ELSEVIER

Biophysical Chemistry 103 (2003) 251–258

Biophysical Chemistry

www.elsevier.com/locate/bpc

Influence of cation–π interactions in different folding types of membrane proteins

M. Michael Gromiha*

Computational Biology Research Center (CBRC), AIST, 2-41-6 Aomi, Koto-ku, Tokyo 135-0064, Japan

Received 17 June 2002; received in revised form 9 September 2002; accepted 9 September 2002

Abstract

Cation–π interactions play an important role to the stability of protein structures. In this work, we analyze the influence of cation–π interactions in three-dimensional structures of membrane proteins. We found that transmembrane strand (TMS) proteins have more number of cation–π interactions than transmembrane helical (TMH) proteins. In TMH proteins, both the positively charged residues Lys and Arg equally experience favorable cation–π interactions whereas in TMS proteins, Arg is more likely than Lys to be in such interactions. There is no relationship between number of cation–π interactions and number of residues in TMH proteins whereas a good correlation was observed in TMS proteins. The average cation–π interaction energy for TMH proteins is −16 kcal/mol and that for TMS proteins is −27 kcal/mol. The pair-wise cation–π interaction energy between aromatic and positively charged residues showed that Lys–Trp energy is stronger in TMS proteins than TMH proteins; Arg–Phe, Arg–Tyr and Lys–Phe have higher energy in TMH proteins than TMS proteins. The decomposition of energies into electrostatic and van der Waals revealed that the contribution from electrostatic energy is twice as that from van der Waals energy in both TMH and TMS proteins. The results obtained in the present study would be helpful to understand the contribution of cation–π interactions to the stability of membrane proteins.

Keywords: Cation–π interactions; Membrane protein; Electrostatic; van der Waals

1. Introduction

Membrane proteins play an essential role in several biological functions including, cell–cell signaling and mediating the transport of ions and solutes across the membrane. Membrane proteins are of two kinds, one that they span the cytoplasmic membrane with α-helices [1] and the other that consists of β-strands in the outer membranes of gram-negative bacteria [2]. The membrane assembly of transmembrane strand (TMS) proteins is more complex than transmembrane helical (TMH) proteins due to the difference in the amino acid sequences of the transmembrane part strands and helices.

Recently, the characteristic features of membrane protein structures have been studied extensively owing to the achievement of solving several crystal structures of TMH [3–7] and TMS

Abbreviations: TMH, transmembrane helical; TMS, transmembrane strand.

*Tel.: +81-3-3599-8046; fax: +81-3-3599-8081.

E-mail address: michael-gromiha@aist.go.jp (M. Michael Gromiha).

0301-4622/03/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0301-4622(02)00318-6

[8–11] proteins at high resolution. The investigations include the amino acid compositions in the periplasmic and cytoplasmic loops [12], development of mutation data matrices [13] and conformational parameters [14,15], hydrophobic distribution and spatial arrangement of residues [16], important amino acid properties in determining the secondary structures [17] and the role of medium and long-range interactions in membrane proteins [18].

Protein structures are stabilized with various non-covalent interactions, such as, hydrophobic, electrostatic, hydrogen bonds and van der Waals interactions [19]. In addition, the cation–π interaction is recognized as an important non-covalent binding interaction in structural biology [20,21]. Recently, the importance of this interaction has been stressed by several investigators in determining the helicity of α-helical peptides [22], folding of polypeptides [23] etc. Gallivan and Dougherty [21] surveyed the nature of cation–π interactions in a set of globular protein structures. Further, the role of cation–π interactions to the stability of thermophilic proteins has been reported [24]. However, the influence of cation–π interactions in stabilizing the structures of membrane proteins is yet to be explored.

