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Why do the outer membrane proteins OmpF from *E. coli* and OprP from *P. aeruginosa* prefer trimers? Simulation studies

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ABSTRACT

Porins are water-filled protein channels across the outer membrane of gram-negative bacteria. They facilitate the uptake of nutrients and essential ions. Solutes are filtered by a constriction loop L3 at the mid of a pore. Porins are heat-stable and resistant to toxic agents and detergents. Most porins are trimer, but no clear explanation why trimeric form is preferable. In this work, we thus studied effects of oligomerization on porin structure and function in microscopic detail. A well-studied OmpF (general porin from *Escherichia coli*) and well-characterised OprP (phosphate-specific pore from *Pseudomonas aeruginosa*) are used as samples from 2 types of porins found in gram-negative bacteria. MD simulations of trimeric and monomeric pores in pure water and 1 M NaCl solution were performed. With a salt solution, the external electric field was applied to mimic a transmembrane potential. Expectedly, OprP is more stable than OmpF. Interestingly, being a monomer turns OmpF into an anion-selective pore. The dislocation of D113's side chain on L3 in OmpF causes the disruption of cation pathway resulting in the reduction of cation influx. In contrast, OprP's structure and function are less dependent on oligomeric states. Both monomeric and trimeric OprP can maintain their anion selectivity. Our findings suggest that trimerization is crucial for both structure and function of general porin OmpF, whereas being trimer in substrate-specific channel OprP supports a pore function.

1. Introduction

Porins are water-filled, pore-forming protein channels across the outer membrane of gram-negative bacteria. They allow diffusion of nutrients and metabolites across outer membrane with various grades of selectivity [1–3]. Especially, porins also serve as an entryway for many antibiotics [4–7]. Porins can roughly be classified as general and substrate-specific pores. General porins (e.g. OmpC and OmpF) filter solutes based on their molecular size, while substrate-specific porins, such as OprP, have specific binding site for certain molecules [8]. Generally, porins have a β-barrel structure connected with extracellular loops and intracellular turns. Most of them have the extracellular loop L3 (constriction loop) folds back into the β-barrel lumen creating a constriction region.

Most porins are homotrimers (e.g. OmpF [9], OmpC [10], OprP [11], and PhoE [12]) and some exist as a monomer such as OccD1 [13], OmpG [14], and NanC [15]. The previous studies found that the multimerization was important for function and structure in soluble proteins. The oligomerization gives shape to active sites, increases affinity of protein complexes for ligand binding, and promotes protein stability [16,17]. However, present understanding of protein oligomerization is mostly originated from studies of soluble proteins. It is unclear whether the same principles can be applied to membrane proteins, especially porins. In a single porin, the oligomerization was found to be not a requirement for stability and membrane insertion [18], whereas the role of multimerization in a triplet-pore porin remains unclear. To better understand the significance of being trimer, in this study, an example of general porins, OmpF, and substrate-specific pores, OprP, were studied in comparison. Both are from different groups of porins and adequate details on structure and function are available. The study of both porins will help us better understand the nature of most trimeric porins.

OmpF is a well-studied porin prevalently found in *Escherichia coli* (*E. coli*). It consists of 16 antiparallel β-strands connected by

Abbreviations: OprP, outer membrane protein P; OmpF, outer membrane protein F; MD, molecular dynamics.

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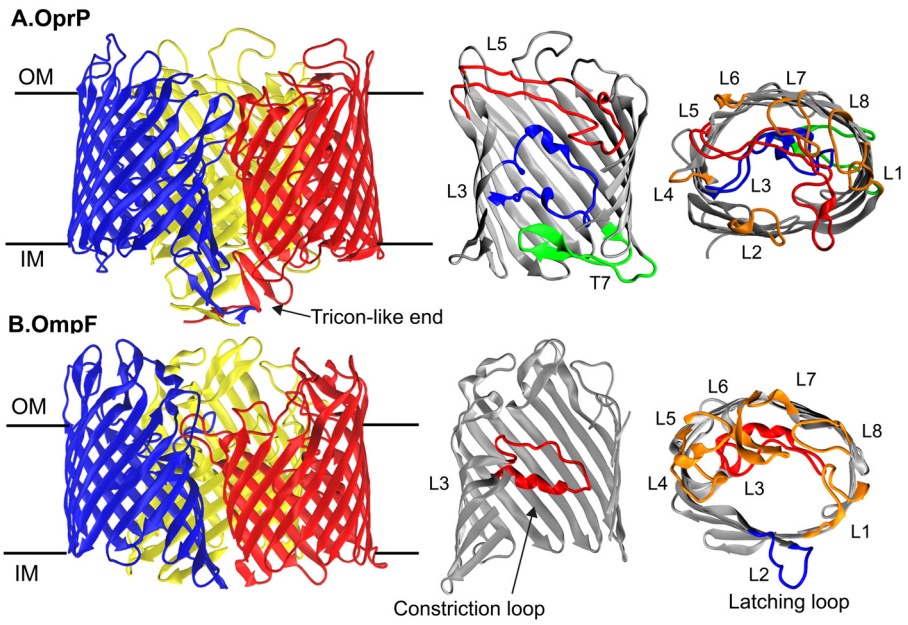

