Structural features of transmembrane helices

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Abstract A total of 160 transmembrane helices of 15 non-homologous high-resolution X-ray protein structures have been analyzed in respect of their structural features. The dihedral angles and hydrogen bonds of the helical sections that span the hydrophobic interior of the lipid bilayer have been investigated. The Ramachandran plot of protein channels and solute transporters exhibit a significant shift Δ (ϕ- and ψ-angles) of Δ mean (+4.5° and −5.4°), compared to a reference group of 151 α-helices of the same average length derived from water-soluble globular proteins. At the C-termini of transmembrane helices structural motifs equivalent to the Gly-caps of helices in globular proteins have been found, with two third of the transmembrane Gly-caps taking up a primary structure that is typically not found at helix termini exposed to a polar solvent. The structural particularities reported here are relevant for the three-dimensional modelling of membrane protein structures.

Key words: Transmembrane; α-Helix; Helix cap; Dihedral angle; Hydrogen bond; Channel

1. Introduction

Membrane proteins fundamentally rule all the different biochemical processes that take place at lipid bilayers. About one third of the presently mapped gene sequences encode for membrane proteins. In contrast, only a minor fraction (1/150) of the protein structures deposited in the protein data bank (PDB) belong to this structural class [1]. The three-dimensional (3-D) modelling of membrane proteins is therefore a key task of bioinformatics [2,3]. It has been ruled out that the packing of α-helices and the interference of these helix bundles with the lipid bilayer is fundamental for the stabilization and function of all membrane proteins [4–6]. Because hydrogen bonds are strengthened by the high permittivity value of the hydrophobic interior of the lipid bilayer the formation of intra- and interhelical hydrogen bonds is essential for the entire folding process [7,8]. Accordingly, the secondary structure of a predestined transmembrane segment is formed, when the helices insert into the membrane and before the helices associate. In this regard transmembrane helices can be interpreted as independent folding units [9]. The 3-D structure modelling of membrane proteins consequently follows these ontogenetical steps [10]. The first step is to define standard values of the transmembrane helical conformation; the next step is to predict the helix orientation relative to the membrane or to a neighboring transmembrane helix [11]. Finally, the loops and extramembranous parts have to be modelled.

Here we summarize the results of a detailed comparison between the geometrical features of transmembrane helices and long α-helices of globular proteins. Following the concept of 'hydrophobic mismatch' a transmembrane helix tilts in order to solvate the side chains in the appropriate milieu [12]. Thus the borders between the hydrophobic interior of the membrane and the polar lipid head groups basically have been established by the accumulation of exposed polar residues near the transmembrane helix ends. Helices of globular proteins frequently start and terminate with structural motifs called helix caps [13]. Because there was no comprehensive analysis of these 'super-secondary structures' in membrane proteins we investigated the helix termini, too. We determined the occurrence of the most abundant C-terminal cap and evaluated whether the amino acid composition implies some information about its position relative to the polar lipid head groups.

In order to understand the structure–function relationships that underlie the transport of metabolites and the transduction of signals through the membrane much effort has been made [5,6]. The present progress in the crystallization and elucidation of helical membrane protein structures highlights that the different membrane protein functions are realized by certain protein architectures [14–16]. Although there is no membrane protein where the crystal structure of both, the open and the closed or the activated and the non-activated conformation is known, it has been suggested that solute transporters or ion channels open throughout a gating mechanism that presupposes broad molecular rearrangements of their transmembrane domains [15,16]. For the mechanosensitive channel e.g. it has been postulated that the presence of small amino acids at every fourth position, in regions that participate in helix–helix packing, may underpin the iris-like opening of the channel, affecting the position of all transmembrane helices [14]. In light-triggered receptors or metabolic driven proton pumps, however, rearrangements seem to occur on a smaller scale: In bacteriorhodopsin a gating mechanism has been proposed, where basically the helical section above the π-bulge (working as a hinge) in helix G swivels out of the proton channel [17]. In the activated rhodopsin the cytoplasmic termini of helices C and F are supposed to be more distant from each other, what could be caused by a conformational change of helix F only during activation [18]. The basis

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Abbreviations: DSSP, define secondary structure of proteins; PDB, protein data bank

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for these different functionalities has to be found in the assembly and flexibility of the transmembrane domains [6]. To verify this hypothesis we assorted 15 high-resolution structures of non-related membrane proteins either as 'membrane gates' (transport of larger solutes, e.g. ions) or 'membrane coils' (coiled coils, transport of small solutes, e.g. protons) and subjected them to further analysis.

