Temperature factor analysis of outer membrane β-stranded porin crystal structures suggests the pore is not uniformly rigid

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Abstract

Outer membrane beta-stranded porins form a diverse and complex set of proteins which allow passage of molecules across the membrane interface have been analyzed here from a biophysical and structural perspective using atomic temperature factors or B-factors. Generally atomic temperature factors of molecules from crystal structures indicate the degree of mobility or disorder seen in the crystal structure. Structures of six porins (four 16 stranded beta barrel porins and two 8 stranded beta barrel porins) were taken from the PDB for the analysis based on resolution (better than 3.0 Å) and R-factor (< 0.23). The residue distribution and mobility distribution was found to be characteristic of each of the porins. The mobility and residue distribution amongst the secondary structural elements were found to follow the level of homology at the sequence and structural level. The loops (L2 and L3) that had defined functional roles in structural terms were found to have lower temperature factors than the other loops. The turn regions that are thought to face the periplasmic region in the cell, showed higher temperature factors. For both the 16 stranded and the 8 stranded barrels it was found one

part of the barrel (the lower wall or 'inner' wall comprising the trimer interface in the case of the 16 stranded barrels) was more rigid and the other half of the barrel (the higher or 'outer' wall) showed more mobility as seen from the temperature factors. This seems to be an intrinsic structural component of the beta barrels.

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Porins are integral membrane proteins that are found in the outer membrane of Gram-negative bacteria, eukaryotic mitochondria and chloroplasts. They act as molecular sieves to ensure that the unhindered diffusion of nutrients and waste materials of size <600 Da into the periplasmic space while protecting the cell from hostile substances like degrading enzymes, bile salts, antibiotics, toxins, phages and abrupt changes in osmotic pressure. These are homotrimers of intimately associated subunits. Each subunit contains 250-450 amino acid residues that form a completely antiparallel β barrels in which all strands are hydrogen bonded to their next neighbors along the chain [1-2]. β –strands β1- β5 and β15- β16 forms the inner wall facing towards the trimer interface while β7- β13 forms the outer wall that interacts with lipids *in vivo* or detergents in crystals and β6 and β14 forms the boundary between inner and outer walls (shown in fig 1(a)). Loops are at the extra cellular ends of the porins (rough end); they start from odd-numbered β barrels and the loops have average 8-10 residues. Turns are the periplasmic ends of the porins (smooth end); they start from even-numbered β-strands and they have average 4-5 residues. Porins have generally very few α-helices. The porins seems unusual for membrane proteins because their sequence are predominantly polar and contain no long hydrophobic segments like those found in membrane-spanning a helices of bacteriorhodopsin and the bacterial photosynthetic reaction center[3]. Monomeric 8-stranded porins have similarity in topology (fig 1(c)) and function to 16-stranded porins [4-6].

The atomic temperature factors (B values) determined by high-resolution crystallographic techniques show smearing of electron densities around their equilibrium positions due to thermal motion and positional disorder [7]. So B-values can indicate regions of protein with more conformational mobility or flexibility. B

value distribution analysis has been used earlier for analyzing structural and functional characteristics of protein structures. For example, in picornaviruses (such as Mengo virus) it was shown that well- defined structural elements have lower temperature factors than that of lesser-defined structural elements [8]. The observed B values have been used to derive flexibility indices for amino acid residues [9-11]

More recently it was found for globular proteins the temperature factor is lower for buried residues[12-13]. The temperature factors show a large variation from one structure to another but the standard deviation from average of temperature factor at for C_α – position show a characteristic frequency distribution[14]. The same group have shown that plausible differences in the dynamics of thermophilic and mesophilic proteins and suggest amino acid substitutions that are likely to change thermal stability using the temperature factor distribution[15]. This paper presents the temperature factor analysis of outer membrane beta-stranded porin crystal structures, which suggests the pore is not uniformly rigid.

Materials and Methods:

Selection of High Resolution Porin Structures

Six high-resolution crystal structures of porins were chosen from PDB [16-17] entries released till April 2001(total 14964 structures) as shown in table I. These structures have resolutions better than 3.0 Å and R factors < 0.23.

Programming Procedure

Shell program with Awk filter was developed to calculate average B-factor and corresponding standard deviation with following parameters- (a) atom types: all atoms, backbone atoms (N, CA, C and O), side-chain atoms (others except backbone atoms) and (b) residue types: polar, non-polar, charged and aromatic residues. For this

program the header portion was manually deleted from PDB co-ordinate files and that was used as input data (only co-ordinate file). The data generated by above program was further tabulated using a C program.

Visualizing Porin Structures

Structures of porins were viewed using Rasmol[19] and Biosym with INSIGHT II 95.0[20]

Results and Discussions:

B-factor mobility for 16-stranded porins

It is found that in all porins considering all atoms, both backbone atoms and side chain atoms, the B-value is lower for β1- β5 and β15- β16 that forms inner wall while it starts increasing from β7 and goes till β14 that are in outer wall as shown in fig.2. This indicates that outer wall of porins is more flexible than inner wall.

