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Stability and membrane interactions of an autotransport protein: MD simulations of the Hia translocator domain in a complex membrane environment

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ABSTRACT

Hia is a trimeric autotransporter found in the outer membrane of *Haemphilus influenzae*. The X-ray structure of Hia translocator domain revealed each monomer to consist of an α-helix connected *via* a loop to a 4-stranded β-sheet, thus the topology of the trimeric translocator domain is a 12-stranded β-barrel containing 3 α-helices that protrude from the mouth of the β-barrel into the extracellular medium. Molecular dynamics simulations of the Hia monomer and trimer have been employed to explore the interactions between the helices, β-barrel and connecting loops that may contribute to the stability of the trimer. In simulations of the Hia monomer we show that the central α-helix may stabilise the fold of the 4-stranded β-sheet. In simulations of the Hia trimer, a H-bond network involving residues in the β-barrel, α-helices and loops has been identified as providing stability for the trimeric arrangement of the monomers. Glutamine residues located in the loops connecting the α-helices to the β-barrel are orientated in a triangular arrangement such that each forms 2 hydrogen bonds to each of the corresponding glutamines in the other loops. In the absence of the loops, the β-barrel becomes distorted. Simulations show that while the trimeric translocator domain β-barrel is inherently flexible, it is unlikely to accommodate the passenger domain in a folded conformation. Simulations of Hia in an asymmetric model of the outer membrane have revealed membrane–protein interactions that anchor the protein within its native membrane environment.

1. Introduction

Haemophilus influenzae is a human pathogen responsible for a wide range of clinical diseases including sinusitis, bronchitis and pneumonia and less commonly, serious systemic disease such as meningitis and septicaemia. The initial step in infection involves colonisation of the upper respiratory tract. The autotransporter protein Hia promotes adherence of the bacterium to the human respiratory epithelium. To mediate adhesion with host cells, the adhesin must be presented on the surface of the pathogen [1,2]. For Gram-negative bacteria such as *H. influenza*, this involves translocation of the protein across the inner and outer membranes. This is generally achieved *via* one of five different protein secretion pathways. The autotransporter or type V pathway is a relatively simple protein secretion mechanism [1,3]. All proteins secreted *via* this mechanism share a common structure, namely: (a) an N-terminal amino signal sequence for transport across the inner membrane; (b) a secreted functional protein, or 'passenger' domain; and (c) a C-terminal translocator domain, which forms a pore in the outer membrane (Fig. 1). The N-terminal signal domain directs transport of the precursor protein across the inner membrane *via* the Sec pathway, it is subsequently cleaved by signal peptidase I. Transport across the inner membrane is followed by insertion of the C-terminal translocator domain into the membrane, where it forms a β-barrel. The passenger domain is then translocated to the surface of the organism where it is usually cleaved.

Autotransporters can be divided into two subfamilies, (1) conventional autotransporters and (2) trimeric autotransporters. Hia is a member of the latter subfamily. Each monomer is composed of a 50 residue N-terminal signal domain, an 857 residue passenger domain and a 75 residue C-terminal domain. The X-ray structure of the Hia translocator domain revealed each monomer to contribute four β-strands and one α-helix to form a trimeric 12-stranded β-barrel containing three α-helices within the central channel [4,5] (Fig. 1). The α-helices are connected to the β-barrel by a long loop (α1–β1 loop), which extends 2/3 of the way into the pore.

The passenger domain of Hia is not cleaved upon secretion and remains cell-associated [6]. While the structure of the entire passenger domain is unknown, Yeo et al. reported the structure of the high-affinity binding domain, HiaBD1, one of the two homologous binding sites in the passenger domain. HiaBD1 contains three receptor binding sites arranged in a novel trimeric architecture with three-fold symmetry [7]. The structures of the translocator domain and HiaBD1 of the passenger domain have provided important insights into the structure

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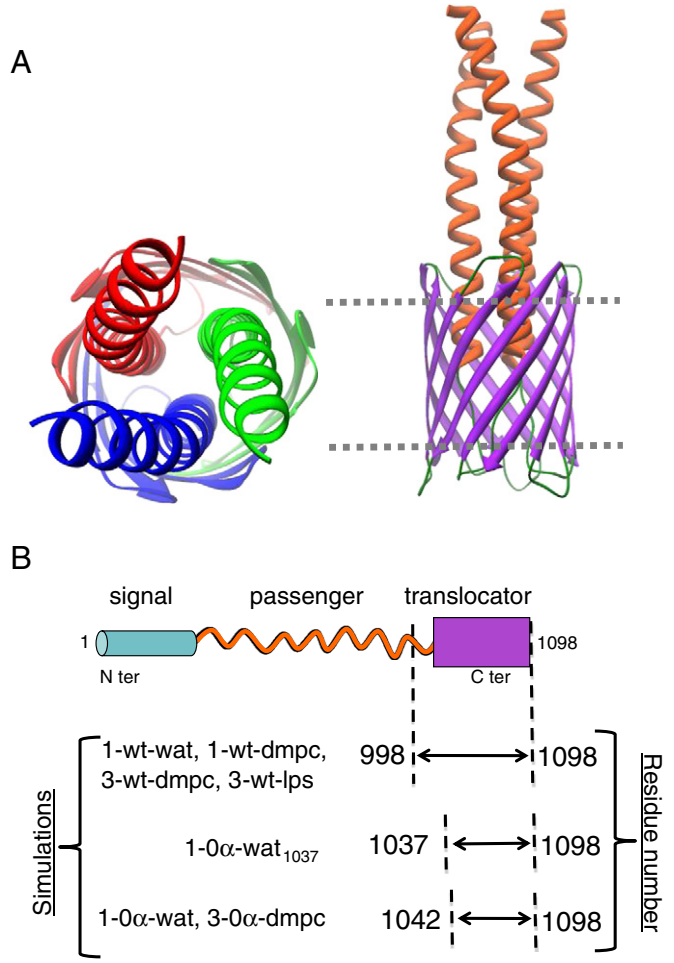