2. Data set and methods

The 15 high-resolution structures of non-related helical membrane proteins are listed as described in Table 1. We first proved whether the architectures of the two membrane protein data sets are indeed different. With the help of a program kindly provided by Senes et al. [19], we manifested that the 'membrane gates' markedly differ from the other membrane proteins in regard of their helix–helix crossing angles: 65% of the helix crossings (as defined by [20]) are right-handed within this data set. In the remaining group of transmembrane helices 64% of the helix crossings are left-handed. In accordance to their preponderant coiled coils architecture [21] the latter group was called 'membrane coils'. In order to quantify the influences of the different milieu, solely those parts of the transmembrane helices have been selected that are located within the hydrophobic part of the membrane:

1. Here we assume that the 'membrane borders' are almost planar and parallel. For monomeric structures the membrane normal was calculated as the average axis of all transmembrane helices; for multimers the symmetry axis was taken.
2. If determined, we used lipids to define the dimensions of the hydrophobic parts of the membrane. The position of the polar lipid head groups was outlined by water molecules or solvent-exposed electron donors and acceptors [22] that lack a hydrogen-bonding partner. The aromatic belt (indicated in Fig. 1) was alternatively used to define the expansion of the lipid bilayer.
3. We picked three Cα atoms on both sides of the bilayer in order to define two parallel planes at a distance range of 20–27 Å. The helical residues with their Cα atoms between the membrane borders were stated as transmembrane helices (http://www.charite.de/bioinf). The statistical evaluations used for the following analysis are also described in detail on this web page.

For the investigation of torsion angles residues with ϕ-, ψ-values $>0°$ or $<-100°$ (e.g. kinks, π-bulges) were excluded. The reference group of long α-helices was taken from 25 non-homologous globular protein structures (Table 2, footnote). The secondary structures were defined by the define secondary structure of proteins (DSSP) criteria [23].

Hydrogen atoms were added to the X-ray crystal structures using the Biopolymer program (Biopolymer, Insight II, version 2000, Accelrys, San Diego, CA, USA). The main chain amide hydrogen atoms were placed on the bisector of the angle C–N–Cα, and in the plane defined by C, O, N, assuming a standard N–H bond length of 1.03 Å. To be comparable to other studies of C═O–H–N main chain hydrogen bonds we used a cut-off value of 2.6 Å [24,25]. This cut-off value is also adequate to recognize the bifurcation of hydrogen bonds in helices [26]. For the exact measurement of geometrical features as the rise per residue (rpr) and mean deviation from the straight axis, local helix axes spanning only 11 residues and global axes spanning the entire transmembrane helix were defined, respectively. The $\arccos(rpr_{global}/rpr_{local})$ expresses the deviation in degree from the straight axis.

In order to detect Gly-caps what are the most abundant cap motifs found at the C-termini of helices in globular proteins, we implemented an automated and simple search algorithm previously applied by [27]. For a Gly-cap a Gly with a positive ϕ is obligatory at the position succeeding the C-terminus [13]. Because the definition of precise helix termination by DSSP is sometimes ambiguous the positions n+1, n+2 and n+3 from the C-terminus (position n) defined by DSSP were also taken into consideration. Because of the minor data set our definition was less restricted than in the analysis mentioned above, where the Schellman motif is defined as a specification of a Gly-cap [13]. These authors postulate as a rule of thumb that the side chains in the $n-2$ or $n-3$ positions of this C-terminal motif are generally polar and solvent exposed. The question arises whether this rule is also valid for Gly-terminated helix caps in membrane proteins.

3. Results

The average length of the 160 membrane helices that span the hydrophobic part of the bilayer is 17.3 residues or 26 Å (standard deviation, S.D.: ±3.1, ranging from 6 to 25). The average rise per residue is 1.50 Å (3.7 residues per turn) in both types of transmembrane helices. The average length of the 151 reference helices of globular proteins is 18.3 (±8.2, 10–50) residues or 28 Å, ascending 1.52 Å per residue (3.6 residues per turn). Transmembrane helices shorter than 10 residues are exclusively found in membrane channels. When

