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Polarity conserved positions in transmembrane domains of G-protein coupled receptors and bacteriorhodopsin

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Abstract

The polarity of residues at certain positions in the transmembrane domains of G-protein coupled receptors (GPCR) is found to be conserved, and to indicate the pattern of specific helix–helix packing of the helices. A concept of polarity conserved positions (PCP) is proposed to describe this conserved property, and is applied to obtain insight into the structural features of the transmembrane proteins. The common pattern of PCPs for GPCRs indicates that they share a similar packing arrangement of their transmembrane helix bundles. For proteins in the bacteriorhodopsin family the PCP pattern suggests a common packing arrangement that differs from that of GPCRs, in agreement with experimental data. This difference in the packing arrangement underscores the shortcomings of a BR template for the construction of molecular models of GPCRs.

Key words: Membrane receptor; Membrane-protein structure; Structural analysis; Sequence alignment; Conserved polarity

1. Introduction

Guanine nucleotide-binding protein coupled receptors (GPCRs) transfer external signals into cells [1]. Their amino acid sequences have been shown to include 7 hydrophobic regions [2] which, by analogy to the transmembrane protein bacteriorhodopsin [3] (BR), are assumed to form a 7-transmembrane-helix bundle [4–9]. The significant sequence homology in the transmembrane regions of the GPCRs suggests that their three-dimensional (3D) structures are similar. However, the sequence identity in these regions of various types of GPCR is much smaller, with only 3 or 4 out of 160–190 residues being fully conserved [2,10]. If the stringency is reduced to 80% occurrence, the number of residues shared by the GPCR sequences increases to about 14 [10]. Within one family of receptors, e.g. the 5-HT receptor subtypes, the residue identity can be much greater, as has been described [11]. Nevertheless, the extent of overall sequence identity among 25 cationic neurotransmitter receptors [2] is less than 15% in the putative transmembrane regions, although pairwise sequence comparisons reveal percentage identities as high as 48% even between unrelated GPCRs. In contrast, percentage identities in pairwise comparisons of cationic neurotransmitter GPCRs and members of the BR protein family are generally very low, and do not exceed 15% (see Fig. 3. in [9]). Recent structural information for a member of the GPCR family of transmembrane proteins, rhodopsin, indicates that the packing arrangement of the helix bundle is different from that of BR [12]. Thus, the extent of sequence identity alone is not sufficient to define the structural similarity. There must be other identifiable conserved properties that contribute to the specific packing of helices into the bundles, and these must be shared by the different proteins with similar structures.

To identify the properties underlying the structural organization of the GPCRs, previous investigations have focused on the alignment of the sequences [5,13,14], the angular periodicity of hydrophobic and conserved residues [15,16], and the tendency of helix sides to face the protein interior or the membrane lipid [10,15–17]. We report here on an analysis of the polarities of residues at certain positions in the transmembrane regions, and use the results to determine structural similarities among members of a transmembrane protein family based on properties that relate to specific packing of helices into bundles. These polarities are shown to be conserved in the protein families of GPCRs and BR, but at different positions. As this conserved property is shown to relate to helix-helix packing in BR, it is also proposed as a simple criterion for evaluating structural similarity of the transmembrane helix bundles. To this end, a concept of polarity conserved position (PCP) is defined and applied to examine the structural similarity within the family of

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Abbreviations: GPCR, guanyl nucleotide-binding protein coupled receptors; BR, bacterio-rhodopsin; TMH, transmembrane helix; PCP, polarity conserved position.

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GPCRs, and between GPCRs and the BR family of proteins.

