putative virulence factors of *M. pneumoniae*. In this study, the bioinformatics itself can be defined as exploiting large databases of biological information with specific *in silico* tools complementing with whole genome transcriptional profile and two-dimensional proteome map of *M. pneumoniae*, which are now available (Dandeker *et al.*, 2000; Regula *et al.*, 2000; Weiner *et al.*, 2003). Then, the structure of putative virulence factors (protein) was built by molecular modeling method. Finally molecular docking and molecular dynamic (MD) approaches were then be used to study the interactions

between *M. pneumoniae* virulence factors and its counterpart proteins that distribute within hBBB. The specific interactions between *M. pneumoniae* and host target protein may allow this organism infection strategy for the pathogen to adapt, and target individual cell or tissue upon infection. An understanding these interactions at a structure level leads to a possible key in combating a particular infection at a very early stage.

Objectives

1. To define putative *M. pneumoniae* virulence factors.
2. To model 3-dimensional structure of putative *M. pneumoniae* virulence factors.
3. To determine appropriate surface proteins on human brain microvascular endothelial cells, leading to possible interaction with *M. pneumoniae* virulence factors.
4. To analyze interaction between *M. pneumoniae* virulence protein and endothelial cell surface protein.
5. To analyze potential route(s) of *M. pneumoniae* entering human blood brain barrier.

