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A new "hydrophobic template" method detects segments forming transmembrane α-helical bundles in ion channels*

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Abstract. We present a "hydrophobic template" method enabling recognition of α-helix bundles in membrane channels from sequence analysis. Inspection of hydrophobic properties of pore-forming helices in proteins with known structure (A-B_5 toxins) permits delineation of a common polarity motif: two hydrophobic surface stretches separated by polar areas. The bundles are stabilized by nonpolar interhelical contacts. A number of transmembrane segments were checked for presence of this motif, and it was detected for pore-forming helices of several ion transporters (segments M2 of acetylcholine and $GABA_A$ receptors, α5 peptide of δ-endotoxin), which reveal five α-helix bundle architecture. Applications of the method to modeling of membrane channels are discussed.

Key words: Hydrophobic interactions – Molecular modeling – Molecular hydrophobicity potential – Helix-helix contacts – Protein fold recognition

1 Introduction

Understanding the molecular events accompanying transport of ions across a cell membrane requires knowledge of spatial structure, hydrophobic and electrostatic properties of pore-forming domains of proteins, that is, ion transporters. However, structural information about membrane proteins is difficult to obtain with experimental techniques, and only a few three-dimensional (3D) structures are known [1]. The membrane pores are often formed by bundles of α-helices arranged parallel or antiparallel to each other [2]. In addition, several globular proteins, like cholera (CT), shiga (verotoxin, VT), and pertussis (PT) toxins, reveal pore-forming five α-helix bundles (5HB) surrounded by β-barrels [3]. While the toxins do not serve to pump ions (although B-pentamer of CT forms ion channels upon reconstitution in lipid bilayers [2]), analysis of this spatial motif is important because similar folds have been proposed for some membrane ion transporters. For example, 5HB structure was discovered for cartilage oligomeric matrix protein (COMP) [4] and membrane parts of the nicotinic achetylcholine receptor (AChR) [5] and δ-endotoxin [6]. Molecular models with five transmembrane (TM) helices lining an ion pore have been developed for some other proteins and channel-forming peptides [2].

A number of attempts have been made to investigate the principles of α-helix arrangement in membrane protein domains [7–9] and to assess hydrophobic organization of TM helix bundles [10]. These studies provide considerable insight into the problem but atomic-scale ab initio prediction of the structure of multi-helix bundles is still a challenging task. Major factors driving helix association must yet be delineated, for example by a detailed analysis of hydrophobic organization of high-resolution 5HB structures.

In this study we pursued the following goals: (1) to assess hydrophobic properties of pore-lining α-helices in known 5HBs and to delineate a common (if any) hydrophobic template for them; (2) to check various TM helices for the presence of a similar motif; (3) based on sequence analysis, to develop a method for recognition of the 5HB fold in channels with unknown structure.

2 Method of calculation

α-Helical segments used in the calculations are listed in Table 1. Their coordinates were either taken from the Brookhaven Protein Data Bank (PDB) [11] or generated using dihedral angles that occur most frequently in α-helices of known protein structures. All the segments (with added hydrogens and neutral termini) were relaxed

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Table 1. Types of α-helical peptides used in the calculations and their boundaries in the amino acid sequence

α-Helical peptide	PDB code	Notation in text	Boundaries in the sequence
Helix A of verotoxin-1 (*E. coli*)	1BOV	VT-A	36–46
Helix D of cholera toxin (*Vibrio cholerae*)	1CHB	CT-D	59–78
Helix S2 of pertussis toxin (*Bordetella pertussis*)	1PRT	PT-S3	146–160
Helix A of cartilage oligomeric matrix protein (*Rattus norvegicus*)	1VDF	COMP	30–66
Helices A-D of ectatomin (*Ectatomma tuberculatum*)	1ECI	ECI-A, ECI-B	5–20, 25–35
		ECI-C, ECI-D	43–58, 63–72
Helices A, B of myohemerythrin (*Themiste zostericola*)	2MHR	MHR-A, B	19–37, 41–64
Helix M2 of AChR (*Torpedo californica*, α-subunit)		ACHA-M2	243–261
Helix M2 of AChR (*Torpedo californica*, β-subunit)		ACHB-M2	271–292
Helix α5 of δ-endotoxin (*Bacillus thuringiensis*)		DTOX-A5	193–214
Helix M2 of $GABA_A$ receptor (*Rattus norvegicus*)		GABA-M2	35–52
TM helix of M2 coat protein (*Influenza A virus*)		VMT2	24–44
H5 region of ROMK1 K^+ channel (*Rattus norvegicus*)		ROMK1	127–149
H5 region of Shaker K^+ channel (*Drosophila melanogaster*)		SHAKER	430–450
H5 region of Kv2.1 K^+ channel (*Rattus norvegicus*)		KV2.1	361–381

via 100 cycles of steepest descents minimization using the Discover program and the CVFF force field [12]. The molecular hydrophobicity potential (MHP) on the surface of isolated helices was calculated as described elsewhere [9, 13]. The surface MHP was visualized by means of two-dimensional (2D) plots in the coordinates (α, *Z*), where α is the rotation angle about the helix axis *Z*. Calculation of one-dimensional (1D) MHP plots and other details have been described earlier [13]. 1D-MHP plots were shifted along the horizontal plot axis to provide the best fit (maximal correlation coefficient) with the 1D-MHP plot of helix A of VT (VT-A) [14].