In loops, L2 and L3 show comparatively lower temperature factor values than other loops and these loops are functionally more defined. Loop L2 is involved in latching onto the neighboring monomers and L3 in eyelet formation that restricts pore size (fig 3). This suggests that these functionally active loops and show lesser conformational mobility. There are 7 turns in *Rhodobacter capsulatus* and *Rhodopseudomonas blastica* porins while 8 turns in case of OmpF and PhoE. Turns T3 to T6 are associated with outer wall and turns T1, T2 and T7, T8 are associated with inner walls. T3 to T6 have higher average B-factor than turns T1, T2 and T7, T8 (fig.4).

For PhoE, B-value is constantly lower than in other porins even though its resolution is lowest amongst all four porins. This suggests that this relative lack of mobility as evidenced by the B-factors reflects functional significance rather than crystallographic errors. *Rhodobacter capsulatus* porin has a consistently higher B-

value distribution. PhoE and *Rhodobacter capsulatus* porin belong to 2 different groups of porins as seen by the sequence alignments where these two porins have less sequence similarity and identity [21-22]. This is corroborated by the nature of the temperature factor distributions being different in the two cases.

B-factor mobility for 8-stranded porins

β1- β2 and β7- β8 forms the inner wall while β3- β6 forms outer wall for 8-stranded porins. The inner wall atoms have lower B-values than those of the outer wall (Table 2). Though 8-stranded porins do not form trimers, still the lower walls have lower B-values (in case of 16-stranded porins the inner wall forms the trimer interface and has lower B-values). This indicates that atoms of the inner wall in both 8 stranded and 16 stranded cases have more interactions than atoms of the outer wall. The trend that inner wall atoms have lower B-values than outer wall atoms in all cases, suggests that there is intrinsic flexibility in outer wall that has nothing to do with other crystallographic parameters (like crystal packing etc.).

Consistently, the B-value is higher for OmpA than OmpX; this may be because the OmpA has lower resolution (2.4Å) than that of OmpX (1.9Å). The loops show higher mobility differences in both OmpA and OmpX as they have difference in loop length. OmpA has bigger loops while OmpX has smaller ones. This might be the reason why they show higher mobility differences. Both 16-stranded porins and 8-stranded porins show similarity at the level of conformational mobility as evidenced by the temperature factor distribution.

Conclusions:

It is found that average B values for beta-strands are lower than that of loops and turns; this indicates that beta strands have lower conformational mobility than loops and turns, which confirm that defined secondary structures, have lesser conformational mobility [8]. The temperature factor distribution in the 16 stranded homotrimeric porins and the 8-stranded monomeric porins suggests similarity at the level of mobility of the beta strands. The outer wall has more intrinsic mobility than the inner wall suggesting that the dynamics of the pore diameter should be considered for functional characterization. The evolutionary relatedness seen at the sequence and structural level amongst *Rhodopseudomonas, Rhodobacter* and *E.coli* porins [21-22] is found to be maintained in the conformational mobility also.

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Legends to Figures

Fig.1: Structures of porins showing outer and inner wall; loops and turns (a) A typical 16- stranded porin e.g. *Rhodopseudomonas blastica* porin (1PRN)[18] monomer is shown and viewed from trimer interface. (b) A typical 16-stranded porin viewed from top showing trimer and dimer interface; loops L2 that latch out to neighboring monomer and L3 that forms eyelet to restrict pore size. (c) 8-stranded monomeric porin e.g. *E.coli* OmpA (1BXW)[4-5]. These views are generated using Rasmol[19] where color indicates temperature factor distribution (blue indicates lowest value while red is for highest value)

Fig.2: Comparison of average B value mobility for four 16-stranded porin in β-strands using atom type parameters- (a) all atom; (b) backbone atom (N, C. CA and O only); (c) side chain atoms (rest except backbone). In all cases the inner wall (β1-β5 and β 15- β16) shows lower B-values than that of outer wall (β7- β13).

Fig.3: Comparison of average B value mobility for four 16-stranded porin in loops using atom type parameters- (a) all atom; (b) backbone atom (N, C. CA and O only); (c) side chain atoms (rest except backbone). Loops L2 and L3 show comparatively lower B value than rest loops.

Fig.4: Comparison of average B value mobility for four 16-stranded porin in turns using atom type parameters- (a) all atom; (b) backbone atom (N, C. CA and O only); (c) side chain atoms (rest except backbone).

