Uncoupling Hydrophobicity and Helicity in Transmembrane Segments

α-HELICAL PROPERTIES OF THE AMINO ACIDS IN NON-POLAR ENVIRONMENTS

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Although the chains of amino acids in proteins that span the membrane are demonstrably helical and hydrophobic, little attention has been paid toward addressing the range of helical propensities of individual amino acids in the non-polar environment of membranes. Because it is inappropriate to apply soluble protein-based structure prediction algorithms to membranes, we have used de novo designed peptides (KKAAAXAAAAAWAAXAAKKK-amide, where X indicates one of the 20 commonly occurring amino acids) that mimic a protein membrane-spanning domain to determine the α-helical proclivity of each residue in the isotropic non-polar environment of n-butanol. Peptide helicities measured by circular dichroism spectroscopy were found to range from \( \theta_{222} = -17,000 \) ° (Pro) to \(-38,800 \) ° (Ile) in n-butanol. The relative helicity of each amino acid is shown to be well correlated with its occurrence frequency in natural transmembrane segments, indicating that the helical propensity of individual residues in concert with their hydrophobicity may be a key determinant of the conformations of protein segments in membranes.

Membrane-spanning segments of proteins are overwhelmingly α-helical (1–5). Previous studies of protein transmembrane (TM) segments have therefore tended to regard their helicity as a given parameter and focused principally on their hydrophobicity properties (6, 7). Yet considering the array of residues occurring in TM domains, including those considered classically as helix breakers (i.e., Gly, Ile, Val, Thr, and Pro) in aqueous-based proteins, it appears unlikely that all residues will possess identical helical propensities in the membrane environment. Previous studies of peptide/protein secondary structures in aqueous solution indicate that individual amino acids have distinct conformational preferences (α-helical and β-sheet) that influence protein structure and folding (8–13). However, in membranes, fatty acyl chains of lipid molecules present a hydrophobic environment to embedded proteins, and consequently, protein folding in membranes is subject to different rules versus those governing proteins in the aqueous milieu (14–16). The need for such information has become more crucial, because the genomes of various organisms recently analyzed indicate that large proportions (20–40%) of the genes correspond to membrane proteins.

We have now examined this situation using a set of de novo designed Ala-based peptides. Features of these peptides mimic a prototypical single-spanning membrane domain with respect to length, hydrophobicity, and residue occurrence. An excellent correlation is found between the experimentally measured helicity in the non-polar phase and the occurring frequency of a given residue computed from a data base of native protein TM helices. These findings identify a novel pathway through which the amino acid composition of a membrane protein can assist its accommodation in a hydrophobic environment.

MATERIALS AND METHODS

Peptide Synthesis—Peptides were synthesized by the continuous flow Fmoc solid phase method (17). C termini of peptides were aminated after cleavage from NovaSyn KR 125 resin. Purification of peptides was carried out on a reverse-phase Vydac-C4 semi-preparative HPLC (10 × 250 mm, 300 Å), using a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Purified peptides were characterized by analytical HPLC, amino acid analysis, and mass spectrometry. The aggregation states of peptides were monitored by CD measurements and by size exclusion HPLC, from which it was found that through the concentration range of 5–250 μM, peptides remained monomeric in all experimental media. Concentrations of peptides were determined in triplicate through quantitative amino acid analysis using Ala recovery as the standard and BCA protein assay (enhanced protocol). Peptides were stored as solid powders at −20 °C. To avoid the complexity of synthesizing a multiple Cys-containing peptide, only the central X residue was substituted by Cys, and the other two X residues were replaced by Leu.

Spectroscopic Measurements—CD measurements were performed on a Jasco-720 spectropolarimeter using a 1-mm path length quartz cell at 25 °C. Each spectrum was the average of four scans with buffer background subtracted. Curves reported are based on triplicate measurements; standard deviation is ± 1%. Peptide concentration was typically 30 μM in aqueous buffer and in n-butanol. The aqueous buffer was prepared from 10 mM Tris-HCl, 10 mM NaCl, pH 7.0.