3 Results and discussion

3.1 Hydrophobic template for 5HB

Spatial hydrophobic properties of pore-forming helices in 5HB folds, expressed in terms of 2D isopotential contour maps of MHP and 1D-MHP plots, are illustrated by the example of VT-A (Fig. 1). The pore-lining parts of the surface are indicated by grey hatching. Only the most hydrophobic zones, that is, high MHP, are shown. They correspond to the residues Leu 36, 39, 40, 41, Ile 45, while hydrophilic regions form two patterns spanning the whole helix length and are observed in the vicinity of Gln 37, 44, Ser 38, 42, and Thr 46. The polar areas are disposed on opposite helix faces and separate two hydrophobic sides. This is pictorially illustrated by the 1D-MHP plot (Fig. 1, bottom, solid line). Interestingly, the pore region corresponds exactly to one of the polar stretches ($80° < \alpha < 160°$), whereas the second one faces the β-sheet surrounding the 5HB in the B-pentamer of VT. Nonpolar areas form interfaces between neighboring helices in the bundle. We also assessed the surface MHP of VT-A induced by atoms of the B-pentamer, excluding atoms of the helix A itself. The resulting "external" 1D-MHP plot (Fig. 1, bottom, dotted line) correlates well with that calculated for the isolated helix, except at the helix/sheet interface ($220° < \alpha < 280°$). This confirms that the helices are packed via strongly hydrophobic sides, while the helix/sheet contacts are less favorable.

The same analysis was done to assess hydrophobic properties of pore-forming helices in other 5HB proteins

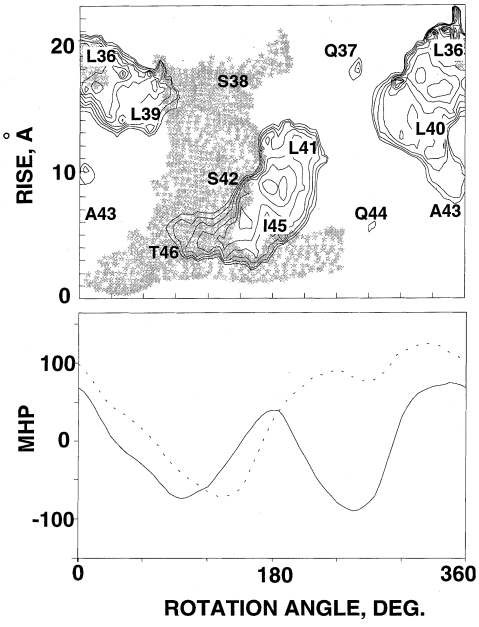

Fig. 1. Hydrophobic properties of the pore-forming α-helix A in B-pentamer of verotoxin-1. (Top) 2D isopotential map of the molecular hydrophobicity potential (*MHP*) on the peptide surface. The horizontal axis is the rotation angle α about the helix axis; the vertical axis is the distance along the helix axis *Z*. Only the hydrophobic areas with MHP > 0.09 are shown. Contour intervals are 0.015. The positions of residues are indicated by letters: L = Leu, I = Ile, Q = Gln, S = Ser, T = Thr. The *grey shading*, represents the pore-lining surface determined from experiment. (Bottom) *Solid line* The MHP as a function of helical angle created by the peptide atoms on its surface; *dotted line* MHP created by the neighboring protein parts on the surface of helix A as a function of helical angle. The values of MHP are summed inside the sectors with angular size 90°

(CT, PT, COMP). They have a polarity distribution on the surface very similar to that of VT-A (Fig. 2A). We should emphasize that, although the MHP analysis was done for isolated helices, the distribution of their hydrophobic properties was then superimposed on the experimental structures of 5HBs. We have thus shown