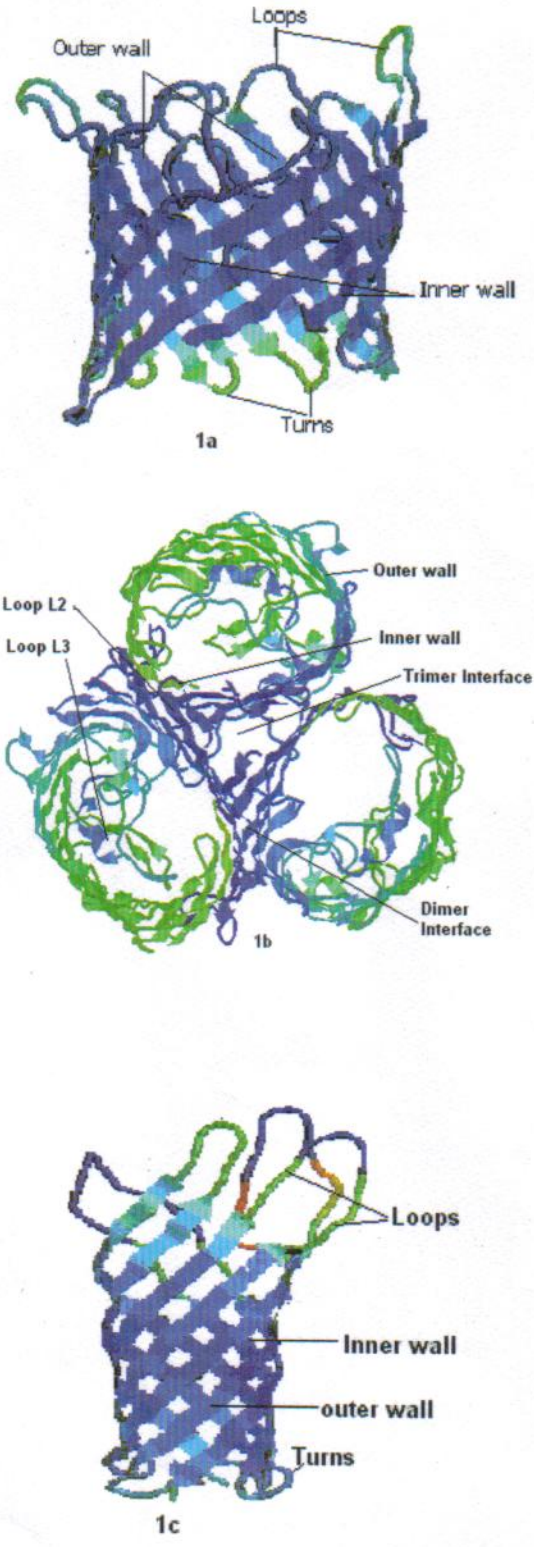
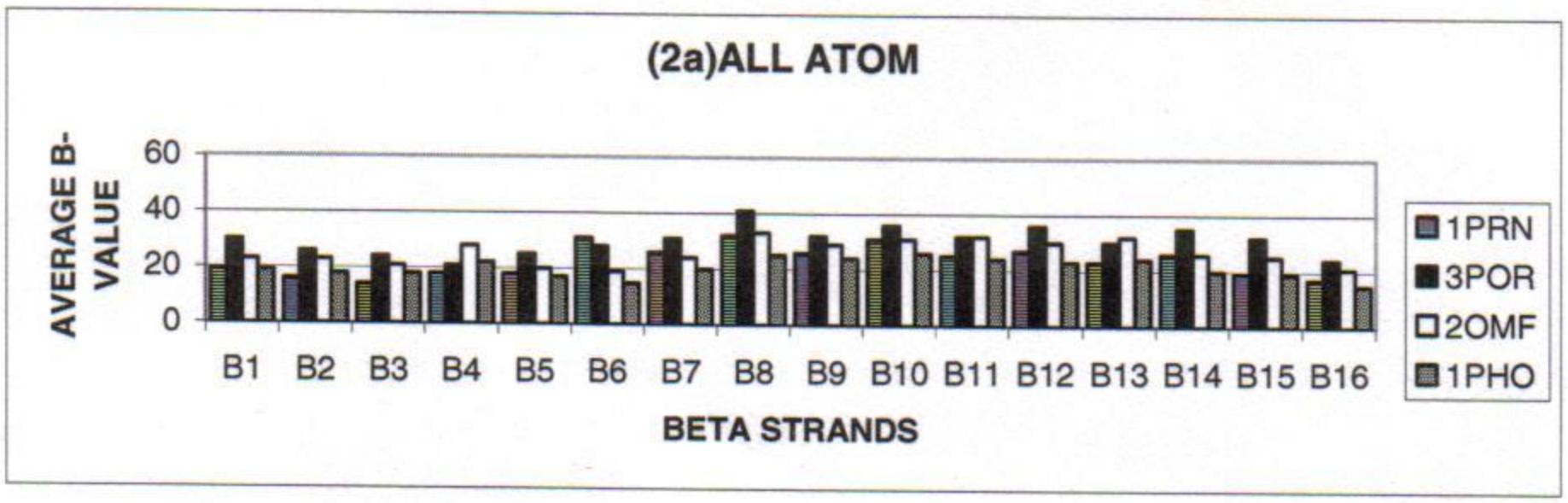
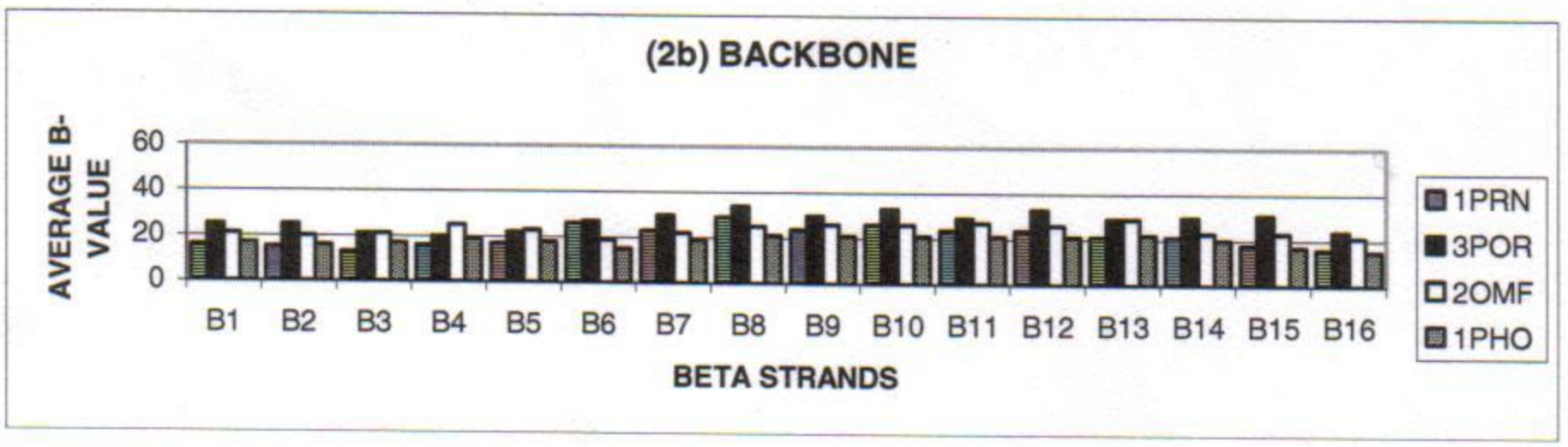