Fig. 1. Trimeric porins (A) OprP (B) OmpF. The locations of key loops are shown in cross-section and top views.

turns at the periplasmic side and loops at the extracellular side [9,19–23]. Like other OMPs, it has an extracellular loop (L3) folded into the lumen in each monomer acting as a molecular filter (Fig. 1). Besides, there is the so-called latching loop (L2) reaching over to the neighbouring monomers to stabilize a trimeric form (Fig. 1B). OmpF is defined as a general or non-specific porin even though it is slightly cation-selective. Despite the observed trimeric form of OmpF, dimeric and monomeric states have also been observed experimentally [8,24–26]. Dimer was found to act as an intermediate in trimerization [24]. Moreover, key residues that are crucial for protein-protein interaction interface were also identified [8].

OprP from *Pseudomonas aeruginosa* (*P. aeruginosa*) is a well-characterised phosphate-selective homotrimeric pore. OprP plays a key role in high-affinity phosphate uptake under the condition of phosphate starvation. In an absence of phosphate, OprP can also conduct common anions. Each pore adopts 16 antiparallel β-strands lined by three positively charged loops (L3, L5, and T7) folded into its lumen (Fig. 1A). These folded loops create an arginine ladder and lysine cluster which are the key features for phosphate selectivity. Unlike other porins, OprP has an extended periplasmic 'tricon-like' end that is involved in stabilizing trimer [11]. Due to its high selectivity for phosphate, almost all OprP studies are devoted to phosphate selectivity and translocation mechanism [27–31]. No previous work emphasizes the role of oligomerization on structural and functional properties.

In this study, Molecular dynamics (MD) simulations were performed to explore the effect of oligomerization state on structure and function in microscopic level. MD simulations have been widely used in many earlier studies to investigate the dynamic properties of OmpF. Studies of trimeric OmpF revealed deviations of dynamical structure relative to the crystal structure. They also showed that L3 flexibility affected a change in pore cavity [32,33]. MD simulations were also successfully used to observe behaviour and solute (such as antibiotics) passage through OmpF in comparison with experiments [2,33–37]. MD simulations were also conducted to reveal a mechanism of phosphate transport by OprP [29–31]. In this study, we then used MD simulations to reveal the importance of being trimer in OmpF and OprP. To generate an ion flow, we apply the external electric field across a membrane. Recently, MD simulations with applied external electric field become a popular tool for studying ion channels [38–40]. Despite concerns about a degree of artificiality, recent studies have been shown that a constant external electric field is a valid representation of the influence of an electromotive force, exerted by a voltage difference [38,41]. Despite the fact that OprP is phosphate-specific, only a common salt (NaCl) was used in this study so as to compare results with general OmpF pores. Understanding structural and functional properties affected by different oligomeric states here can facilitate further studies on the structural biology of outer membrane proteins (OMPs) and the development of nanopore technology.

Table 1
8 systems set up in this study.

Name	Condition		Time (ns)
	Pure water (MD)	1 M NaCl (1 M)	
TF	Trimeric OmpF	Trimeric OmpF	30
MF	Monomeric OmpF	Monomeric OmpF	30
TP	Trimeric OprP	Trimeric OprP	30
MP	Monomeric OprP	Monomeric OprP	30

2. Method

2.1. Molecular dynamics simulations

The trimeric OprP (PDB ID: 2O4V) and OmpF (PDB ID: 2OMF) crystal structures consisting of 411 and 340 amino acids in each monomer respectively were downloaded from the Protein Data Bank (www.rcsb.org). The protonation states of all charged amino acids were set at physiological pH. To study the effects of oligomeric states on structure and function, trimeric and monomeric systems of both OmpF and OprP in electrolyte solution (1 M NaCl) and pure water were set. There were 8 simulations performed as seen in Table 1 TF stands for a trimeric OmpF and TP is a trimeric OprP. TF1–TF3 represent OmpF monomer 1–3 and TP1–TP3 are OprP monomer 1–3. MP and MF are for stand-alone OprP and OmpF, respectively.

Each system was embedded in a pre-equilibrated dimyristoylphosphatidylcholine (DMPC) bilayer (pre-equilibrated by running a 2 ns simulation). The solvent-accessible molecular surface of both