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LETTER TO THE EDITOR

Computational profiling of pore properties of outer membrane proteins

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Introduction

The β-strand forming outer membrane proteins (OMPs) are found in the outer membrane of the Gram negative bacteria, eukaryotic organelles of prokaryotic origin, mitochondria, and chloroplasts. The OMPs present in the outer membrane play a vital role in the maintenance of cell homeostasis. Two classes of OMPs were recognized within the outer membrane, one is peripheral and another is integral membrane proteins with unique β-barrel fold architecture. In Gram negative bacteria, OMPs are classified into six families based on the function (Koebnik, Locher, & Van Gelder, 2000). There are general porins (OMPC, OMPF, and PhoE), passive transporters (LamB, ScrY, FadL, and TodX), active transporters (siderophores transporters FepA, FecA, Fhu A, and vitamin B₁₂ transporters BtuB), enzymes (phospholipase OmpLA or protease OmpT), defensive proteins (OmpX), and structural proteins (OmpA).

Understanding the structure and function of OMPs is of critical importance, in the antibiotic design and development. In an antibacterial therapy, one of the main steps for the antibiotic is to cross the outer membrane through OMPs like OMPF present in S. typhi (Balasubramaniam, Arockiasamy, Kumar, Sharma, & Krishnaswamy, 2012). Most of the antibiotic targets are present within the bacterial cell; the antibiotics are known to take two routes to reach the targets. The hydrophobic antibiotics pass through the lipid and the hydrophilic molecules translocate through water-filled pores. The lipid and protein compositions of the outer membrane have a strong impact on the permeability of antibiotics and drug resistance. So, studies conducted on physico-chemical properties of amino acid residues along the pores and by considering the lipid–OMP interactions may provide valuable insights into the antibiotic resistance. Liposome-swelling assay is used to qualitatively characterize antibiotic translocation through porin. Later quantitative electrophysiology measurements were used. The OMP-mediated antibiotic permeability is still not clearly understood. The availability of high resolution porin structure and further investigation on this reveals that antibiotic translocates by interacting with the surface of the channel. Ampicillin-binding site in the channel of OMPF is identified. Facilitated diffusion through a binding site was observed in maltoporin (Danelon, Brando, & Winterhalter, 2003). Computational molecular dynamics (MD) studies in the past have shed light on the antibiotic translocation process (Kumar et al., 2010). Moreover, the alteration or modification of porin expression is associated with antimicrobial resistance. The emergence of porin-related antibiotic resistance is a challenge for designing better antibiotics (Beceiow, Tomas, & Bou, 2013; Nikaido, 2003). There are limited studies on relating computational modeling/dynamics with experiments. A search on pubmed results in only 150 hits in which very few are interesting. An interesting work by Kumar et al., demonstrates the structural and dynamical properties of the two major porins (OmpF and OmpC) in Escherichia coli, using MD simulations and showed the relationship between pore amino acid properties and antibiotic transport (Kumar, Hajjar, Ruggerone, & Ceccarelli, 2010). They presented the results of transport properties using accelerated MD simulations to probe the diffusion of norfloxacin (a fluoroquinolone antibiotic) through the two porins OmpF/OmpC.

Porins perform various functions by depending upon channels, regulation of porin expression and its structure. Although OMPs are commonly made of β-strands, it is interesting to observe the variety of completely different functions, that is been performed such as diffusion pores, substrate-specific transporters, signal transduction, and enzymatic activity. The transport of various substances
across membrane depends on water-filled channels and pore dimensions which determine the exclusion limit. Mostly, porins form a tightly assembled trimer to produce a water-filled channel. The polypeptide chains of the subunit of porins are assembled in such a manner, to produce a significant narrowing of the channel. This narrow channel is responsible for several physiological functions of porins to exclude toxic compounds and to influx-required nutrients (Kumar, Bhandari, & Krishnaswamy, 2015). Among various parameters responsible for physiological functions of porin, attention in the present study is focused on pore size and dimension. The residues at the pore mouth and interface are believed to be essential for bacterial pathogenesis and studies of antigenic variations due to variation in pore parameters have implications in development for porin-based vaccines. There are many reviews that focus on the OMPs structure and function. (Koebnik et al., 2000). However, this literature does not make explicit review on the pore properties relating with the function, considering the vast amount of 3D structural data of OMPs available in the protein data bank (PDB).