2. Materials and methods

Conserved properties are identified by examining aligned amino acid sequences. 25 sequences of cationic neurotransmitter receptors (the GPCR set) (sequences 4–28 in Fig. 2 of [2]) are used to generate the data. Sequences of 6 members of the BR protein family (the BR set) identified in the SwissProt data bank [18] include the following proteins (name followed by SwissProt file name in parenthesis): bacteriorhodopsin precursor (Bacr_Halha), archaerhodopsin 1 precursor (Bac1_Hals1), archaerhodopsin 2 precursor (Bac2_Hals2), natronobacterium pharaonis halorhodopsin precursor (Bach_Natph), halobacterium sp halorhodopsin precursor (Bach_Halsp), sensory rhodopsin I (Bacs_Halha). The two sets of protein sequences examined here have comparable numbers of identical residues, 22 in the GPCR set, and 24 in the BR set.

Sequence alignment, as well as assignment of transmembrane regions of GPCRs are based on [2]. Gaps in the original sequence alignment are filled by shifting the gaps to the regions of connecting loops, in directions up or down the sequence stream determined by the position of the gap relative to the conserved residue selected for each transmembrane helix (TMH). Using the sequence of the 5-HT_2 receptor as numbering reference [19], the first and last residue of each individual TMH, and the conserved residues are identified in Table 1. The sequences of the BR set were aligned with the program Pileup of the GCG sequence analysis software package [20]. TMHs were assigned according to [3]. Gaps in transmembrane regions in the aligned sequences were filled by shifting them to the nearest connecting loops.

For simplicity, the 20 natural amino acids were divided into two groups: polar and apolar. The polar residues include: Asp, Asn, Glu, Gln, Arg, Lys, His, Cys, Ser, Thr and Tyr. An index P is defined to reflect the extent of conservation of residue polarities at a given position. P is the fraction of polar residues found at a given position in the sequences of a protein family aligned according to sequence homology. A position in the aligned sequences is considered as a polarity conserved position (PCP) if more than 80% of the residues found at that position are either all polar, or all apolar. Thus, the position with $P \geq 0.8$ is considered as a conserved polar position. Similarly, $P \leq 0.2$ indicates a conserved apolar position.

The distribution patterns of PCPs in the compared protein families are examined by plotting the P values versus relative residue numbers (Fig. 1), and by projecting conserved polar positions in helix wheels viewed from the extracellular side with the rotational angle of successive residues taken as 100° (Fig. 2). Some of the structural roles of polar residues at conserved polar positions and at other positions are examined from the proximity table (Table 2) which lists residues within 5 Å of the side chain atoms of the polar residues in the structure of BR [3].

The statistical significance of the PCP identifications (Fig. 1) and of the tendency we observed for polar residues at PCPs to cluster with polar residues and conserved residues in adjacent TMHs (Table 2), was evaluated with a comparative procedure against randomized sequences. This approach is analogous to that used in assessing sequence similarities [21]. In this approach, the original procedure (see above) is repeated for sequences obtained by randomizing the original set of sequences. The set of random sequences of BR or GPCR are then aligned, and the PCPs (polar or apolar) that occur by chance are counted. The average number and standard deviation of the PCPs occurring by chance in the seven TMHs were calculated from 100, 200, 500 and 1000 randomizations (Table 3). These averages and standard deviations were then used to evaluate the statistical significance of PCPs observed originally in the authentic sequences aligned according to homology.

Similarly, the statistical significance of the tendency for polar residues at PCPs to interact with polar residues and conserved residues in adjacent TMHs (Table 2), identified as outlined above, is also evaluated against random rearrangements of the sequences and compared to the interaction patterns of polar residues at non-PCPs (see above). The fraction of polar and conserved residues in BR (termed f), and the average number of residues from adjacent TMHs within 5 Å of the side chain of any residue (termed W) were calculated from the known structure of BR. Taking the average number W as a window, the number of polar residues and conserved residues in this window were considered to follow a binomial distribution, with a mean $W \times f$ and a standard deviation obtained as the square root of $[W \times f(1-f)]$ [22]. These average results for a random structure were compared to the average number of contacts of polar residues at PCPs (Table 2 – upper panel), and of those not at PCPs (Table 2 – lower panel) calculated for the authentic structure.