In this work, we analyzed the role of cation–π interactions in the two major classes (TMH and TMS) of membrane proteins. The energetic contribution due to cation–π interactions has been brought out for each protein and for all the six pairs of residues forming such interactions. We observed that TMS proteins have more number of cation–π interactions than TMH proteins. The cation–π interaction energy for Lys–Trp and Arg–Trp is higher in TMS proteins than TMH proteins whereas an opposite behavior is observed for Arg–Phe, Arg–Tyr and Lys–Phe. Further, the contribution from electrostatic energy is twice as that from van der Waals energy in both TMH and TMS proteins.

2. Materials and methods

2.1. Database

A database of membrane proteins was derived from the information about their three-dimensional structures available in Refs. [1,25]. The PDB codes of the proteins used in the present study are, for TMH proteins, 1PRCL, 1PRCM, 1OCCA, 1OCCC, 2BRD, 1E12, 1F88, 1PSSL, 1PSSM, 1QLAC, 1AR1A and 1BGYC, and for TMS proteins, 1A0SP, 1BXWA, 1BY5A, 1E54A, 1EK9A, 1FEPA, 1OPF, 1OSMA, 1PHO, 1PRN, 1QD6C, 1QJ9A, 2MPRA, 2POR and 7AHLA. The coordinates of all the membrane protein structures have been taken from the Protein Data Bank [26].

2.2. Computation of amino acid composition

The amino acid composition for each amino acid residue that are involved in cation–π interactions was computed using the standard formula,

$$\mathrm{comp}(i) = n(i)/N \quad (1)$$

where $n(i)$ is the number of amino acids of type i and N is the total number of amino acids in a protein. We have calculated the composition for all the five residues (Lys, Arg, Phe, Trp and Tyr) that are involved in cation–π interactions in both TMH and TMS proteins.

2.3. Estimation of cation–π interactions

The number of cation–π interactions in each protein has been calculated using the program, CAPTURE developed by Gallivan and Dougherty [21] available at http://capture.caltech.edu. We have considered only the energetically significant interactions in the present study. The percentage composition of a specific amino acid residue contributing to cation–π interactions is obtained by the equation,

$$\mathrm{comp}_{\mathrm{cat}-\pi}(i) = n_{\mathrm{cat}-\pi}(i) \times 100/n(i), \quad (2)$$

where i stands for the five residues, Lys, Arg, Phe, Trp and Tyr, $n_{\mathrm{cat}-\pi}$ is the number of residues involved in cation–π interactions and n is the number of residues of type i in protein structures.

2.4. Energetic contribution due to cation–π interactions

We have computed the energetic contribution of cation–π interactions for each protein in TMH

Table 1
Frequency of occurrence of aromatic and positively charged residues in TMH and TMS proteins

PDB code	Lys	Arg	Phe	Tyr	Trp
TMH proteins					
1PRCL	1.8	3.3	9.5	3.6	5.5
1PRCM	1.6	4.0	7.7	4.6	5.6
1OCCA	1.8	1.5	8.2	3.7	3.3
1OCCC	1.2	1.9	9.2	4.2	4.6
2BRD	3.2	3.2	5.4	5.0	3.6
1E12	0.4	4.6	4.2	2.9	4.2
1F88	3.3	2.1	9.2	5.3	1.5
1PSSL	2.3	3.0	9.0	4.5	5.3
1PSSM	0.7	4.1	8.8	2.7	6.8
1QLAC	7.1	3.5	9.1	3.9	3.5
1AR1A	1.5	2.3	9.1	5.1	3.2
1BGYC	2.6	2.1	6.3	4.2	3.2
Average	2.29 ± 1.68	2.97 ± 0.95	7.98 ± 1.67	4.14 ± 0.79	4.19 ± 1.38
TMS proteins					
1A0SP	4.1	4.6	5.8	4.6	2.7
1BXWA	4.1	3.5	2.3	8.1	2.9
1BY5A	5.1	4.6	5.1	5.9	1.3
1E54A	5.0	4.1	5.0	4.7	0.9
1EK9A	3.8	4.7	1.9	4.7	0.2
1FEPA	4.3	5.5	2.4	4.5	3.0
1OPF	5.3	3.2	5.6	8.5	0.6
1OSMA	4.9	2.7	5.5	8.5	0.9
1PHO	7.0	3.7	6.4	6.7	0.9
1PRN	1.4	2.8	5.5	6.6	1.4
1QD6C	3.5	4.6	3.8	9.2	3.8
1QJ9A	4.1	4.1	4.7	10.8	1.4
2MPRA	5.2	3.8	3.6	5.5	4.8
2POR	3.3	2.3	5.0	5.3	0.3
7AHLA	9.6	3.4	3.4	4.8	2.7
Average	4.73 ± 1.75	3.84 ± 0.85	4.40 ± 1.36	6.56 ± 1.93	1.85 ± 1.33