Table 1
High-resolution structures of non-related membrane proteins

Data set, protein name	PDB	ϕ	ψ
Membrane coils			
Bacteriorhodopsin	1c3w	−65.1	−39.7
Rhodopsin	1f88	−66.7	−40.2
Photosynthetic reaction center	1aig	−68.0	−37.9
Photosystem 1	1jb0	−63.7	−42.4
Light harvesting complex	1lgh	−64.1	−40.4
Cytochrome *c* oxidase	2occ	−63.3	−42.0
Cytochrome *bc*1	1ezv	−65.7	−39.3
Fumarate reductase	1qla	−64.1	−42.7
Mean value (S.D.)		−64.5 (±8.1)	−41.1 (±9.4)
Membrane gates			
Ion channels			
Potassium channel	1jvm	−54.2	−51.7
Mechanosensitivity channel	1msl	−59.2	−45.7
Chloride channel	1kpl	−61.3	−44.6
Mean value (S.D.)		−60.3 (±12.2)	−45.5 (±14.2)
Transporters			
Aquaporin	1j4n	−62.7	−43.1
P-type ATPase	1eul	−64.1	−41.3
ABC transporter	1l7v	−58.3	−47.0
Multi-drug efflux transporter	1iwg	−59.4	−45.1
Mean value (S.D.)		−60.9 (±11.2)	−44.3 (±13.8)
Mean value (S.D.)		−60.7 (±11.7)	−44.7 (±13.0)

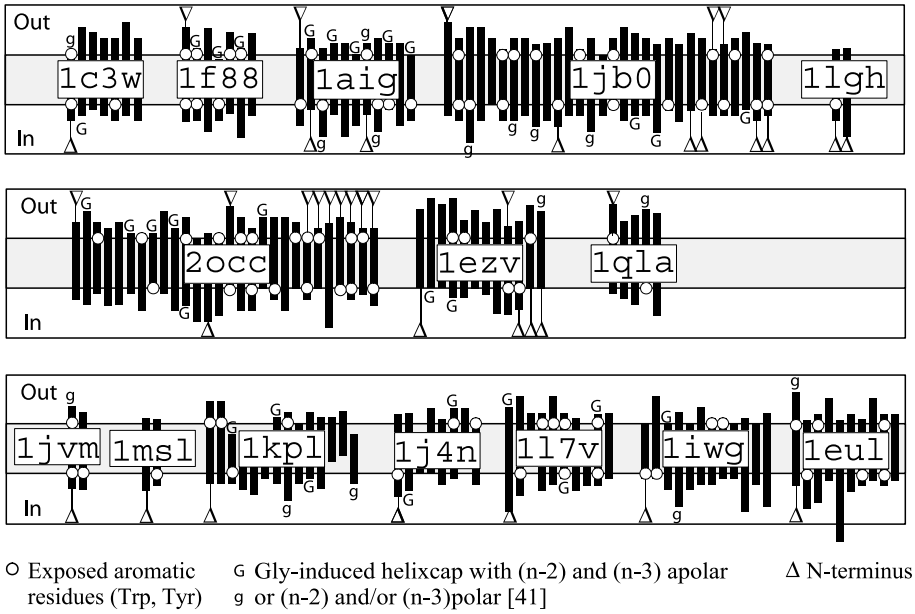

Fig. 1. Arrangement of transmembrane helices within the lipid bilayer. The lengths of the helical sections that span the hydrophobic interior of the membrane (gray) vary with their tilt angles (not imaged). Helices terminated by Gly are indicated according to the polarity of amino acid types in the $n-2$ and $n-3$ position [13]. Trp and Tyr that label the border to the aqueous milieu are additionally denoted as are the N-termini of the protein subunits.

Table 2
Amino acid composition of the different data sets of transmembrane helices