Fig.1





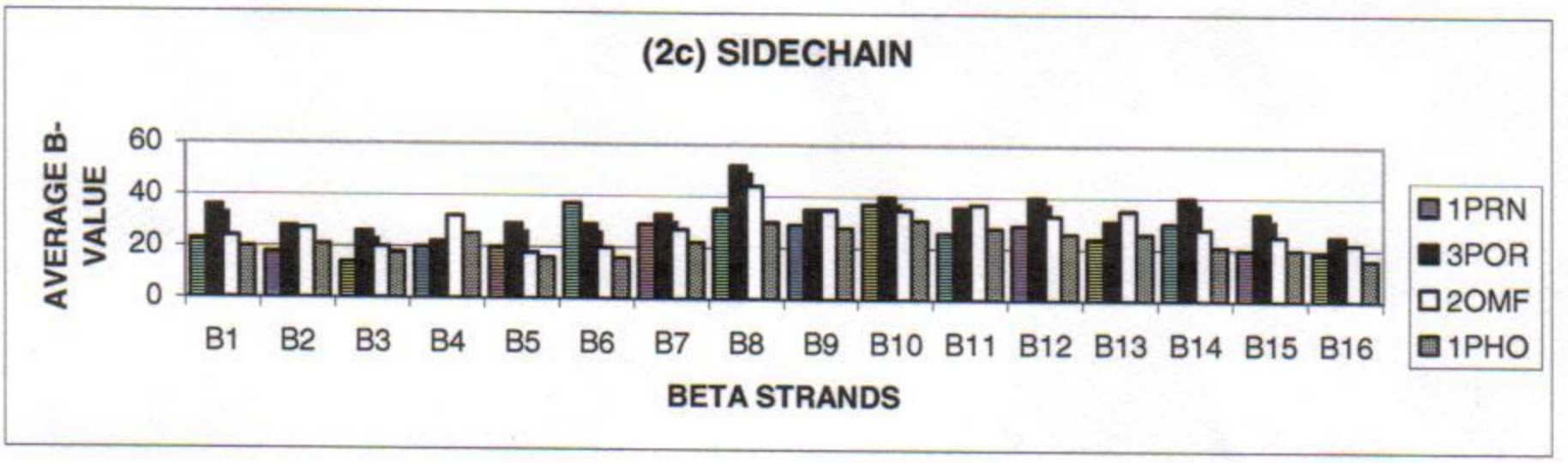