and TMS group and for all possible pairs of positively charged—aromatic amino acids. The total cation–π interaction energy ($E_{cat-\pi}$) has been divided into electrostatic (E_{es}) and van der Waals energy (E_{vdw}) and the results are discussed. The electrostatic and van der Waals energies were obtained from the program CAPTURE, which has implemented a subset of OPLS force field [27] to calculate the energies. The electrostatic energy (E_{es}) is calculated using the equation, $E_{es} = q_i q_j e^2 / r_{ij}$, where q_i and q_j are, respectively, the charges for the atoms i and j, and r_{ij} is the distance between them. The van der Waals energy is given by $E_{vdw} = 4\varepsilon_{ij} [(\sigma_{ij}^{12}/r_{ij}^{12}) - (\sigma_{ij}^{6}/r_{ij}^{6})]$, where $\sigma_{ij} = (\sigma_{ii}\sigma_{jj})^{1/2}$ and $\varepsilon_{ij} = (\varepsilon_{ii}\varepsilon_{jj})^{1/2}$; σ and ε are, respectively, the van der Waals radius and well depth.

3. Results and discussions

3.1. Occurrence of Lys, Arg, Phe, Trp and Tyr in membrane protein structures

We have analyzed the frequency of occurrence of amino acid residues that are involved in cation–π interactions. The results for TMH and TMS proteins are presented in Table 1. We observed that in TMH proteins, Phe has the highest frequency of occurrence and the other aromatic residues,

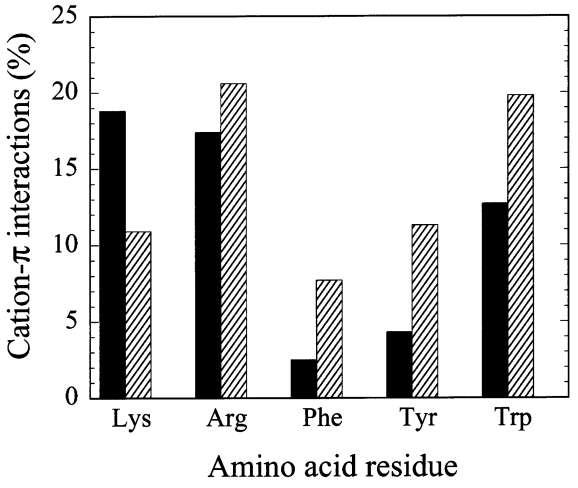

Fig. 1. Histogram showing the percentage of aromatic and positively charged residues contributing towards cation–π interactions in TMH and TMS proteins, filled column: TMH proteins; slant column: TMS proteins.

Trp and Tyr have similar amino acid composition. There is no significant difference in the occurrence of Lys and Arg. In TMS proteins, the frequency of occurrence of Tyr is approximately 3.5 times higher than that of Trp. Further, the composition of Trp in TMH proteins is significantly higher than that in TMS and globular proteins. This might be due to the importance of it for the function and stability of TMS proteins [28]. Although there is no significant difference between the amino acid composition of Lys and Arg, more number of Lys is present in TMS proteins than Arg.