There are computational studies to profile pore or channel properties (Pellegrini-Calace, Maiwald, & Thornton, 2009) for a small set of β-barrel membrane proteins and for other proteins like bovine heart Cytochrome c oxidase, GABA_A Ion Channel (Vijayan et al., 2012).

In the present backdrop here, we systematically analyze the pore-forming OMPs, with a special attention on the pore region. Considering the above facts and availability of high-resolution porin crystal structures in PDB prompted us to explore in detail about the different conformational properties of pore-lining residues in channels like amino acid composition, pore size, diameter, volume, solvent accessibility surface area (SASA) etc., in OMPs. Profiling the pore features, across the different OMPs would allow us to understand the nature’s way of devising channels and would benefit us in the task of designing more efficient antibiotics for combating the infectious diseases and emerging resistance.

Materials and methods

The OMP structures were retrieved from the PDB, using the key word ‘outer membrane porin’ and ‘outer membrane protein.’ Combining the results obtained from above search, we have got more than 500 hits that include alpha-helical and beta-sheet forming OMPs. Out of these structures, only beta sheet forming outer membrane proteins form the source for present study. The mutants structures solved through NMR and cryo-electron microscopy were not considered in this study. After data cleaning, 78 protein structures were considered and used for the further computation. Total number of OMP structure retrieved from PDB, used in this study and the organism it belongs is provided vide Table 1.

The substrate for each OMP was identified from the PDB and/or literature. The 3D structure of the ligand was downloaded from the pubchem database using keyword search. The ligands were prepared in Accelrys Insight II software, and these prepared structures were used to calculate descriptors, available in MOE software. Only, 49 ligands were identified, the properties calculated from this 49 ligand structures were used for the correlation studies. The principle component analysis (PCA) and correlation study on the OMP pore properties and substrate descriptors were developed using statistical software statistica 7.0, with default settings.

The CASTp is an online server, used for the detection of the possible protein cavities (Dundas et al., 2006). It calculates solvent accessible surface area (SASA) and solvent accessible surface volume (SASV) for the cavities and mouth region of these cavities. CASTp server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. A probe radius of 1.4 Å was used for the calculation of the cavity surface area and volume.

PoreWalker, online server is used to identify the channels of transmembrane proteins (Pellegrini-Calace et al., 2009). It provides vital information like pore diameter profile at regular interval along the channel, size, shape, regularity, and the pore-lining residues. We have used the PoreWalker server to calculate these above said parameters for the OMPs.

Results and discussion

Composition of pore amino acid residues

The OMPs constitute general porins and substrate-specific porins like maltoporin and sucrose-specific porins. The sugars like maltose are bound to a binding site of the maltoporin in order for translocate. Functional studies by mutation of specific residues in LamB and ScrY have changed the functions of these two substrate-specific porins. Site-directed mutation of several residues in the E. coli OMPF pore region was shown to produce alteration in minimum inhibitory concentration (MIC) of various antibiotics. Hence, amino acid composition of the pore region plays a vital role in the translocation of small molecules in the cell. So we have extracted the pore-lining residues from the 78 OMP structures and furnished the percentage of the occurrences in Figure 1(A). It is interesting to note that Glycine (7.69%), a small hydrophobic residue and serine, a small polar residue (7.65%) are predominant in all the OMP pores. It has been already stated that the sequence of transmembrane porin segments is different than the other transmembrane segments. The flexible nature of glycine in various aspects of protein structure is already discussed (Saravanan & Krishnaswamy, 2015). The polar cysteine residue is the least occurring...
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amino acid with 0.07%. Arginine (positively charged) and Aspartic acid (negatively charged) residues comparatively occur in higher numbers at 7.57 and 7.49%, respectively. The presence of charged residues (ASP, GLU, LYS, ARG, and HIS) alone is an impressive rate at 27.19%. The presence of positively charged residues (LYS, ARG and HIS) with a 14.1% is slightly better than the negatively charged residues (ASP and GLU) at 13.09%. Intriguingly, the composition of charged and polar amino acids in the pore region is 64% and remaining 36% constitute nonpolar amino acids. These results show that the pore-forming amino acids are more favorable to translocate charged and polar molecules across the membrane. The occurrence of polar amino acids is higher than the nonpolar amino acids in the water-filled pores of OMPs, the difference, observed here is a 10%. This observation makes obvious that the OMPs are the entry channels for hydrophilic molecules.