	Amino acid	Membrane proteins					Globular proteins ($N = 2686$)[a] (%)
		All ($N = 2677$) (%)	Coils ($N = 1807$) (%)	Gates			
				All ($N = 870$) (%)	Ion channels ($N = 274$) (%)	Solute transporters ($N = 596$) (%)	
Non-aromatic	Ile	9.6	8.9	11.1	9.9	11.7	6
	Val	11	9.6	13.8	13.1	14.1	6.3
	Leu	17.3	16.7	18.5	16.4	19.5	11.2
	Met	4.6	5.1	3.3	3.6	3.2	3.3
	Ala	12.5	11.6	14.6	15	14.3	10.9
	Gly	8.9	8.3	10.1	13.1	8.6	3.1
Aromatic	Phe	9.2	11.0	5.5	9.1	3.9	4.9
	Trp	2.8	3.7	1.0	1.1	1.0	2.2
	Tyr	2.6	3.2	1.5	1.1	1.7	4.7
	Σ_1	14.7	**17.8**	8.0	11.3	6.5	11.8
	Σ_2[b]	78.5	78.0	79.5	**82.5**	77.9	52.7
Indifferent	Cys	1.2	0.9	1.7	1.1	2	1.1
	Pro	2.2	2	2.8	1.8	3.2	1.5
	Thr	5.5	5.7	4.9	6.9	3.9	4.1
	Ser	4.9	5.2	4.1	2.9	4.7	4.5
	His	2.6	3.7	0.3	0	0.5	2
	Σ_3	16.3	**17.4**	13.9	12.8	14.3	13.3
Polar	Glu	0.8	0.6	1.4	0.7	1.7	9.2
	Asn	1.3	1.3	1.4	1.1	1.5	3.2
	Gln	0.8	0.6	1.1	0.4	1.5	5.1
	Asp	0.6	0.6	0.8	0.7	0.8	4.3
	Lys	0.9	0.8	1	1.5	0.8	5.7
	Arg	0.7	0.7	0.8	0.7	0.8	6.5
	Σ_4	5.2	4.5	6.6	5.1	7.2	**34.1**

[a] 1alv, 1c3c, 1cm4, 1ezj, 1go3, 1h1r, 1i2t, 1iwo, 1jqc, 1k04, 1kp4, 1kqf, 1l2p, 1mhy, 1mty, 1mxe, 1n1b, 1n45, 1qsa, 1shk, 1taf, 1vrk, 2a0b, 3fap, 119l (resolved at high resolution ≤ 2 Å, R-value $\leq 20\%$, sequence homologs removed at 90% sequence identity, helical content $\geq 60\%$, helix length ≥ 10 residues).
[b] Sum of hydrophobic amino acids. The amino acids are grouped into different categories and listed according to the hydrophobicity scale of [41]. Highest (lowest) mean values are hallmarked bold (underlined).

these helices were excluded from statistics the average transmembrane helix comprises a length of 17.7 residues or 27 Å. Helices of membrane coils deviate from the straight axis by a mean of 7.2° what is slightly more than the helices of membrane gates (1.8°).

The investigated transmembrane domains protrude at an average of 4.7 (S.D.: ±3.5) residues, or 1.3 turns into the polar milieu (Fig. 1). These sections consist of only 60% hydrophobic residues (not shown). About a quarter of the helices of the transmembrane domains and of globular proteins are terminated by a Gly-cap, which corresponds to the fraction found in globular proteins [27]. However, two third of these helix caps in globular proteins and only one third in transmembrane domains are 'polar Gly-caps' (Fig. 1). Most (80%) of the transmembrane domains with a polar helix cap protrude more than one helix turn (seven residues at average) into the polar milieu.

Transmembrane helices are predominantly composed of hydrophobic residues (78.5%). When we compared the two membrane protein data sets we found that only 8% of the amino acids in the membrane gates have aromatic side chains (Table 2). This is less than half the number found in the data set of membrane coils (17.8%). On the other hand the former group contains clearly more polar residues (6.6% compared to 4.5%), a higher amount of the small residues Gly and Ala (24.7% compared to 19.9%) and of the β-branched residues (29.8% compared to 24.2%). Compared to the transmembrane helices, α-helices of globular proteins have an explicitly poorer content of hydrophobic residues (52.7%).

The conformation of the α-helix can be characterized by the backbone torsion angles ϕ and ψ. The ω-angles exhibited no significant differences between the different data sets. When we considered the Ramachandran plots of the three data sets we noticed that the torsion angles around the N–Cα (ϕ-angle) and the Cα–C bonds (ψ-angle) cumulate differently (Fig. 2a–c). Accordingly, the mean ϕ- and ψ-angles of the transmembrane α-helices of membrane gates (−60.7° and −44.7°) and of α-helices in globular proteins (−65.2° and −39.3°) are significantly different (Student's test, $t_{f,0.995}$, S.D. given in Table 2). This discrepancy is evident when the distributions of the ϕ- and the ψ-angles are compared in discrete plots (Fig. 3a,b). That is even more pronounced when the transmembrane helices of the ion channels are separately compared with the reference data (values given in Table 2). The torsion angles of transmembrane helices of membrane coils do not deviate markedly from those of helices in globular proteins (Table 1).