3.2. Relative contribution of amino acids involved in cation–π interactions

We have estimated the percentage of aromatic and positively charged amino acids that are involved in cation–π interactions in membrane protein structures (Eq. (2)). The relative contribution of each of the five amino acid residues in TMH and TMS proteins are depicted in Fig. 1. We found that all the aromatic residues in TMS proteins have higher tendency to form cation–π interactions than those in TMH proteins. The percentage of residues involving cation–π interactions are 2.54, 4.27 and 12.74 for Phe, Tyr and Trp, respectively, in TMH proteins and 7.66, 11.25 and 19.80 in TMS proteins. The side chain of Lys (18.82%) is more likely than that of Arg (17.43%) to be in cation–π interactions in TMH proteins whereas an opposite trend is observed in TMS proteins (10.85% for Lys and 20.64% for Arg) and in globular proteins [21].

3.3. Energetic contribution of cation–π interactions in TMH and TMS proteins

The number of cation–π interactions in each of the TMH and TMS proteins and their energetic contribution are presented in Table 2.

3.3.1. TMH proteins

An average of three cation–π interactions are found in TMH proteins. The number of cation–π interactions varies for different proteins; it is zero in 1E12 and seven in the L chain of 1PRC. There is no correlation between the number of amino acid residues and number of cation–π interactions (Fig. 2a); although the protein length is similar in the C chain of 1OCC and L chain of 1PSS, the former one contains only one while the latter one contains five cation–π interactions. Further, the strength of cation–π interaction differs in protein structures; it is −2.69 and −7.37 kcal/mol, respectively, for the A and C chains of 1OCC, both of them contain a single cation–π interaction. However, we found a good correlation between number of cation–π interactions and their energetic contributions. The decomposition of energies into two components, electrostatic and van der Waals showed that the electrostatic energy is twice stronger than van der Waals energy, similar to globular proteins [21].

Detailed analysis on the location of amino acid residues involving cation–π interactions in TMH proteins showed that there is no specific preference to be in membrane or outside. The residues in membrane spanning helices and outside the membrane are having similar tendency of forming cation–π interactions. We noted that cation–π interactions are also formed between membrane bound and surface amino acid residues. The residue pairs within membrane spanning helices acquired more cation–π interaction energy than

Table 2
Energetic contribution due to cation–π interactions in TMH and TMS proteins

PDB code	$N_{cat-\pi}$	$-E_{es}$	$-E_{vdw}$ (kcal/mol)	$-E_{cat-\pi}$
TMH proteins				
1PRCL	7	21.70	13.36	35.06
1PRCM	6	19.47	12.59	32.06
1OCCA	1	2.32	0.37	2.69
1OCCC	1	4.97	2.40	7.37
2BRD	2	11.58	3.38	14.96
1E12	0	0.00	0.00	0.00
1F88	1	3.29	0.82	4.11
1PSSL	5	21.79	6.69	28.48
1PSSM	5	15.69	11.72	27.41
1QLAC	1	3.24	0.92	4.16
1AR1A	3	12.72	5.09	17.79
1BGYC	3	9.94	6.61	16.55
Average	2.9±2.2	10.56±7.52	5.33±4.71	15.89±11.91
TMS proteins				
1A0S	6	22.63	14.01	36.64
1BXWA	4	9.87	6.34	16.21
1BY5A	12	42.49	19.65	62.14
1E54A	7	25.59	15.83	41.42
1EK9A	2	5.33	3.95	9.28
1FEPA	11	46.74	21.91	68.65
1OPF	4	10.95	4.48	15.43
1OSMA	5	17.25	6.51	23.76
1PHO	2	6.94	2.51	9.45
1PRN	2	7.03	4.16	11.16
1QD6C	5	18.84	12.29	31.13
1QJ9A	0	0.00	0.00	0.00
2MPRA	9	35.17	18.67	53.84
2POR	1	3.85	1.00	4.85
7AHLA	3	9.81	6.14	15.95
Average	4.9±3.5	17.50±13.92	9.16±6.99	26.66±20.79

$N_{cat-\pi}$, number of cation–π interactions in a protein. E_{es}, E_{vdw} and $E_{cat-\pi}$ are, respectively, electrostatic, van der Waals and total cation–π interaction energy.

that in surface and the high contribution is mainly attributed with electrostatic energy.

