(1)Even when the hydrophobicity of the segments is not high enough, they may become transmembrane core, if they include a long noncharged segment. (2) Even when the hydrophobicity of the segments is sufficiently high, they may not become transmembrane core, if they include large number of charges. These assumptions seemed physically sound and actually improved the results of the analysis.

Maturation of transmembrane helix: Transmembrane helices bind with each other through the polar interactions in the native structure.[1] Therefore, some part of a transmembrane helix, become more polar than the core regions, particularly near the ends of helices. In other words, some part of a transmembrane helix has amphiphilicity and become stable in membrane through the formation of the tertiary structure. In this stage, we have taken two factors into account for the determination of the ends of helices. First, the amino acid segments neighboring the core region and having periodicity of about 3.6 residues become helical in membrane. Second, the total length of a transmembrane helix including the core and the extended regions is not so different from the thickness of membrane. The power spectral density of helical periodicity was calculated by a maximum entropy method of Fourier transformation and used for the extension of the transmembrane core. In order to set the limit of the total length, a filter corresponding to the thickness of membrane was multiplied to the sequence of the power spectral density around the transmembrane core. Then, the ends of transmembrane helices were determined by the following equation,

$$(AverageHydrophobicity) + (Filter) \times (HelicalPeriodicity) > (Threshold). \tag{1}$$

This system was applied to the amino acid sequences of membrane proteins whose atomic coordinates are given in protein data bank (pdb): bacteriorhodopsin, photoreaction center and light harvesting complex II. Amino acid sequences of soluble proteins in pdb were also analyzed as reference. The results were satisfactory: All transmembrane helices were predicted correctly and the error in the ends of helices was about only two residues in average. False positive prediction was observed for several soluble proteins. However, the ratio of such examples was smaller than the results by other systems.

The present system is based on the physicochemical mechanism of transmembrane helix formation, i.e. the three-stage model, and may be applied to any amino acid sequence, providing the transmembrane helical regions with accuracy of about two residues.

Reference

[1] S. Mitaku, K. Suzuki, S. Odashima, K. Ikuta, M. Suwa, F. Kukita, M. Ishikawa, and H. Itoh, PROTEINS: Structure, Function, and Genetics, 22 (1995) 350-362.

Prediction of Transmembrane Helical Regions by Three-stage Model

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The formation of transmembrane helices is the first process of the folding of membrane proteins. Because of the hydrophobic environment of membrane, transmembrane helices are usually quite hydrophobic. This fact leads to a simple method for the prediction of transmembrane segments, in which only one parameter, the average hydrophobicity of partial sequences, is used for the determination of such amino acid regions. However, it is pointed that this simple method is not good enough for the next process of the tertiary structure prediction, because not only some helices are missing but also the position of ends is sometimes erroneous in the simple analysis.

The methods for the prediction of transmembrane helical regions should satisfy three conditions: (1) False positive prediction, that is the transmembrane helix in a soluble protein, has to be as rare as possible. (2) The transmembrane helix in a membrane protein should be predicted certainly: True positive prediction should be high and the false negative one should be zero. (3) The end position of transmembrane helices should be as accurate as possible.

We have developed a new method under physicochemical consideration, trying to satisfy all three requirements by assuming three-stage model of transmembrane helix formation: the binding to membrane surface, the formation of transmembrane core region, and the maturation of helix due to the tertiary structure formation in membrane.

Binding to membrane: As suggested from the amino acid sequences of signal peptides and venom peptides such as melittin, the existence of polar groups appears important for the binding to membrane. Therefore, the difference between the maximum and the minimum hydropathy in a window of nine residues was used as a parameter of the binding signal.

Formation of transmembrane core region: The most important interaction for the formation of transmembrane helices is apparently the hydrophobic interaction. Therefore, among the segments that may bind to membrane we have selected segments with the hydrophobicity higher than some threshold value. Complementary assumptions were made in this stage.

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