The establishment of hydrogen bonds is the driving force for helix formation in the hydrophobic milieu [7]. During folding the i, i+3 hydrogen bond typical for the 3_{10}-helix evolves to the i, i+4 hydrogen bond typical for the α-helix and usually persists in α-helices [28,29]. Therefore the hydrogen bond of an α-helix is a hybrid of both. In the transmembrane sections of membrane gates nearly one third (31%) of the amino acids are engaged in bifurcated main chain hydro-

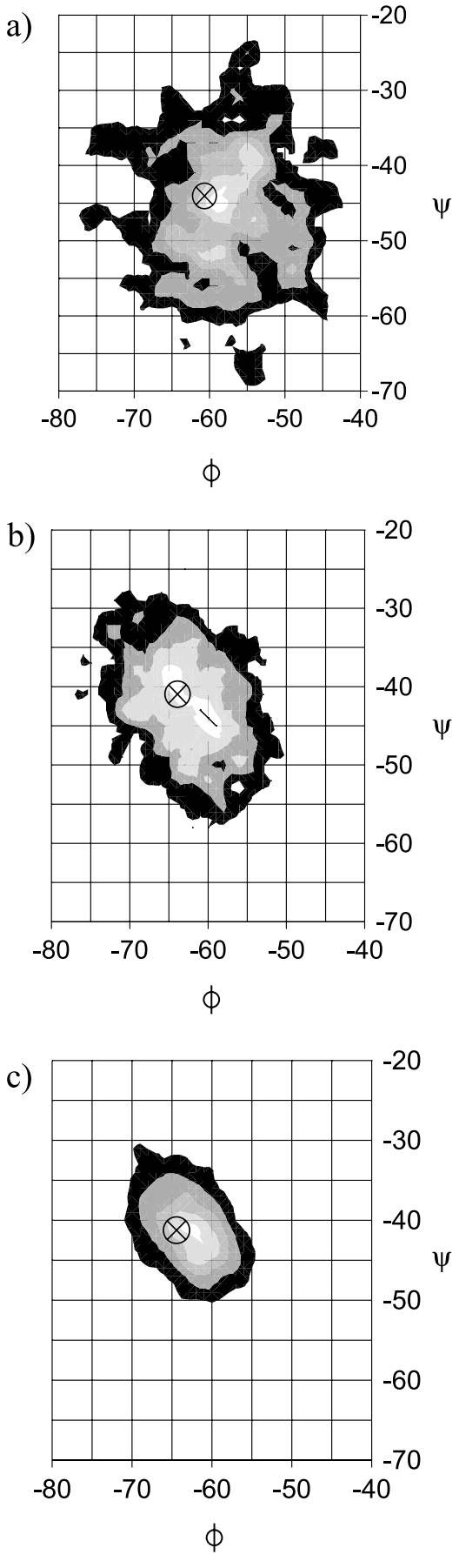

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Fig. 2. Ramachandran 2-D density plot of main chain ϕ-, ψ-angles from transmembrane helices of a: membrane gates (914 residues), b: membrane coils (1884 residues) and c: long α-helices in globular proteins (2753 residues). The shaded regions map the graduated appearances of ϕ-, ψ-angles, while the marks point at the respective mean values.

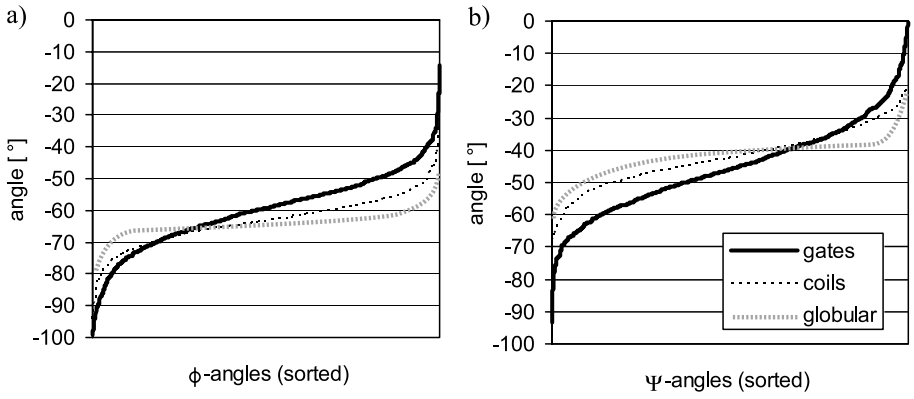

Fig. 3. Main chain torsion angles ϕ (a) and ψ (b) from transmembrane helices of membrane gates, membrane coils and long α-helices in globular proteins, arranged according to angle size.

gen bonds. In membrane coils, however, only one fifth (21%) and in helices of globular proteins solely one sixth (17%) of the main chain hydrogen bonds are bifurcated.