3.3.2. TMS proteins

In TMS proteins, we found that each protein contains an average of five cation–π interactions, higher than that in TMH proteins. This might be due to the higher occurrence of aromatic and positively charged amino acid residues in the membrane part of TMS proteins than that of TMH proteins, which is mainly dominated by a stretch of hydrophobic amino acid residues. Essentially, one cation–π interaction is observed for every 74 residues of protein length in TMS proteins, as seen in globular proteins [21]. 1FEP has the maximum of approximately −69 kcal/mol due to cation–π interactions. The electrostatic energy is stronger than van der Waals energy in all the proteins. Unlike the TMH proteins, we found a good correlation between number of amino acid residues and number of cation–π interactions in TMS proteins (Fig. 2b). We also observed a strong correlation between number of cation–π interactions and total energy.

Further analysis on the location of amino acid residues involved in cation–π interactions showed

that there is no preference to be in membrane spanning β-strands and outside the membrane. In TMS proteins, total energy is higher for the cation–π interaction formed by the residues in the surface than others.

3.4. Electrostatic and van der Waals energy for different pairs of amino acid residues

We have computed the average electrostatic and van der Waals energy for all the six possible pairs of amino acids, Arg–Phe, Arg–Tyr, Arg–Trp, Lys–Phe, Lys–Tyr and Lys–Trp and the results are given in Table 3. In TMH proteins, the energy is appreciable for the aromatic residues paired with Arg, and Lys–Phe. It is less for the other two combinations, Lys–Tyr and Lys–Trp. The van der Waals energy is very less (−0.71 kcal/mol) for Lys–Trp.

In TMS proteins, both the positively charged residues paired with Trp have strong energetic contribution. The energies of all the other pairs are weaker than these two pairs. Comparing TMH and TMS proteins, the pairs Arg–Trp and Lys–Trp have more energy in TMS proteins than TMH proteins. The energy is similar in both TMH and TMS proteins for Lys–Tyr. The average energy is between −6 and −7 kcal/mol for Arg–Phe, Arg–Tyr and Lys–Phe in TMH proteins and that is between −4 and −5 kcal/mol in TMS proteins.

Table 3
Average energetic contribution for each amino acid pair experiencing cation–π interactions

Amino acid pair	$-E_{es}$		$-E_{vdw}$		$-E_{cat-\pi}$	
	TMH	TMS	TMH (kcal/mol)	TMS	TMH	TMS
Arg–Phe	3.90	2.70	2.70	2.15	*6.60*	4.85
Arg–Tyr	3.68	2.56	2.80	2.32	*6.48*	4.88
Arg–Trp	4.96	5.13	2.59	2.86	7.55	7.99
Lys–Phe	3.59	3.00	2.92	1.15	*6.51*	4.15
Lys–Tyr	2.32	2.54	1.08	0.91	3.40	3.45
Lys–Trp	2.90	5.39	0.71	1.20	3.61	6.59

3.5. Comparison among TMH, TMS and globular proteins

The comparative analysis among TMH, TMS and globular proteins reveals the following insights: (i) in TMS proteins, the frequency of Tyr is 3.5 times that of Trp. By contrast, with TMH proteins, Phe dominates and with globular proteins

