Position Dependent Amino Acid Propensity in the Transmembrane Region for Topology Prediction of Membrane Proteins.

Koji Kadota kadota@cc.tuat.ac.jp
Takatsugu Hirokawa hirokawa@cc.tuat.ac.jp
Shigeki Mitaku mitaku@cc.tuat.ac.jp

Department of Biotechnology, Tokyo University of Agriculture and Technology
Nakacho 2-24-16, Koganei, Tokyo 184, Japan

Abstract

Amino acid propensity in the central and the end regions of transmembrane helices was compared to that in the loop region, using the data of membrane proteins whose 3D-structure is already known. Polar residues showed characteristic distribution in transmembrane helical regions, depending on the type of amino acids. The polar residues with large side chains (Lys, Arg, His, Glu and Gln) were located in the end region more than the small polar residues (Asp, Asn), suggesting that the side chains having the detergent-like characteristics stabilize the end of transmembrane helices. The positively charged residues were more preferable in the inside end region than in the outside. However, this tendency was significant for the proteins in the inner membrane of mitochondria from mitochondrial DNA, in contrast to the symmetrical distribution of the large polar residues for the membrane proteins from genomic DNA. The physical factors for the determination of membrane protein topology was discussed on the position dependent propensity.

1 Introduction

The propensity of amino acids has good correlation to the structural characteristics of the polypeptide segments in membrane proteins. The most significant correlation in membrane proteins is that the transmembrane helical regions are mainly comprised of hydrophobic residues, while the loop segments have many polar residues. The transmembrane regions are usually predicted by using this feature of the amino acid propensity. The topology of membrane proteins has correlation with the distribution of positively charged residues, as first proposed by Heijne et al[1]. They pointed out that the loop segments of membrane proteins in the inside of a cell have more positively charged residues than the outside.

Recently, it was reported that the end region of transmembrane helices is not so hydrophobic and contains many polar residues particular large polar residues[2]. These characteristics of the amino acid distribution in membrane proteins raise a question about the mechanism of the topology of membrane proteins: Is the topology of membrane proteins really determined by the positively charged residues in the loop segments? In order to reveal the contribution of polar residues in the end regions to the topology determination of membrane proteins, we have calculated the propensity of amino acids in five regions around transmembrane helices, using the membrane proteins whose molecular structure is already known.

2 Method

We have used the amino acid sequence of 19 polypeptides from bacterial photoreaction center[3], bacteriorhodopsin[4], light harvesting complex[5] and bovine cytochrome c oxidase[6] whose molecular structure is known. The amino acid sequence around a transmembrane helix was divided into five areas: central region of 13 residues long, inner and outer end regions of a transmembrane helix, and inner and outer loop regions of 10 residues next to the transmembrane. The propensity was calculated by normalizing the number of each type of amino acids by the total number of residues.

3 Results and Discussion

The average propensity of amino acids in the five regions around transmembrane helices of 19 polypeptides clearly indicated that the end region contain as many polar residues as the loop region, although the propensity of hydrophobic residues are much larger than that in loop region. Significant difference of propensity is observed between the inner and the outer end regions for several kinds of polar residues. Positively charged residues are more abundant in the inside of a cell or organella than in the outside. Very similar distribution was observed for glutamic acid and glutamine, whereas aspartic acid and aspargine showed symmetric distribution. This characteristic distribution of polar residues in the end regions indicates that the so-called "positive inside rule" is also valid in the transmembrane regions. Because the end region of transmembrane helices is considered to interact strongly with the polar head groups of lipid bilayer, the concept that the topology is determined by the partitioning of polar residues according the membrane potential should be changed partly.

The proteins used in this work may be classified into two categories: the proteins in cytochrome c oxidase which are synthesized by genomic DNA and all other proteins. The latter category is characterized by the fact that the position of biosynthesis of the protein is inserted from the inside of a cell or organella, while the proteins of the former category are synthesized in the outside of the compartment. The propensity of the large polar residues did not show the unbalance between the inside and the outside of the compartment. This fact cannot be understood by naive mechanism of the "positive inside rule" for the membrane protein topology. Consequently, there have to be two factors for the topology of membrane proteins: the partitioning of positive charges according the membrane potential and the binding of polar residues in the end region of transmembrane helices with the polar head group of lipid molecules.

References

[1] G. von Heijne and Y. Gavel, "Topogenic signals in integral membrane proteins," *Eur. J. Biochem.*, 174:671-678, 1988.

[2] F. A. Samatey, C. Xu, J. L. Popot, "On the distribution of amino acid residues in transmembrane alpha-helix bundles," *Proc. Natl. Acad. Sci. U.S.A.*, 92:4577-4581, 1995.

[3] D. C. Rees, H. Komiya and T. O. Yeates, "The bacterial photosynthetic reaction center as a model for membrane proteins," *Annu. Rev. Biochem.*, 58:607-633, 1989.

[4] N. Grigorieff, T. A. Ceska, K. H. Downing, J. M. Baldwin and R. Henderson, "Electron-crystallopraphic Refinement of the Structure of Bacteriorhodopsin," *J. Mol. Biol.*, 259:393-421, 1996.

[5] G. McDermott, S. M. prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell and N. W. Isaacs, "Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria," *Nature*, 374:517-521, 1995.

[6] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono and S. Yoshikawa, "The Whole Structure of the 13-Subunit Oxidized Cytochrome c Oxidase at 2.8 Angstroms," *Science*, 272:1136-1144, 1996.