RESULTS AND DISCUSSION

De Novo Peptide Design—The rationale for the design of the peptides used in the present study, of sequence KKAAAXAAAAAWAAXAAKKK-amide (where X indicates one of the 20 commonly occurring amino acids) (17, 18), is given as follows: (i) The hydrophobic segment of peptide is comprised of 19 amino acids, which when folded into an α-helical conformation is of sufficient length to span a phospholipid bilayer (19). (ii) Distributions of the three “guest” residues X have been designed to preserve both angular and longitudinal symmetry around the helix, thereby minimizing any bias from amphipathic character that may arise when X is a polar or charged residue. In addition, triple substitutions of guest residue X in the hydrophobic core serve to amplify the effect of guest replacements, ensuring that the spectroscopic measurements can detect their structural impact. (iii) Ala, the most appropriate background residue as demonstrated by previous studies (20,
Peptide concentrations were typically 30 μM. The aqueous buffer was prepared from 10 mM Tris-HCl, 10 mM NaCl, pH 7.0. Curves obtained only minimum background absorbance at the lower wavelength region (>200 nm) of the CD measurements. Experiments with selected peptides in the above four solvents demonstrated that the CD spectra for a given peptide were essentially superimposable, indicating that the measured ellipticity is a property largely determined by the amino acid sequence of the peptide.

Organic solvents with dielectric constants between pure water and the hydrocarbon interior of biological membranes have been widely used to mimic the non-polar environments of membranes (22–25). To create a quasi-membranous yet homogenous (isotropic) environment and to eliminate the complexities involved in protein/peptide-lipid interfacial interaction (viz. partitioning, electrostatics), peptides were dissolved in n-butanol, a moderate non-polar solvent of dielectric constant 17.8 at 25 °C (26), and their CD spectra were recorded. n-Butanol was chosen as a membrane-mimetic environment on the basis that (i) it effectively dissolved all 20 peptides, and (ii) in comparison to iso-propanol, n-propanol, and iso-butanol, n-butanol produced only minimum background absorbance at the lower wavelength region (<200 nm) of the CD measurements. Experiments with selected peptides in the above four solvents demonstrated that the CD spectra for a given peptide were essentially superimposable, indicating that the measured ellipticity is a property largely determined by the amino acid sequence of the peptide.
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In the preceding turn of the α-helix, permitting such residues to occur in helices buried within a hydrophobic milieu (33–35).

Implications of Residue Helical Propensities for Protein Transmembrane Segments—Several workers have analyzed the membrane inclusion preference of individual amino acids based on known membrane proteins (36–38), analogous to the C.-F. algorithm for globular proteins (8); for example, Jones et al. (38) used a dynamic programming algorithm to provide a membrane topology model from single sequence information. To obtain a membrane-based predictor comparable with the conventional C.-F. Pα parameter, we converted the “log likelihood” parameter of Jones et al. (38) to $P_x(TM) = qi$, where $P_x(TM)$, the preference of an individual amino acid for occurrence in a TM helix, is the ratio of $qi$, the relative frequency of occurrence of the amino acid $i$ in a particular structural class, to $p_i$, the relative frequency of occurrence of the amino acid $i$ in all the sequences in the data set. The resulting values of $P_x(TM)$ derived from the “helix middle” of single-spanning membrane proteins are listed in Table I. There is good correlation ($r = 0.93$ when Pro was excluded) between the $\theta_{222}$ (n-butanol) and the $P_x(TM)$ (Fig. 2B).

Thus, rather than simple identification of high-hydrophobic segments in the primary sequences of membrane proteins, the uncoupling of hydrophobicity from helicity in transmembrane domains allows a clearer delineation as to where helices are highly probable as a function of both protein sequence and environment. The correspondence observed between the experimentally determined helical propensity for individual amino acids and their non-random occurring frequency in protein TM helices suggests that the high frequency of occurrence in membranes of residues such as Leu, Val, Ile, and Phe derives not only from their hydrophobicity but also from their intrinsic propensity to form the α-helical conformation in the non-polar environments of membranes.

REFERENCES


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