Fig. 1. A — Two views of the X-ray structure of Hia. Looking down the principal axis from the extracellular end (left), here the three monomers are coloured individually (red, green and blue) and side view (right), here the β-barrel is purple, the loops are green and the α-helices are red. B — Schematic of domain organisation. The rulers indicate the portion of the Hia structure that was used in each simulation of this study.

and function of the Hia autotransporter. However, the exact mechanism of translocation remains unclear. In particular key questions remaining unanswered include:

1– What is the role of the α-helices?
2– Is the β-barrel rigid or flexible?
3– How does the protein interact with the complex environment of the outer membrane?

Hia remains the only trimeric autotransporter to have its translocator domain solved experimentally, yet it shares similarities with the other monomeric autotransporters: EspP and Hbp from *Escherichia coli* [8,9], EstA from *Pseudomonas aeruginosa* [10], and NalP from *Neisseria meningitidis* [11]. The translocator domain of the monomeric autotransporters is a 12-membered β-barrel, with a single N-terminal α-helix located within the hydrophilic pore. The diameter of the Hia pore with the central α-helices removed, as revealed by the X-ray structure, is 1.8 nm compared to 1.1 nm in the X-ray structure of EspP and 1.2 nm in the X-ray structure of NalP. Thus it might be expected that the autotransporters share a common translocation mechanism. However, experimental and simulation studies have suggested a plug-like role for the α-helix in NalP, while it does not seem to be implicated in maintaining the integrity of the β-barrel [11,12]. In contrast, experimental studies have shown that the α-helices located within the Hia β-barrel and in particular the loops that connect the α-helices to the β-barrel (α1–β1 loop) are key in maintaining the structural integrity of the trimer [4].

The translocation mechanism is likely to involve complex conformational rearrangements, however before exploring what these conformational changes may be, it is useful to reflect upon the X-ray structure of the trimeric translocator domain in detail and consider the factors that contribute to its stability. The interface between the monomers is formed largely through helix–helix, strand–strand and loop–loop (α1-β1 loop) interactions. The strength of the stabilising interactions at these interfacial regions is likely to play a key role in mediating the conformational flexibility of the trimeric protein. While helix–helix interactions are usually driven by small, polar interactions in transmembrane regions [13–15], in the aqueous environment the driving force comes from burial of hydrophobic residues [16,17]. Thus it is not surprising, as it extends from the β-barrel into the aqueous phase, that the helix–helix interface of the Hia translocator domain is largely hydrophobic [4,5]. The interface between the α1–β1 loops is largely polar, therefore hydrogen-bonding and electrostatic interactions are likely to contribute to the stability of the protein in this region. Indeed, polar side-chain interactions of contributing to the stability of enclosed loops have been previously reported from simulation and structural studies of autotransporters [8,18].

An understanding of the role of the protein–protein interactions that have been revealed by the static X-ray structure, by consideration of the conformational dynamics of Hia is fundamental to gaining further insights into the translocation mechanism. Molecular Dynamics (MD) simulations provide a computational route to analysing the conformational dynamics of membrane proteins and have been successfully applied to evaluate possible transport mechanisms of ABC transporters [19,20] and of lactose permease [21,22]. Thus, we employ MD simulations of the Hia translocator domain to demonstrate that the α1–β1 loop plays a key role in maintaining the stability of the β-barrel. In addition, we investigate the conformational dynamics of the Hia translocator domain in an OM model membrane containing a realistic mixture of lipopolysaccharide (LPS), also known as lipooligosaccharide, and phosphatidylethanolamine (PE) lipids. Analysis of these simulations suggests that positive residues at the base of the α-helices interact with the LPS inner core to help anchor Hia within the outer membrane.

2. Methods

2.1. General simulation details

All simulations in this work were performed using the GROMACS simulation package, version 4.5.1 [23–25]. The water model used in the simulations was the SPC model [26]. Simulations were undertaken in the NPT ensemble, with the Nosé–Hoover thermostat [27,28] with a time constant of 0.5 ps and the Parrinello–Rhaman barostat [29,30] with a time constant of 5.0 ps used to maintain a temperature of 310 K and a pressure of 1 bar. Long-range electrostatic interactions were treated using the smooth particle mesh Ewald method [31] and a long-range dispersion correction was applied to the energy and pressure beyond the cut-off. The neighbour list was updated every 5 steps during the simulations. All bonds were constrained using the LINCS algorithm [32] allowing a 2 fs timestep to be applied in all simulations. Energy minimisations were performed using the steepest descent method for 1000 steps. Simulations in which positional restraints were used involved the application of a harmonic restraint force of 1000 kJ mol^{-1} nm^{-2} to non-hydrogen atoms. The Hia crystal structure used in the simulations was the 2.0 Å model of Prince et al. (PDB code 2GR7). The protonation states of all titratable residues of Hia were assigned using the standard protonation states at pH 7 and checked with the H++ webserver [33–35]. Repeats of all of the simulations were performed using different randomly assigned starting velocities. Table 1 shows a summary of the simulations described in this work.