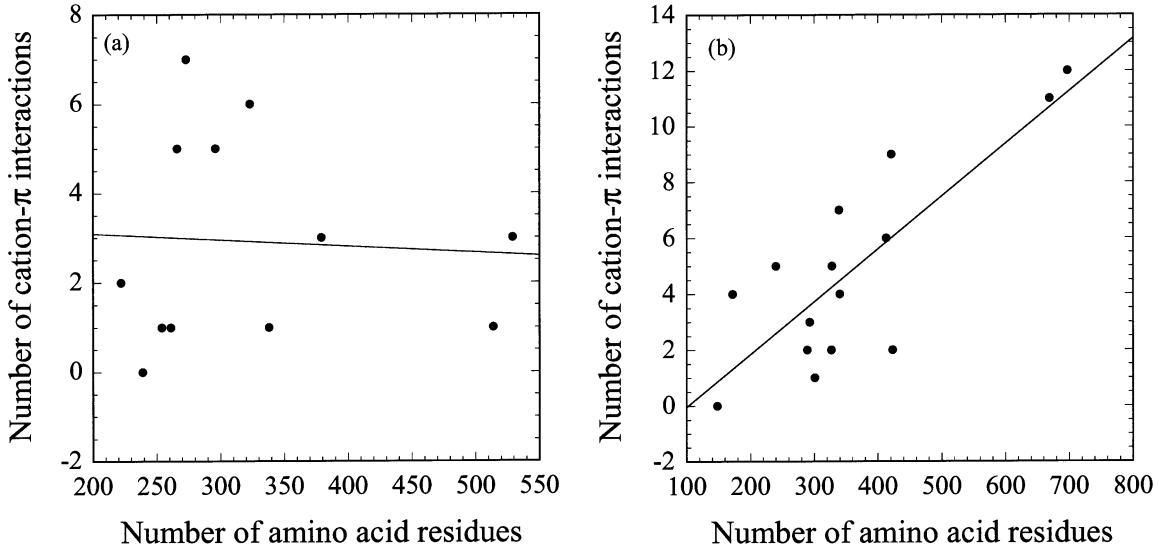

Fig. 2. Relationship between number of amino acid residues and number of cation–π interactions (a) TMH proteins; (b) TMS proteins.

Tyr and Phe have higher frequency of occurrence than Trp, (ii) Trp would experience more number of energetically significant interactions than Phe and Tyr in all types of proteins, (iii) Lys prefers to contribute more cation−π interactions than Arg in TMH proteins whereas Arg has more influence than Lys in TMS and globular proteins, (iv) a good correlation is observed between number of residues and number of cation−π interactions in TMS and globular proteins while no correlation was observed in TMH proteins, (v) electrostatic energy is stronger than van der Waals energy in all types of proteins, and (vi) the residue pair, Arg−Trp has the highest contribution for cation−π interactions in TMH, TMS and globular proteins.

4. Conclusions

We have analyzed the contribution of cation−π interactions in the structures of membrane proteins. TMH and TMS proteins have an average of three and five cation−π interactions, respectively. There is no relationship between number of residues and number of cation−π interactions in TMH proteins. The cation−π interaction energy for Lys−Trp and Arg−Trp is stronger in TMS than TMH proteins whereas TMH proteins have high contribution for the other pair of residues. The average energetic contribution of Arg−Phe, Arg−Tyr and Lys−Phe is approximately −6.5 kcal/mol in TMH proteins and it is approximately −4.5 kcal/mol in TMS proteins. The contribution from cation−π interaction energy may be incorporated with other non-covalent interactions for protein structure prediction. Hence, the results obtained in the present study would be very useful to understand the folding and stability of membrane protein structures.

Acknowledgments

The author wishes to thank Dr S. Selvaraj for reading the manuscript. Dr Yutaka Akiyama and Dr Makiko Suwa are acknowledged for encouragement.