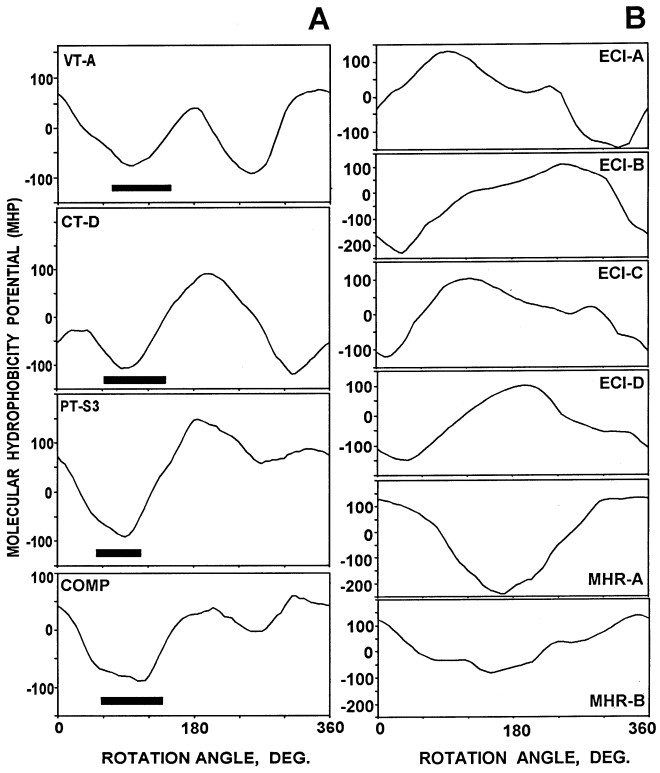

Fig. 2. Angular distribution of MHP on the surface of pore-forming helices in proteins with five- (**A**) and four- (**B**) helix bundle folds. **A** Helix A of verotoxin-1 (*VT-A*), helix D of cholera-toxin (*CT-D*), helix S3 of pertussis toxin (*PT-S3*), cartilage oligomeric matrix protein (*COMP*). The plots are aligned relative to that of VT-A. Known pore exposure is shown by the horizontal bars. **B** Helices A–D of ectatomin (*ECI-A–ECI-D*), A, B of myohemerythrin (*MHR-A*, *MHR-B*)

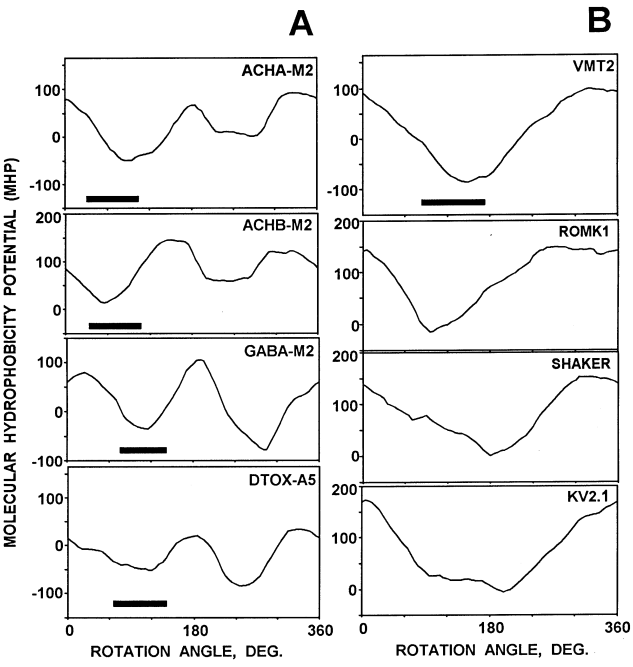

Fig. 3. Angular distribution of MHP on the surface of transmembrane pore-forming segments in ion channels with (**A**) and without (**B**) a putative five-helix bundle fold. The plots are aligned relative to that of VT-A. Known pore exposure is shown by the *horizontal bars*. M2 helices in α- and β-subunits of nicotinic acetylcholine receptor (*ACHA-M2*, *ACHB-M2*); M2 helix of $GABA_A$ receptor (*GABA-M2*); α5 segment of δ-endotoxin (*DTOX-A5*); TM helix of influenza virus M2 coat protein (*VMT2*); H5 regions of ROMK1, Shaker, and Kv2.1 K^+ channels (*ROMK1*, *SHAKER*, *KV2.1*)

that the MHP characteristics of individual segments complement each other in the bundle and thus provide strong packing of the oligomer: helices interact through their strongly nonpolar surfaces, while relatively polar sides are exposed inside and outside the bundle. By contrast, the distributions of electrostatic potential on their surfaces are different (data not shown): they neither correlate with the orientation of the helices in the bundle nor reveal a common electrostatic motif for 5HB. The hydrophobic 5HB template is quite different from those usually employed in models of ion channels (often built on coordinates of A-B_5 toxins): α-helices are oriented to the pore with their polar sides, while the hydrophobic regions are turned outside. As follows from the present data, this does not correspond to the hydrophobic organization of known 5HB structures.