Pore diameter

The limited pore diameter of diffusion channels of OMPs present in the outer membrane plays a selective barrier. Earlier, research work had established a size exclusion limit for porins, with diffusion of hydrophilic molecule of <600 Da. However, no attempt was made to profile the pore diameter of the OMPs. The size of the pore is also one of the major determinants that regularize the entry of small molecules with specific sizes. We have

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calculated average pore radius from the regular interval pore radius obtained from the PoreWalker. The small pore diameter (average) found for PDB 1UYN (2.38 Å³), is a translocator domain of the autotransporter NaIp from Neisseria meningitidis and largest pore diameter (average) found in PDB 2JK4 (20.46 Å³), is a human voltage-dependent anion channel (VDAC), also known as mitochondrial porin. The pore diameter (average) has been grouped into several bins, which are depicted in Figure 1(B). From the figure, it is clear that, about 32 OMP structure has a pore diameter (average) within the range of 5–10 Å³. Only one OMP structure has the widest pore in the range of 20–25 Å³. Nearly, 67% of the OMP pore diameter (average) fall within the range of 5–15 Å³, suggesting that medium-sized pore is more prevalent than the larger and smaller channels.

**Pore shape**

Pore shape symmetry is an important feature that affects the translocation of antibiotics through OMPs. MD study on the translocation of ampicillin through E. coli porin (Ziervogel & Roux, 2013) highlights the importance of the pore amino acid symmetry. PoreWalker predicts the shape of the pore and classifies the pore shape into three primary categories, they are denoted as ‘D,’ ‘U,’ and ‘S.’ ‘D’ indicates a conical frustum generated by decreasing pore diameter values, i.e. the diameter of the lower base of the frustum is bigger than the diameter of its upper circle. ‘U’ indicates the opposite conical frustum, i.e. lower base diameter is smaller than the upper base diameter. ‘S’ indicates a cylinder. Depending on the shape of the pore, PoreWalker defines a pore region by a single letter code or a combination of all the three in different sequence. In short, the sequence of these letters simply describes the overall pore shape. There are also some commonly recognized pore shape, they are (i) DU = Hourglass, (ii) UD = Diamond, and (iii) UDU/UDU = Hourglass-Diamond-Complex.

Through shape analysis, we observe that 78 OMPs share 33 types of pore shape or shape sequences. The frequency of occurrence of pore shape sequence is provided in Figure 2. Among the 33 types of shape, ‘UD’ (Diamond) shape sequence is observed for 18 OMP pore, a maximum number. Out of this, four PDB structure (2QTK, 4GEY, 1XKW and 3T20) belong to Pseudomonas Sp. and two each for osmoporin (1OSM and 2J1 N) and maltose-specific porin (1MPR and 1AF6), respectively. Another recognized pore shape ‘DU’ (Hourglass) is found only in one OMP structure (PDB 4D51) out of 78 OMPs studied. It is noteworthy to mention here that 17 OMPs have unique pore shape that is not shared by any other OMPs. Four OMPs (3JTY, 4FRX, 3WI4 and 3EMO) have only one shape feature i.e. either ‘D,’ or ‘U,’ or ‘S’. Two OMPs (3JTY, 4FRX) possess pore feature ‘D’ and one each for ‘U’ and ‘S’. A majority number of 61 OMPs pore shape shares a small number of 16 pore shape features and each shape occurs in more than one OMP.

**Pore straightness**

Pore straightness is one of the factors that affects movement of ligand or ions within the pore, and in turn affects the entry and exit to the cell. Earlier studies on membrane proteins show that ion channels possess a linear pore and channels that translocate organic molecules had an irregular pore (Pellegrini-Calace et al., 2009). Here, we analyze the OMP pore straightness, with respect to the ability of ligand translocation within the pore lumen. The regularity of the pore is deduced from the positions of the pore centers. Based on the pore centers along the channel, the regularity of the pore can be classified into three types. (i) regular and symmetric pore, (ii) non-linear and symmetric pore (iii) non-linear and irregular pore. In PoreWalker, the quality of the pore straightness is assigned in a range from 0 to 1%, a complete pore irregularity mean a value of 0 is assigned and
1 indicate a perfect straight pore. As per the PoreWalker, pore straightness prediction, the most irregular pore is observed for PDB 2HDI with a zero value. The straight pore is observed for PDB 1EK9. The representative OMP structure with straight and irregular pore and percentage of pore straightness are shown in Figure 3. Through the Figure 4, it is evident that only less than five OMP structures fall within the range of .8–1% straightness, suggesting only very few OMP structures channels are straight and fall in the regular and symmetric pore category. Most of the OMP pore is either irregular or moderately regular, thus most of the OMPs fall in the category of either non-linear and symmetric pore or non-linear and irregular pore. These results show that the small molecule that would pass through the OMP pore channel has to go through several energy barriers.