References

[1] S.H. White, W.C. Wimley, Membrane protein folding and stability: physical principles, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 319–365.
[2] G.E. Schulz, β-Barrel membrane proteins, Curr. Opin. Struct. Biol. 10 (2000) 443–447.
[3] J. Deisenhofer, H. Michel, The photosynthetic reaction center from the purple bacterium rhodopseudomonas-viridis, Science 245 (1989) 1463–1473.
[4] D. Picot, P.J. Loll, R.M. Garavito, The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1, Nature 367 (1994) 243–249.
[5] T. Tsukihara, H. Aoyama, E. Yamashita, et al., The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å, Science 272 (1996) 1136–1144.
[6] N. Grigorieff, T.A. Ceska, K.H. Downing, J.M. Baldwin, R. Henderson, Electron-crystallographic refinement of the structure of bacteriorhodopsin, J. Mol. Biol. 259 (1996) 393–421.
[7] M. Kolbe, H. Besir, L.O. Essen, D. Oesterhelt, Structure of the light-driven chloride pump halorhodopsin at 1.8 Å resolution, Science 288 (2000) 1390–1396.
[8] M.S. Weiss, G.E. Schulz, Structure of porin refined at 1.8 Å resolution, J. Mol. Biol. 227 (1992) 493–509.
[9] S.W. Cowan, T. Schirmer, G. Rummel, et al., Crystal structures explain functional properties of two *E. coli* porins, Nature 358 (1992) 727–733.
[10] J.E. Meyer, M. Hofnung, G.E. Schulz, Structure of maltoporin from Salmonella typhimurium ligated with a nitrophenyl-maltotrioside, J. Mol. Biol. 266 (1997) 761–775.
[11] H.J. Snijder, I. Ubarretxena-Belandia, M. Blaauw, et al., Structural evidence for dimerization-regulated activation of an integral membrane phospholipase, Nature 401 (1999) 717–721.
[12] H. Nakashima, K. Nishikawa, The amino acid composition is different between the cytoplasmic and extracellular sides in membrane proteins, FEBS Lett. 303 (1992) 141–146.
[13] D.T. Jones, W.R. Taylor, J.M. Thornton, A mutation data matrix for transmembrane proteins, FEBS Lett. 339 (1994) 269–275.
[14] M.M. Gromiha, R. Majumdar, P.K. Ponnuswamy, Identification of membrane spanning β strands in bacterial porins, Protein Eng. 10 (1997) 497–500.
[15] M.M. Gromiha, A simple method for predicting transmembrane α helices with better accuracy, Protein Eng. 12 (1999) 557–561.
[16] M.M. Gromiha, P.K. Ponnuswamy, Hydrophobic distribution and spatial arrangement of amino acid residues in membrane proteins, Int. J. Pept. Protein Res. 48 (1996) 452–460.
[17] J.M. Koshi, W.J. Bruno, Major structural determinants of transmembrane proteins identified by principal component analysis, Proteins 34 (1999) 333–340.

[18] M.M. Gromiha, S. Selvaraj, Role of medium- and long-range interactions in discriminating globular and membrane proteins, Int. J. Biol. Macromol. 29 (2001) 25–34.
[19] M.M. Gromiha, S. Selvaraj (Eds.), Recent Research Developments in Protein Folding, Stability and Design, Research Signpost, Trivandrum, India, 2002.
[20] D.A. Dougherty, Cation–π interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp, Science 271 (1996) 163–168.
[21] J.P. Gallivan, D.A. Dougherty, Cation–π interactions in structural biology, Proc. Natl. Acad. Sci. USA 96 (1999) 9459–9464.
[22] Z. Shi, C.A. Olson, N.R. Kallenbach, Cation–π interaction in model alpha-helical peptides, J. Am. Chem. Soc. 124 (2002) 3284–3291.
[23] T.P. Burghardt, N. Juranic, S. Macura, K. Ajtai, Cation–π interaction in a folded polypeptide, Biopolymers 63 (2002) 261–272.
[24] M.M. Gromiha, S. Thomas, C. Santhosh, Role of cation–π interaction to the stability of thermophilic proteins, Prep. Biochem. Biotech. 32 (2002) 355–362.
[25] W.C. Wimley, Toward genomic identification of β-barrel membrane proteins: composition and architecture of known structures, Protein Sci. 11 (2002) 301–312.
[26] H.M. Berman, J. Westbrook, Z. Feng, et al., The protein data bank, Nucleic Acids Res. 28 (2000) 235–242.
[27] W.L. Jorgensen, D.S. Maxwell, J. TiradoRives, Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids, J. Am. Chem. Soc. 118 (1996) 11225–11236.
[28] M. Schiffer, C.H. Chang, F.J. Stevens, The functions of tryptophan residues in membrane proteins, Protein Eng. 5 (1992) 213–214.