In the investigated data sets the average *i*, *i*+4 hydrogen bond length is 2.0 Å and the distributions are generally quite similar. Nevertheless in transmembrane helices of membrane gates shorter (≤ 1.9 Å) and energetically stronger hydrogen bonds are more frequent (Fig. 4a). Only 9% and 8% of the residues of membrane gates and membrane coils, respectively, and 3% of the residues in helices of globular proteins lack the *i*, *i*+4 hydrogen bond. The *i*, *i*+3 component typical for 3_{10}-helices is normally weak in long α-helices (here ≥ 10 residues) [29] and short distance interactions are hence scarcely present in all data sets. At the proposed cut-off, nevertheless, more of them are found in transmembrane helices of membrane gates. The average bond length is 2.3 Å (S.D.: ±0.19) in this data set and therefore significantly (Student's test, $t_{f,0.95}$) shorter compared to the non-channel data set (2.4 ± 0.18 Å) and to the data set of globular proteins (2.5 ± 0.13 Å) (Fig. 4b).

The side chain conformers can be considered in case of χ_1 as a dense cluster around the rotamer states g^+ (gauche$^+$), g^- (gauche$^-$) and *t* (trans) [30]. Referring to the limited size of the data set of membrane proteins the delineation of structures into different categories wasn't suitable and our investigations were restricted to the torsion angle around the C_α and the C_β bond (χ_1-angle). Only for the amino acids Met, Trp and small polar amino acids (Asn, Asp) we observed significant deviations from the side chain conformations found in helices of globular proteins (*chi*-square test, $P = 0.95$) (http://www.charite.de/bioinf).

4. Discussion

It has been proposed that membrane channels and solute transporters (membrane gates) can be differentiated from coiled membrane proteins by the specific assembly of the transmembrane helices [6]. Pursuant we ascertained that in membrane gates two third of the helix contacts are right-handed, while left-handed super-coils are equally abundant in membrane coils. Besides, these two data sets have different amino acid compositions. The relatively high abundance of polar amino acids in transmembrane helices of membrane gates already lead to the postulation of an individual folding mechanism [31]. Further it has been noticed that channels and solute transporters are stabilized by certain interaction motifs [19].

Here we demonstrate that the transmembrane domains of membrane gates enclose a particular helix conformation (Figs. 3, 4). The mean ϕ- and ψ-values of these helices differ significantly from those of globular proteins Δ (+4.5° and −5.4°) and of membrane coils Δ (+3.8°, −3.6°). It has been shown that the mean values of the main chain torsion angles of

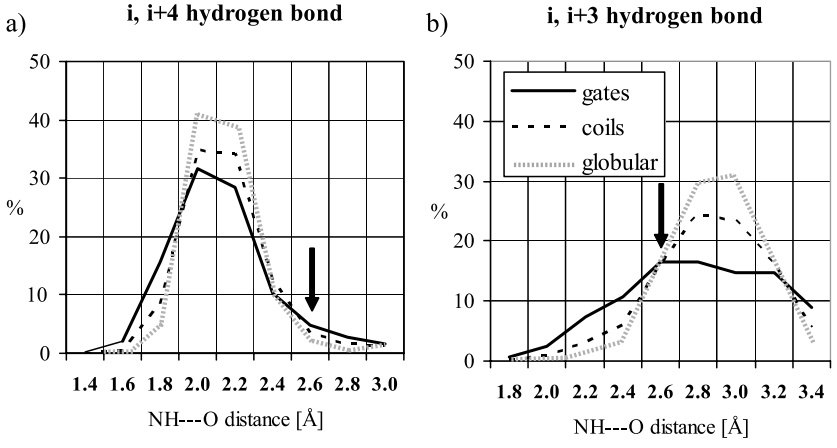

Fig. 4. Relative distribution of main chain hydrogen bonds found in transmembrane helices of membrane gates, membrane coils and α-helices of globular proteins. The black arrows mark the proposed cut-off value at 2.6 Å.

helices in globular proteins enclose the curvature of many of the helices [32]. Here we substantiate that helices of membrane coils comprise nearly the same mean ϕ- and ψ-values as helices of globular proteins and deviate more from a straight axis (by 7.2°) than the helices of membrane gates (by 1.8°). These distinct architectures probably account for the different functions: right-handed contacts generally facilitate interactions of straight helices at a larger crossing angle, allowing the formation of the funnel-shaped folds seen in many channel proteins [6].