3. Results and discussion

In Fig. 1 the P values are plotted against the relative positions of residues in the 7 TMHs of proteins in the set of GPCR (solid lines) and of BR (broken lines). The P plots exhibit some notable features. (1) For both the GPCR and BR sets the plots contain P values equal or close to 1, as well as 0, indicating that sequences of both protein families have PCPs. (2) The distributions of the PCPs are different in the GPCR set compared to the BR set. The differences pertain to the values of P, their locations in TMHs, and the intervals between the positions of PCPs within a given TMH. The PCPs of a protein family identified from the positions with $P \geq 0.8$ as well as $P \leq 0.2$ can be considered as a characteristic pattern of polarity distribution over helix surfaces, because most

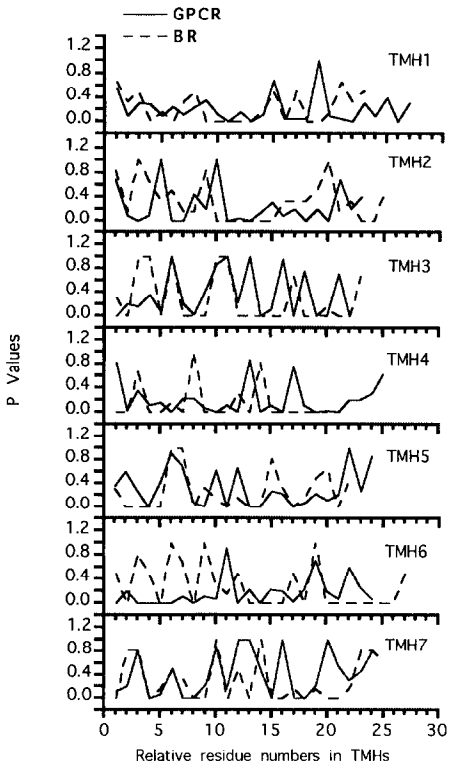

Fig. 1. The distribution of polarity conserved positions (PCPs) in the seven transmembrane helices of the protein family of G-protein coupled receptors (solid lines) and the bacteriorhodopsin protein family (broken lines). TMH1 through TMH7 indicate the individual helices defined as described in the text. The P value is the fraction of polar residues found at the specific position in an alignment of all the sequences in a protein family. The numbering for each TMH, and the definition of PCPs at positions with $P < 0.2$ or $P > 0.8$, are defined in section 2.

Table 1
The first, the last and the conserved residues in each transmembrane helix, selected for the sequence alignment[a]

Helix	First residue	Conserved residue	Last residue
TMH1	Lys^{74}	Asn^{92}	Ser^{100}
TMH2	Tyr^{11}	Asp^{120}	Leu^{133}
TMH3	Ile^{150}	Asp^{155}	Leu^{171}
TMH4	Lys^{191}	Trp^{200}	Leu^{215}
TMH5	Asn^{233}	Phe^{243}	Leu^{256}
TMH6	Leu^{325}	Trp^{336}	Ile^{348}
TMH7	Leu^{361}	Asn^{376}	Asn^{384}

[a]The sequence of the 5-HT_2 receptor [19] is taken as reference for numbering.

members of the protein family will exhibit the same polarities at these positions. This pattern provides information that is not included simply in the identification of conserved residues, because all conserved residues are at PCPs, but not all PCPs are occupied by conserved residues.

The patterns of PCPs identify collective properties of the TMHs and of the helix bundles. They are expected to relate to specific helix–helix packing because the patterns of polarity distributions on helix surfaces should indicate the faces of the helices that are likely to interact. As demonstrated for adrenergic receptors [22–25] and BR [26], as well as for cytochrome b_{562} [27], the role of the connecting loops in determining the structures of the helix bundles are likely to be negligible [28,29]. The packing will depend on protein interaction at interfaces, which involve the positioning of hydrophobic centers of one surface against hydrophobic centers of another surface, and a similar matching of hydrophilic centers [30]. This conclusion is based on observations from the structures of soluble proteins, but is applicable to the helix–helix interfaces of GPCR and BR, based on the significant similarity in some aspects of the structural organizations of membrane proteins and of soluble proteins noted from atomic packing densities and hydrophobic organizations [16,31]. The existence of characteristic patterns of polarity distribution on helix surfaces within the GPCR set and the BR set of proteins indicates that members of each protein family share helix–helix packing patterns and thus similar structures. However, the characteristic patterns are different for the GPCR and BR