**Pore length**

Depending on the length of the OMPs channel, the diffusion of the solute will tend to increase or decrease (Nikaido, 2003). It is interesting to observe the required distance, for a ligand or an ion has to traverse within the pore. Based on the 3 Å increment used in PoreWalker to calculate pore diameter, we calculated the OMPs pore

![Image](image_url)
length. Profiling the pore length of various OMPs will help us to understand the probable path length of small molecule translocation pathway through the pore and the distance required to enter and exit the cell. The frequency of occurrences of pore length in angstrom is furnished in Figure 4(A). In the range of 40–50 Å pore length, there are 30 OMPs structures. Another 17 OMPs fall within the range of 30–40 Å. So it is evident that the majority of the OMPs fall within small to medium range pore length. It is also interesting to note that the smallest pore length is calculated for PDB 1QJP, which is an Outer Membrane Protein A (OMPA) from *E. coli* with a length of 21 Å. The largest pore length is observed for PDB 1EK9.

**Pore mouth SASA and MSA**

The OMPs are channel-forming protein that has a pore or a cavity that spans through the entire membrane with an opening on both sides of the membrane. The opening in both the side of the membrane that is formed by the OMPs is termed as pore mouths. The nature of pore mouth region is very important, as depending on the presence of positively and negatively charged residues, would attract either cationic or anionic molecules into the pore. A classical example of this type of OMP is the Omp32, anion selective porin from *Acidovorax delafieldii* and *Comamonas Acidovorans*. It is the interest of the drug designer to know the SASA and molecular surface area (MSA) properties of the pore mouth and pore region. So that better understanding of the physicochemical properties of the pore mouth and pore channel would result in design of a better permeable drug molecule. Hence, we calculated SASA and MSA for the mouth region and the results are depicted in Figure 4(B). About 82% of the OMPs are having SASA region in the mouth. As expected, the results agree with the assumption that the mouth region should be solvent exposed, since the periplasmic and external mouths are faced towards the solvent-rich region. In contrast, MSA in the 0–800 Å³ range has only 40% OMPs structure. Comparative analysis reveals SASA regions are more than the
MSA region in the pore mouths. In the range of 800–1000 Å³, OMPs mouth with large MSA region and the number of occurrence are high. This is because the majority of the OMPs pore mouths is large in size.

**Pore SASA and MSA**

The SASA of lipid-facing residues which is used to understand protein design rules that govern the lipid-facing residues in the *E. coli* OmpA is studied. This understanding shows that SASA would be a useful property in *de novo* β-barrel membrane protein design (Stapleton, Whitehead, & Nanda, 2015). A study on the translocation kinetics of antibiotics through OmpC porin suggests that solvation within the porin cavity is highly energetically favorable. The cavity volume and SASA properties of the drug targets have been used for designing or prediction of prospective inhibitor molecules (Manoharan, Chennouj, & Ghoshal, 2015). Using the CASTp server, we calculated the SASA, MSA and volume of SASA and MSA for the pore region. The result is depicted in Figure 4(C). The number of OMP structures with pore SASA and the volume of SASA are larger than the MSA and volume of MSA for the 0–1000, 1001–2000, and 5001–6000 Å³ bins. This result suggests that the entire pore region is solvent exposed. In the range of 3001–4000, 4001–5000, and 5001–6000 Å³ bins more OMPs with large pore MSA are present.

**Pore property and function**

Based on the pore property, we have attempted to explain the function of the OMPs. For this we carried out PCA and correlation analysis. In an attempt to delineate the common and different pore feature that is been shared by the OMP structure that is under investigation, we performed PCA. PCA is a multivariate technique used for data reduction and to classify structures based on the variables. Here, we used nine variables (pore radius, pore straightness, pore length, mouth SASA, mouth MSA, pore SASA, pore MSA, pore volume SASA, and pore volume MSA) in the classification of OMP proteins based on their pore properties. The PCA plot for the first two factors is given in Figure 4(D). The factor 1 and factor 2 explains 95.3 and 4.4%, respectively, of the total variance observed in the variables. Out of 78 structures, 75 OMP structures fall into a single cluster, which is highlighted with a red oval in Figure 4(D). This shows that most of the OMP proteins pore features are similar. Only three OMP structures...
Table 2. Correlation coefficient values obtained for the OMP pore properties and ligand properties.