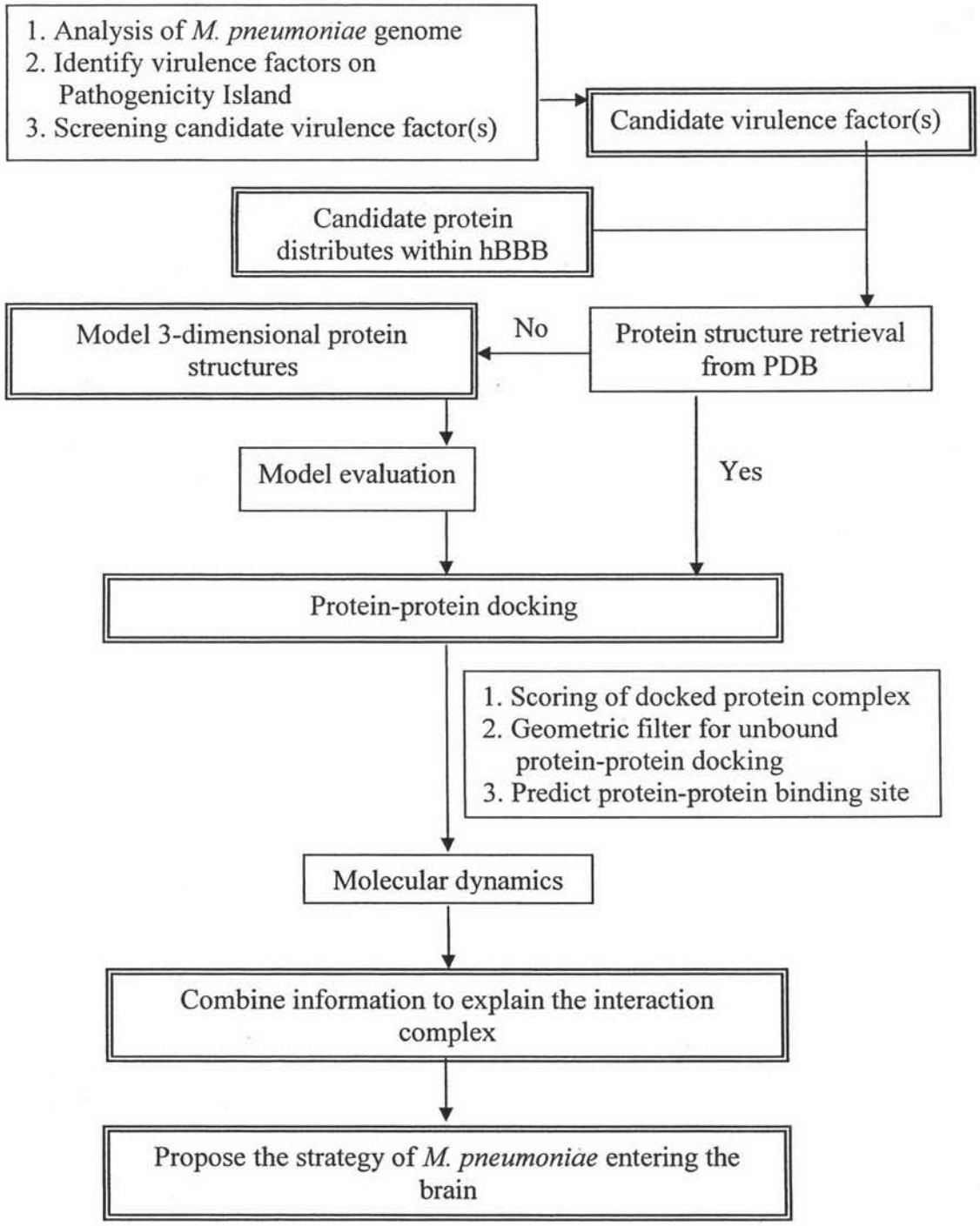
Scope of study

This present study investigated candidate virulence factors by *IslandPath* search tool. To validate selected virulence factor as a potential target in blood brain barrier, specific interactions of target complex were identified. This research describes a structural modeling approach that uses comparative modeling of selected virulence factor and its counterpart protein, which is well validated and documented. Structural algorithms including THREADER, 3D-PSSM, and Phyre web server were used to determine best possible template for comparative modeling. Once template was obtained, MD simulation in NAMD was used in docking experiments, in which the virulence factor model was docked with its known counterpart protein. The decreasing of accessible surface area and binding energy from the docking were summarized. These interactions could potentially be key interaction for of *M. pneumoniae* to invade brain tissue upon infection.

Experimental design

The putative virulence factor of *M. pneumoniae* genome was investigated by both data mining and bioinformatics approach including un-annotated gene was analyzed by Sequence annotation tools. Subsequently, 3D structure of the putative virulence factor (target protein) was be built by computer modeling. The construction of 3D structure is based upon technique of homology modeling and refinement of homology by a variety of criteria is based upon the biophysical properties of proteins (Romero-Arroyo *et al.*, 1999; Seto *et al.*, 2001; Layh-shmitt *et al.*, 2000). MODELLER, a homology modeling tool, (Marti-Renom *et al.*, 2000) is the most advance tool to build high quality model (Wallner and Elofsson, 2005). To start homology modeling using MODELLER, homologous protein of target protein was selected by using THREADER (MacGuffin *et al.*, 2004), 3D-PSSM, and Phyre (Kelley *et al.*, 2000) web server for template finding. The sequence alignment was used as starting point to align amino acid sequence of target protein with template sequence by MODELLER (Marti-Renom *et al.*, 2000). Then 3D model of target protein was derived from the aligned sequence by using MODELLER 8V1 program. Subsequently, the model was validated by evaluate program PROCHECK (Laskowski *et al.*, 1993), Verify 3D (Kirton *et al.*, 2002), and ERRAT (Colovos and Yeates, 1993). The refinement model was completed by energy minimization and NAMD configuration file in implicit water via NAMD2 program (Kal'e *et al.*, 1999). The protein-protein interaction between *M. pneumoniae* virulence factor and human plasminogen kringle domain2 was investigated by Hex4.5, macromolecular docking tool. 3D parametric functions, surface shape and electrostatic charge was also calculated by Hex 4.5 program for the best interaction model selection (Ritchie *et al.*, 2003). Then MD approach was used to investigate protein complex in implicit water and motion environment by NAMD configuration file via NAMD2 with parallel computers. The MD output and DCD trajectory files were then taken to describe RMSD and energy binding. Finally, all information was gathered and analyzed to propose the strategy of *M. pneumoniae* used to enter human brain microvascular endothelial cells.

Flow chart of experimental design for bioinformatic and computational modeling



Contributions of the study

1. Obtain information on proteins that facilitate the entering of *M. pneumoniae* to human brain in order to be targets for drug discovery.
2. Gain a novel basic knowledge of how *M. pneumoniae* trafficking across human blood-brain barrier.