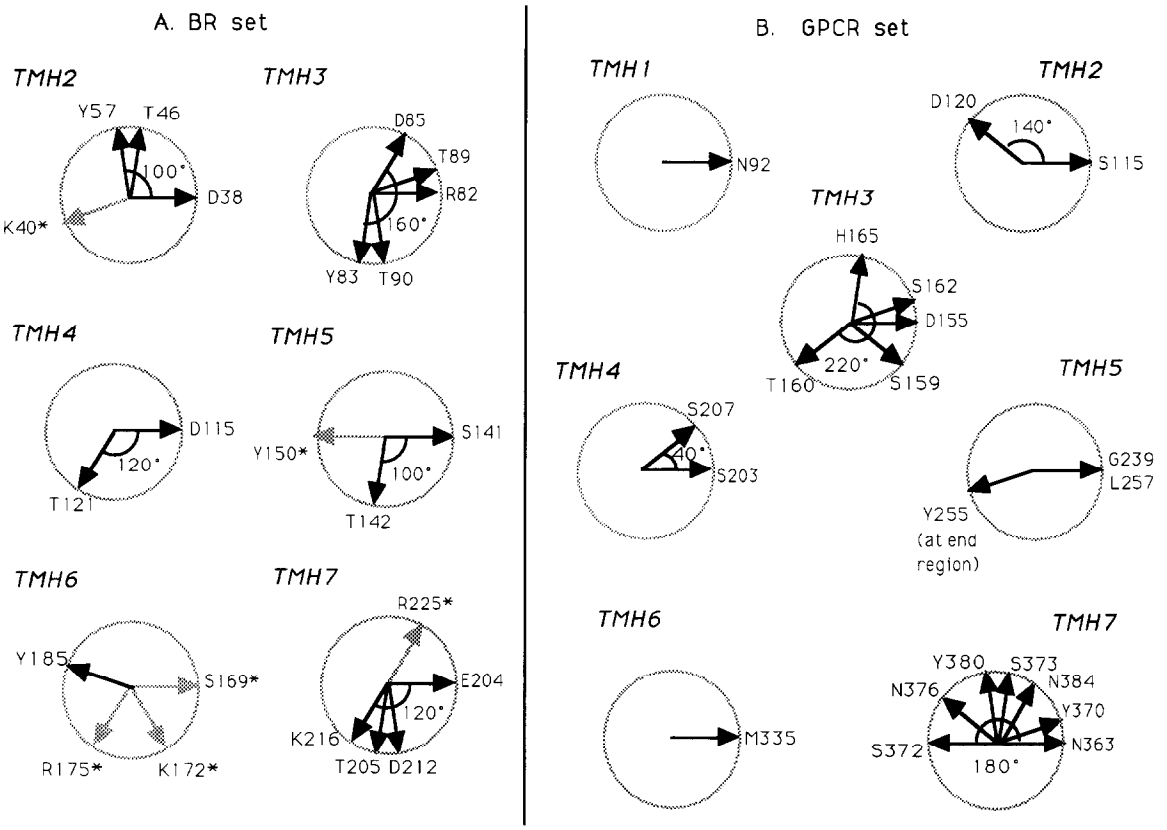

Fig. 2. The angular spread of conserved polar positions in a projection of the transmembrane helices. The helix wheels are viewed from the extracellular side. Each conserved polar position is represented by the end point of an arrow originating at the helix axis and pointing to the helix surface. The rotation angle between successive residues is taken as 100°. The spread is measured by the central angle of the arc over which the conserved polar positions are distributed. (A) For the 6 members of the bacteriorhodopsin protein family, with bacteriorhodopsin (BR) [3] as reference. The arrows in light gray point to the position of residues marked with the symbol (*) that are at the helix ends and face the membrane according to the known structure [3]. (B) For the sets of 25 G-protein coupled receptors (GPCRs), with the amino acid sequence of the 5-HT_2 receptor [19] taken as reference (see section 2).