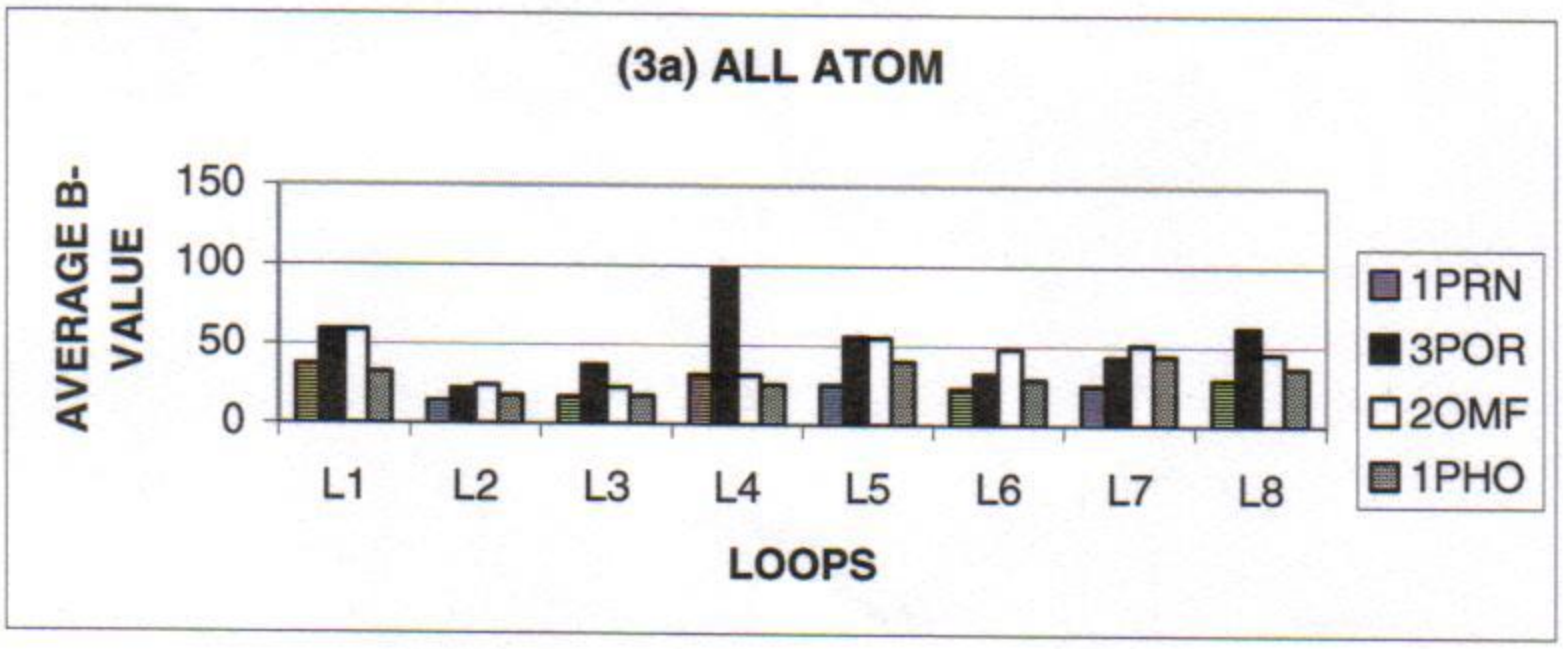
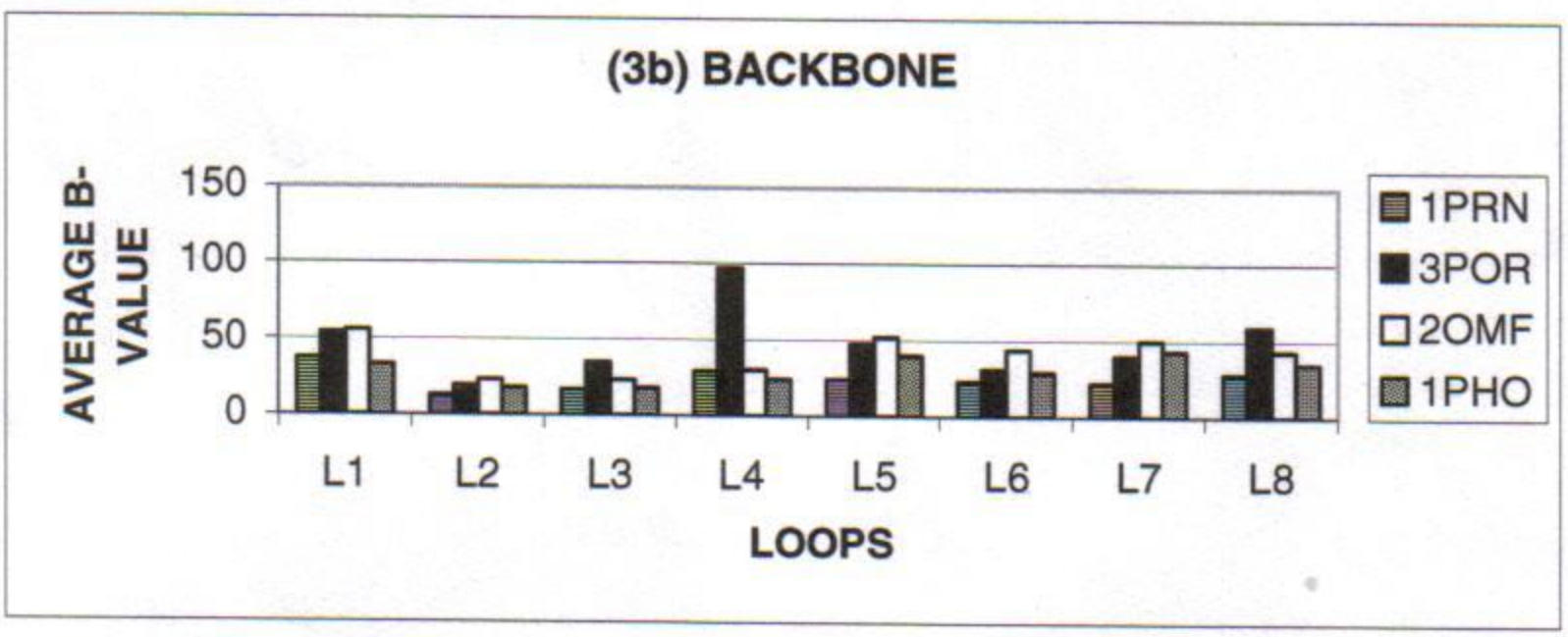