<table>
<thead>
<tr>
<th></th>
<th>Pore length</th>
<th>Pore straightness</th>
<th>Pore radius average</th>
<th>Pore mouth SASA</th>
<th>Pore mouth MS</th>
<th>Pore SASA</th>
<th>Pore MS</th>
<th>Pore volume SASA</th>
<th>Pore volume MS</th>
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<td>.13</td>
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<td>.37</td>
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<tr>
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Note: Correlation values marked in bold letters are significant at p < .05000.

(4K3C, 4K3B and 1EK9) are found to fall outside the cluster. These proteins are found to have additional attachment (alpha helical segment) to the beta-barrel sheets. Because of these features, three OMP proteins are away from the cluster.

We also worked on to establish correlation between the physico-chemical properties of OMP pore with its function, by correlating pore properties with OMP substrate properties. We have considered 13 ligand descriptors in our study which would have functional implications, and the list of descriptors is provided in Table 1 of Supplementary Material. The correlation coefficient obtained for the OMP pore properties and substrate properties are given vide Table 2. The analysis of the correlation matrix obtained between the OMP substrate properties and OMP pore properties, reveals the functional implications of these properties. All the ligand properties except ligand molecular mass show significant correlation with the pore SASA, pore mouth MS, and pore MS. Some of the important observations are the molecular mass of the OMP substrates show significant correlation with the pore radius. Ligand molecular mass simply describes the substrate size, so this observation clearly validates the existing of the size exclusion limits of the OMPs. Only based on the pore size the entry of the ligand is determined. Another important ligand property is the dipole moment; this descriptor is known for the long-range recognition and subsequent binding of ligand to the receptor. MD simulation reports show that antibiotics that pass through the constriction zone should adopt particular orientation, with the dipole of the antibiotic aligned along the pore electric field (Bajaj et al., 2016). A significant correlation of ligand dipole moment with the OMP pore mouth SASA and MS shows that the pore mouth helps the substrates to attract to the OMPs. The outer membrane protein, Omp32, anion-selective porin from Acidovorax delafeldi and Comamonas Acidovorans is one such protein, where the positively charged residues present in the pore mouth of this protein help to attract the anionic substrates to the pore lumen.

It is true that both static and dynamic properties of OMP pores help in antibiotic translocation. The static properties like the amino acid composition of the OMPs pore play a major role in antibiotic uptake. The amino acid composition in the pore dictates the nature of the pore and the kind of molecule that will be translocated. Any physico-chemical changes in the pore affect the antibiotic uptake. Alteration of the pore amino acid composition in OMPC33 created the change in the vector of the electric field of the pore, which results in an additional barrier for the antibiotic translocation (Bajaj et al., 2016). Pore residue substitution in OMPF, OMPC, and the Vibrio cholerae porin OMU decreases cationic selectivity. In our study, we have observed a significant correlation between the ligand molecular mass and pore radius. This is a classical example for the static properties influence in antibiotic translocation.

Conclusion

This is the first study that extensively analyzes the pore properties of OMPs from different species. Analysis of pore parameters of OMPs reveals several interesting observations like highest occurrences of glycine and lowest occurrences of cysteine. The polar and charged residues composition are higher than the hydrophobic residues in the OMP pore. Analysis of pore diameter suggests that prevalence of porins with medium-sized pores (5–15 Å³) than smaller and larger channels. It was found
that channel of very few porins are straight. Further, analysis of MSA and SASA of mouth region and pocket regions of pores reveals the variation in pore parameters in OMPs. A significant correlation observed between the OMP substrate descriptors with OMP pore properties shows that these properties would have impact on ligand translocation. An increased number of OMP substrate property correlations with pore mouth MS, pore SASA, and pore MS shows these properties are very important in antibiotic uptake. Profiling the pore features across the different OMPs would allow us to understand the diversity in pore properties of pores which is a major reason for porins to perform different physiological function. We conclude that the distribution of pore parameters like pore radius, shape, area, volume etc., in OMPs varies to perform its physiological function like translocation of ions and antibiotics. Survey of variation in such parameters presented here from OMP crystal structures may help to design antibiotics with better pore permeability property.

**Supplementary material**

The supplementary material for this article is available online at [http://dx.doi.org/10.1080/07391102.2016.1220329](http://dx.doi.org/10.1080/07391102.2016.1220329).

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**Disclosure statement**

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