Table 2
Interactions of polar residues with other polar and/or conserved residues in the seven transmembrane helices of bacteriorhodopsin (A to G) measured as the proximity of 5 Å among side chain atoms of the polar residues[a]

TMH	# Residue	Inter-helix interactions	Intra-helix interactions
B	Asp[38]	(Not in helix)	
	Lys[40]	(Facing membrane)	
	Thr[46]	C: Asp[96]	Tyr[43], Thr[47]
C	Tyr[57]	A: ***Leu[13]***, Thr[17]; C: Asp[85]; G: **Asp[212]**	
	Arg[82]	G: Glu[204], Thr[205]	**Tyr[83]**, ***Trp[86]***
	Tyr[83]	D: ***Gly[122]***; F: ***Trp[189]***	**Arg[82]**, ***Try[86]***
	Asp[85]	B: **Tyr[57]**; G: **Asp[212]**	**Arg[82]**, ***Trp[86]***, Thr[89]
	Thr[89]	(Binding retinal)	**Asp[85]**, ***Trp[86]***, **Thr[90]**
D	**Thr[90]**	D: **Asp[115]**; F: ***Trp[182]***	***Trp[86]***, Thr[89], ***Pro[91]***
	Asp[115]	**C: *Trp[86]*; Thr[90]**, ***Pro[91]***	***Met[118]***
E	Thr[121]	E: **Ser[141]**	***Met[118]***, ***Gly[122]***
	Ser[141]	D: ***Met[118]***, Thr[121]	Thr[142]
	Thr[142]	F: ***Pro[186]***	**Ser[141]**
F	Tyr[150]		Tyr[147]
	Ser[169]	G: Arg[225]	Thr[170]
	Lys[172]	(Facing membrane)	Ser[169], ***Phe[171]***, Arg[175]
	Arg[175]	E: ***Leu[152]***, Thr[157]	Lys[172], Asn[176]
G	**Tyr[185]**	C: ***Trp[86]***; G: ***Leu[211]***, **Asp[212]**	***Trp[182]***, ***Pro[186]***
	Glu[204]	C: **Arg[82]**	Thr[205]
	Thr[205]	A: ***Leu[13]***; C: **Arg[82]**	Glu[204]
	Asp[212]	B: **Tyr[57]**; C: Asp[85], ***Trp[86]***; F: **Tyr[185]**	***Leu[211]***, **Lys[216]**
	Lys[216]	(Forming Schiff base with retinal)	**Asp[212]**, ***Ala[215]***
	Arg[225]	F: Ser[169]	
A	Thr[17]	B: **Tyr[57]**, Ser[59]	***Leu[13]***
	Thr[24]	B: Thr[47], ***Pro[50]***	
	Tyr[26]		Lys[30]
	Lys[30]		Tyr[226]
B	Lys[41]		Asp[38], Lys[40]
	Tyr[43]		Lys[40], Thr[47]
	Thr[47]	A: Thr[24]	Tyr[43], Thr[46]
	Thr[55]		Ser[59]
	Ser[59]		Thr[55]
C	Asp[96]	B: Thr[46]	
E	Tyr[147]		Tyr[150]
	Thr[157]	F: Arg[175]	
F	Thr[170]		Ser[169]
	Asn[176]		Lys[172], Arg[175]
	Thr[178]		Arg[175], ***Trp[180]***
	Ser[183]		
G	Ser[214]		

[a] *Upper panel:* polar residues at conserved polar positions (PCPs). *Lower panel:* polar residues at polarity non-conserved positions (non- PCPs). **Bold:** indicates conserved polar residues. **Bold** and ***Italics:*** indicates conserved apolar residues.

sets, indicating that the TMHs of the two protein families are packed differently into bundles.