Fig.2





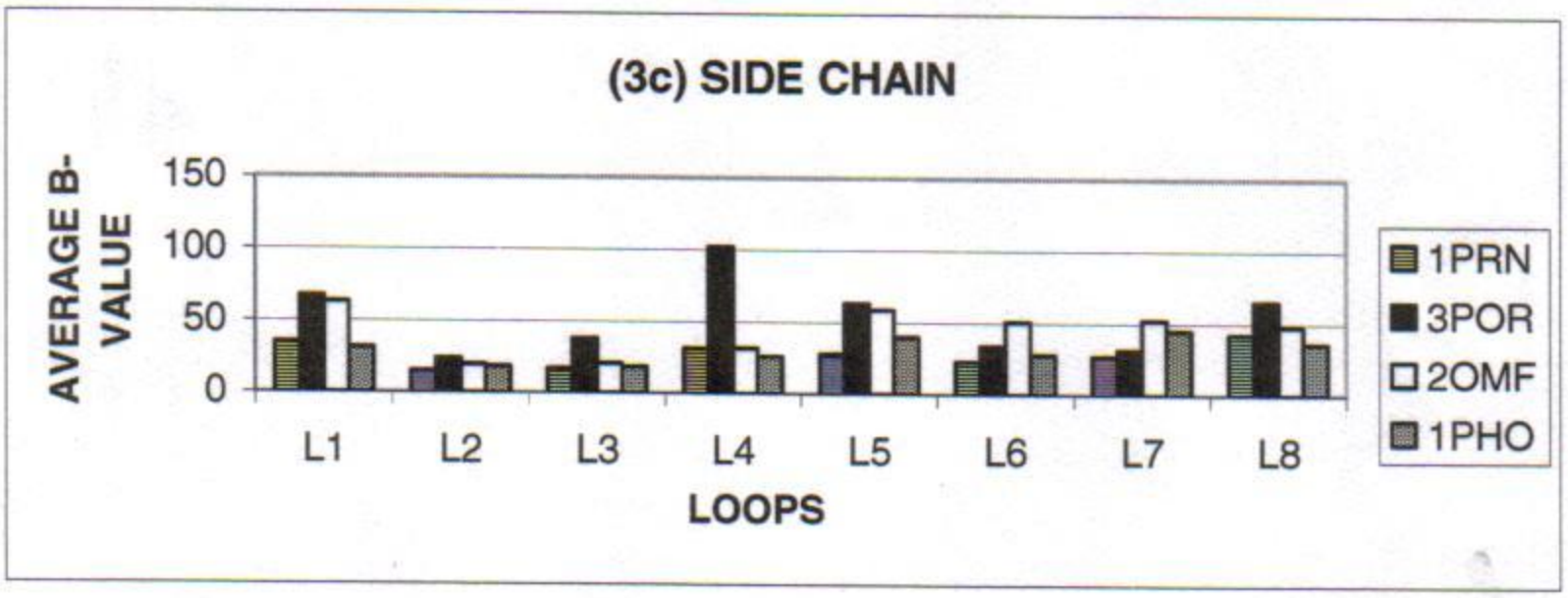