The Ramachandran plots of the transmembrane helices are not as uniform as those of the reference data of long α-helices of globular proteins (Fig. 3). This can be explained by the minor data sets and the lower resolution of the membrane protein structures. On the other hand there is no proof for the assumption that this likewise causes the observed differences of the mean ϕ- and ψ-values, e.g. we found no correlation between the resolution and the deviation of ϕ- and ψ-angles when we compared the different X-ray structures of bacteriorhodopsin that are deposited in the PDB [33]. Besides, the ϕ- and ψ-angles are not directly optimized in the refinement process and therefore provide a sensitive and a trustworthy indicator for the detection of structural specialities in proteins [34].

The side chain rotamer preferences of amino acids in transmembrane helices and helices of globular proteins are nearly identical, what has been stated earlier by Bywater et al. [35]. Only the *t* state is preferred in the side chains of Met, Trp, Arg and Asp ($P = 0.95$) in transmembrane helices. To that effect more structural data are needed in order to correlate the preference of side chain conformation to the specific values we found for the backbone dihedral angles of helices in membrane gates [36]. To ascertain the influence of the different ϕ- and ψ-angles on the helix modelling, we built two poly-Ala helices of 18 residues length, either using the mean values of transmembrane helices in membrane gates or of long helices in globular proteins. Starting from the same initial point the helices end at a Cα distance of 1.9 Å and are superimposed with a root mean square deviation (rmsd) of 0.42.

The breaking of a main chain hydrogen bond within the hydrophobic milieu of the lipid bilayer is extremely unfavorable [6]. Hydrogen bonds therefore provide a sensitive tool to estimate the stability of transmembrane helices (Fig. 4). The relatively high number of residues lacking the *i*, *i*+4 hydrogen bond in transmembrane helices is caused by the frequently found kinks introduced by Pro residues [37,38] or other structural particularities such as π-bulges and 3_{10}-helices [6]. Most of these structural particularities are energetically compensated by tertiary structure interactions [16,39] and support the function of these proteins by implementing structural flexibilities [40]. Our results indicate that aside from this, the residues of the transmembrane helices of membrane gates are stabilized by a higher content (+14%) of bifurcated hydrogen bonds, with stronger *i*, *i*+3 hydrogen bonds. If so, these helices commonly appear more rigid than helices of membrane coils and helices of globular proteins (Fig. 4).

About a quarter of the helices of the transmembrane domains and of globular proteins are terminated by a Gly-cap, what compares well with the fraction found by Preissner and Bork [27] who investigated 456 C-termini of helices in globular proteins. Here we report for the first time that helix caps of transmembrane helices are substantially specific structural patterns that can be clearly distinguished from the classical caps, known from helices in globular proteins. In general solely those transmembrane helices end up with a classical 'polar Gly-cap' that protrude more than one helix turn into the polar milieu. The Gly-caps that are close to the hydrophobic lipid tails are overwhelmingly apolar. The amino acid composition of the helix caps is therefore related to the localization relative to the lipid head groups (Fig. 1). This implies that these super-secondary structural elements are only stable when they are exposed to the appropriate surrounding conditions. According to the variety of lengths, the transmembrane helices have to be tilted within the lipid bilayer to fulfill this criterion. Thus our results confirm the hypothesis of the hydrophobic mismatch, whereupon transmembrane helices are pre-orientated or tilted within the lipid bilayer before they finally associate [12].

We conclude that in order to assess the basic structural principles of membrane proteins with different functions, their subdivision in at least two different groups is an advantageous step. Membrane channels and transporters can be differentiated from coiled membrane proteins by their amino acid composition. According to the analysis given here, transmembrane helices of membrane channels and transporters have to be modelled using different torsion angles, while we propose that the rotamer libraries derived from helices of globular proteins are also valid for transmembrane helices. The Gly-caps found at the C-termini of transmembrane helices are typical for membrane proteins and indicate their relative position to the polar lipid head groups. Our future aim is to apply these findings in order to improve the tertiary structure prediction of at least the membrane-spanning part of helical membrane proteins.

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