The statistical significance of the PCPs (Table 3), explored as described in section 2, supports the importance of the PCP patterns suggested by these findings. The fractions of polar residues in the BR and GPCR sets are about the same, 0.276 and 0.282 respectively. The numbers of polar PCPs are also quite close (Table 3). Yet results in Table 3 clearly show that the PCPs are properties of the sequences and are very unlikely to be found by chance. For example, with the criterion of a 0.8 value for P at polar PCPs, *none* was found among the aligned randomized sequences of TMHs in the set of GPCR. Similarly, in the BR set the number of polar-PCPs in the authentic sequence is about 20 standard deviation away from the mean of 1.18 identified by chance in the randomized sequences. Note that while the similarity in the number of PCPs in the authentic BR and GPCR sets might be a coincidence, it is more likely that it reflects a key architectural feature of the seven TMH helix bundle. With an average length of about 25 amino acids, a TMH incorporates about 7 helical turns. From the data in Table 3, each helical turn in the GPCR transmembrane domains would have an average of 1.3 PCPs and a distribution of polar versus apolar PCPs that is characteristic of a protein family (Fig. 2), therefore defining the helix–helix packing.

Table 2 lists the polar residues and conserved apolar residues within 5 Å of side chain atoms of polar residues in BR structure [3]. Note that polar residues at PCPs (upper panel of Table 2) cluster together with polar and/or conserved residues of adjacent TMHs, as expected if hydrophilic centers are matched between adjacent helices (Tyr[150] which faces the membrane lipid is the only exception). In contrast, polar residues not at PCPs (lower panel of Table 2) generally do not cluster with polar residues of adjacent TMHs, indicating that they contribute little to the specific packing of the TMHs into the bundle. These residues are mostly Ser and Thr which have been shown to form intrahelical hydrogen bonds and to be accepted on the helix face exposed to the membrane [32]. In BR, the Thr and Ser distribute nearly equally between PCPs and non-PCPs, which is consistent also with the finding from the crystal structure of the Photosynthetic Reaction Center that in the transmembrane regions there is no preference for the Ser/Thr residues to face the membrane lipid or the interior of the protein [31]. Inspection of the BR structure [3] indicates that most of these polar Ser/Thr residues actually do face the membrane lipid when they are not at PCPs.

The statistical significance of this organization was explored as described in section 2 in a comparison to random distributions of contacts between residues. Thus, in the BR structure each residue has an average of 2.157 residues within 5 Å of the side chain in adjacent TMHs (this is the window W, see section 2). The total number of polar-PCP and conserved residues is 55, and the total number of residues defined in TMHs is 159, yielding a value of 0.346 for the fraction of polar-PCP and conserved residues (termed f). The mean $W \times f$ of interactions with polar-PCP and conserved residues is

Table 3
Statistics of polarity conserved positions

Data set	PCP	Number in authentic sequence	Average number in randomized sequences[a]			
			100 runs[b]	200 runs[b]	500 runs[b]	1000 runs[b]
BR	polar	24	1.16 (0.98)	1.23 (1.11)	1.19 (1.04)	1.18 (1.06)
	apolar	79	23.08 (4.59)	23.26 (4.42)	23.55 (4.43)	23.72 (4.44)
GPCR	polar	21	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	apolar	42	0.09 (0.29)	0.09 (0.29)	0.07 (0.26)	0.05 (0.23)

[a]The average number of PCPs calculated from the randomized sequences, and the standard deviation – in parentheses.
[b]Number of sequence randomization runs for the calculation of average PCP numbers.

therefore 0.746, with a standard deviation of 0.699. One unit of standard deviation above the mean is 1.445. From Table 2, it can be calculated that the average number of interactions for a polar residue at a PCP in the authentic structure is 1.478, well above the average. For a polar residue not at a PCP, the corresponding value is 0.412. These data support the observed tendency of a clustering preference for polar residues at PCPs, although the small window does not permit a conclusive statistical assessment since residues are considered either to interact or not to interact according to the distance criterion.