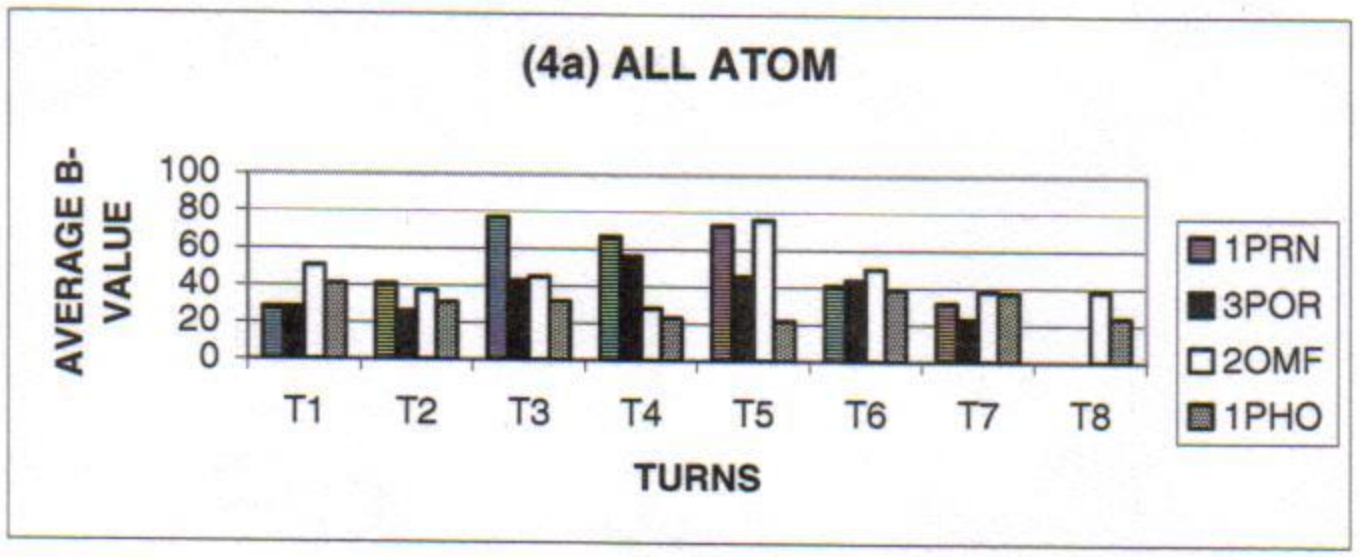
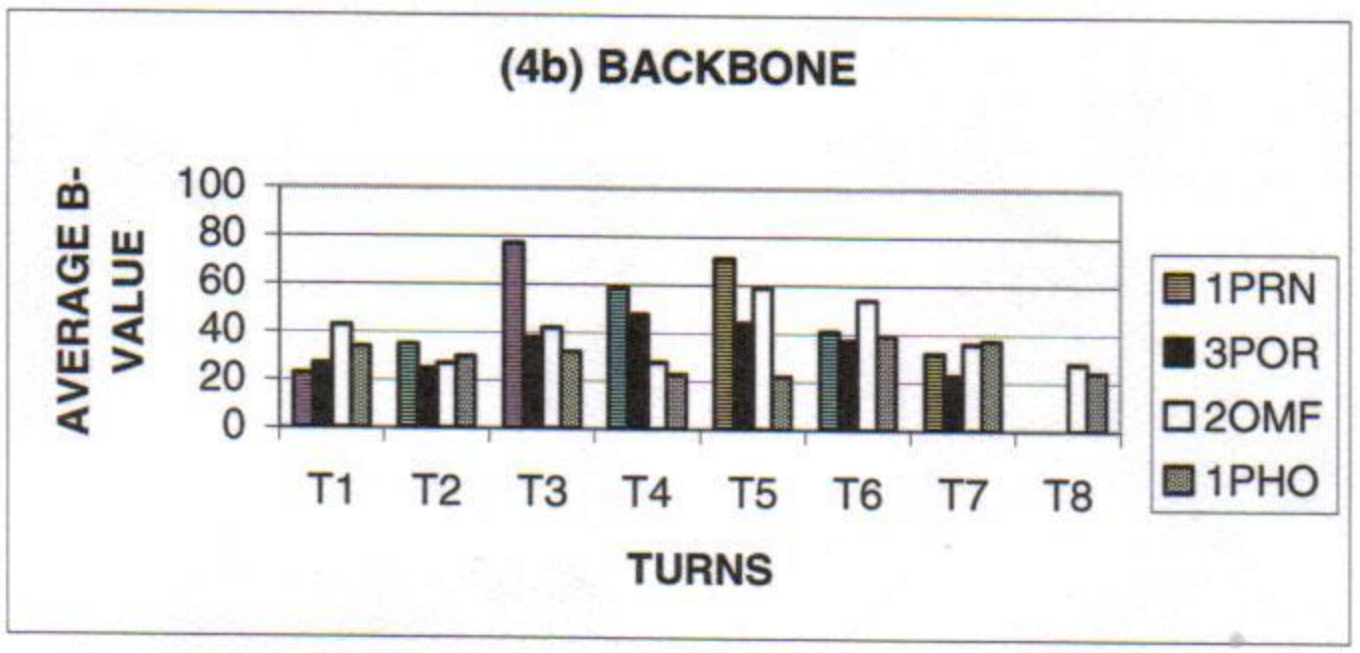