Another fold which occurs frequently in ion channels is a four-helix bundle (4HB) [2]. To check whether this structure has a hydrophobic organization similar to that found for a 5HB fold, we estimated the MHP on surfaces of helices in two "canonical" 4HB proteins – ectatomin (ECI) and myohemerythrin (MHR). Resulting angular MHP distributions (Fig. 2B) correspond to "classical" amphiphilic helices with one polar and one nonpolar side (1 maximum and 1 minimum on the MHP plot). This is different from the pattern with two maxima and two minima in the MHP observed for 5HB proteins (Fig. 2A). Therefore, we propose that the shape of a 1D-MHP plot obtained for individual α-helices permits discrimination between the two types of helix bundles based on sequence information only (see below).

3.2 5HB-hydrophobicity motif in ion channels

It order to check whether the 5HB polarity template can be applied to pore-forming segments of ion channels, we calculated 1D-MHP plots for several of them, for which a body of structural information exists. Thus, TM α-helix bundles and residues involved in channel functioning were proposed for M2 segments of AChR [5] and $GABA_A$ receptors [15], the α5 segment of δ-endotoxin [6] and the influenza virus M2 protein (VMT2) [16]. We also examined the H5 regions of three K^+ channels – ROMK1 [17], Shaker [17], and Kv2.1 [18] – which are believed to oligomerize in membrane-forming channels with β-barrel architecture [19] (although monomeric H5 segments are able to form α-helices in micelles [20]). Corresponding 1D-MHP plots are presented in Fig. 3. Some of them (Fig. 3A) correlate well with those characteristic for 5HB, while the others (Fig. 3B) do not. It is important that all helices shown in part A of Fig. 3 are considered as constituting channels with a 5HB fold. [Interestingly, the hydrophobic properties calculated here for a computer-built model of M2α of

AChR agree fairly well with those found for the 3D structure obtained recently in our laboratory by NMR in SDS micelles (Pashkov et al., in preparation).] Therefore, in spite of different biological functions and the absence of sequence homology in A-B_5 toxins and COMP, pore-forming helices of these channels exhibit similar hydrophobic properties (5HB motif). By analogy with A-B_5 toxins and COMP and by taking into account that helix-helix packing in membranes is driven to a large extent by hydrophobic interactions, we propose that oligomeric forms of these channels have 5HB architecture.

In contrast, the angular distributions of hydrophobicity in transmembrane segment (TMS) of the cation-selective channel VMT2 and the H5 regions of three potassium channels differ from the 5HB motif (Fig. 3B). They correspond to the "classical" amphiphilic pattern: one hydrophobic and one hydrophilic side of the helix. As mentioned before, such a pattern is typical for 4HB proteins. Therefore, our results confirm that VMT2 can form a channel with four but not five α-helices [16]. We also conclude that the ion-conducting regions of the studied K^+ channels do not form a 5HB fold, although we cannot exclude the possibility of the 4HB motif. Additional work is required to discriminate between β-barrel and 4HB architectures for H5 segments.

The similar structural and hydrophobic organization of 5HB proteins and some membrane ion channels suggest that it is possible to detect pore-forming helix bundles from sequence information. The computational protocol would be as follows: (1) delineation of putative TMS in the sequence and assessment of their probable secondary structure; (2) computer-modeling of TM α-helices [if the segment was assigned to an α-helix at stage 1]; (3) calculation of surface MHP for them and comparison of 2D-MHP maps and 1D-MHP plots with those characteristic for a 5HB motif. If there is close correspondence in the MHPs then this argues in favor of a model with five α-helices forming a channel. Moreover, by analogy with known structures of 5HB proteins, the most probable lipid, protein, and channel exposure of the helices might also be determined.

We should also indicate the shortcomings of this method. In some cases (e.g., in the absence of sequence homologs) the secondary structure of TMS is difficult to assess with theoretical methods only. Also, the algorithm fails to discriminate between bundles of five, six, and more helices because the hydrophobic properties of corresponding individual segments are quite similar.

4 Conclusions

1. Despite the lack of sequence homology, α-helices in 5HB reveal similar hydrophobic properties on their surfaces: two polar sides separated by strong hydrophobic stretches. The helices are tightly packed via nonpolar faces, one hydrophilic side is exposed to the central pore, and another one is turned to the exterior of the bundle. This polarity template is rather different from that observed in 4HB proteins.
2. Hydrophobic properties of several channel-forming TM α-helices (M2 of AChR and $GABA_A$ receptors, α5 of δ-endotoxin) are similar to the 5HB polarity template. This confirms that these TM segments can form channels with 5HB architecture.
3. Application of the method to recognition of 5HBs based on sequence information only is proposed. This information is necessary to build molecular models of ion channels because it imposes stringent constraints on the helix orientation in the assembly. In turn, the models provide a basis for rationalization of structural and functional data on the channel as well as for design of further experiments.

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