The results of this analysis show that the PCPs of a particular set of proteins (the BR set) reflect the collective properties of helix surfaces relating to helix–helix packing. Consequently, the application of the PCP concept to the analysis of sequence alignment of protein families suggests a classification of the polar residues in TMHs into two groups. One group includes the polar residues at identified PCPs; these polar residues tend to match hydrophilic centers of other TMHs or interact with head groups of membrane lipid molecules [33]. The other group of polar residues is not associated with PCPs, includes Ser/Thr or Arg/Lys [32,33], and tends to face the membrane. This is illustrated in Fig. 2 which shows the projections of conserved polar positions on helix wheels. The key structural information provided by these projections is the central angle of the arc over which the conserved polar positions are distributed. The angle represents the surface section of the TMH that is likely to be surrounded by other TMHs. This inference about helix packing is based on the consideration that given the hydrophobic nature of the membrane interior, the hydrophilic centers composed of polar residues at PCPs in a TMH can only match hydrophilic centers of adjacent TMHs, except for end regions where polar residues may interact with the head groups of membrane lipid molecules. Consequently, the helix faces that expose polar PCPs will be surrounded by other helices rather than by the lipid.

Analysis of the projections in Fig. 2 suggests several comparative features of the GPCR and BR sets. The central angle of 160° in the helical wheel of TMH3 is the largest one observed for the BR set, indicating that this helix is buried by other TMHs in a central position of the helix bundle of BR [3]. In the GPCR set, however, both TMH3 (with an arc central angle of 220°) and TMH7 (with a central angle of 180°) have a wide spread of conserved polar positions on helix wheels, suggesting that there are two TMHs in the GPCR set that are buried among the other TMHs like TMH3 of BR. This is consistent with the structural features of the projection map of rhodopsin [12]. The implication that TMH3 and TMH7 of the GPCR set have the smallest surface areas exposed to the membrane environment is also consistent with the result of analysis on residue variation of GPCRs among species [10]. The exact topology of the GPCRs is yet to be solved, but the key structural feature placing TMH3 and TMH7 in relative buried positions among the other TMHs emerged in a model of the transmembrane domain of the 5-HT_2 receptor reported recently [34]. The model has a shape similar to that of rhodopsin, with TMH3 and TMH7 in center positions among the other helices in the bundle. Importantly, this organization of the helix bundle was shown to produce a model receptor that responds to the binding of various types of ligands in computational simulations of the signal transduction mechanism, in a manner consistent with the structural inferences obtained from experiments and with the pharmacological properties of the ligands [34,35]. It is unlikely that a proper functional response to ligand binding would be obtained if the helix bundle were not properly organized.

4. Concluding remarks

The new concept of polarity conserved positions offers a tool to evaluate the extent of expected structural similarity among protein families based on explicit physicochemical properties, and backed by observations from known structures. Since the requirement for matching hydrophilic centers is positioning the polar surfaces away from the interface with membrane lipid, the PCP also provides information on the orientation of a TMH with respect to the membrane environment. Consequently, predictions from the PCPs regarding surface

orientation preferences should include any inferences from the analyses of hydrophobic moments [36] and from the periodicity of conserved residues [15,16]. Since the match of hydrophilic centers, as well as of hydrophobic centers among TMHs determines their specific packing, the comparison of PCPs should be useful in evaluating the extent of similarity in the structures of comparable transmembrane protein domains. The difference in the packing arrangement predicted for the family of GPCRs compared to that of BRs further underscores the drawbacks in the use of BR as a template for the construction of molecular models of transmembrane domains of GPCRs (see also [10,34,37,38]. However, de novo modeling efforts should profit from the definition and analysis of the PCPs in the particular family of the modelled GPCR. The matches of hydrophilic centers of helices as guides for packing in the bundle should be obtainable from such analysis. They have to be carried out with special caution if the polar residues are at end regions of the helices, in view of the likely interaction of the charged side chains of Arg and Lys residues with the polar head groups of the phospholipids in the membrane [33].

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