Fig.3





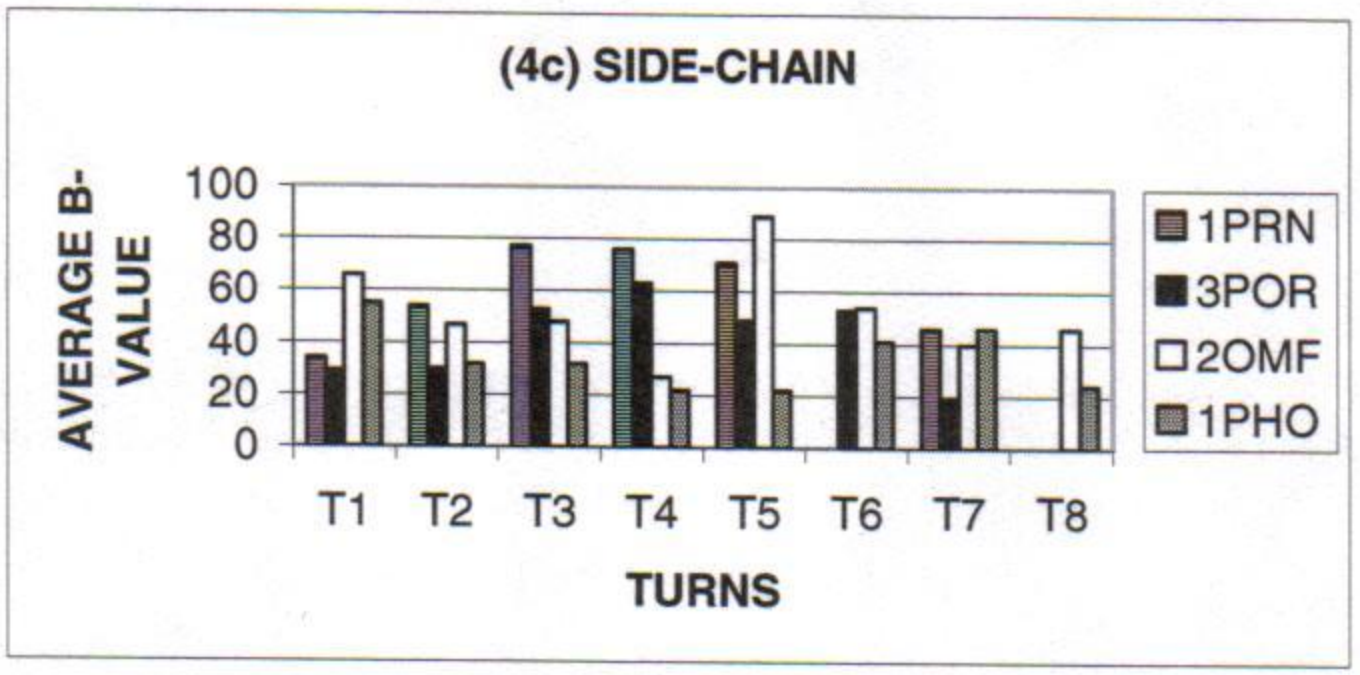


Fig.4

Table 1 Different porins with PDB ID, resolution. R-value, total number o amino acids and source are given below:

Serial No.	Porins	PDB ID	Resolution (in Å)	R-value	Total Amino Acids	Source	References
A.			8- Stranded	Porins			
1.	OmpA	1BXW	2.50	0.189	171	E. coli	4-5
2.	OmpX	1QJ8	1.90	0.204	148	E .coli	6
B.			16-Stranded	Porins			
1.	RB Porin	1PRN	1.96	0.176	289	R. blastica	18
2.	RC Porin	3POR	2.5	0.188	301	R. capsulatus	2
3.	PhoE	1PHO	3.0	0.222	340	E. coli	3
4.	OmpF	2OMF	2.40	0.176	340	E. coli	3

Table 2: Average B-value and standard deviation (in parentheses) for different atom and residue types in beta strands and loops of 8-stranded porins.

Sec Str	PDB ID	All Atom	Backbone atom	Side chain atom
B1	1QJ8	36(13)	35(11)	38(12)
	1BXW	42(14)	39(14)	45(14)
B2	1QJ8	32(15)	30(7)	39(15)
	1BXW	41(13)	38(7)	45(18)
B3	1QJ8	40(24)	36(15)	47(28)
	1BXW	45(17)	38(11)	51(20)
B4	1QJ8	39(13)	34(10)	47(15)
	1BXW	49(20)	40(11)	57(23)
B5	1QJ8	33(20)	36(11)	44(17)
	1BXW	43(15)	39(14)	47(16)
B6	1QJ8	42(13)	36(9)	49(12)
	1BXW	48(10)	43(13)	55(18)
B7	1QJ8	36(13)	32(6)	42(14)
	1BXW	37(10)	30(6)	39(11)
B8	1QJ8	39(15)	36(10)	42(14)
	1BXW	40(12)	36(11)	44(11)
L1	1QJ8	89(11)	90(8)	87(13)
	1BXW	100(68)	94(69)	106(67)
L2	1QJ8	73(8)	72(8)	76(9)
	1BXW	80(47)	75(44)	86(50)
L3	1QJ8	43(7)	38(5)	47(7)
	1BXW	96(18)	91(16)	102(18)
L4	1QJ8	65(8)	62(6)	68(9)
	1BXW	49(43)	47(40)	51(46)