The role of Pro/Hyp-kinks in determining the transmembrane helix length and gating mechanism of a [Leu]zervamicin channel

Juan A. Ballesteros and Harel Weinstein
Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, New York 10029 USA

INTRODUCTION

[Leu]zervamicin ([L]-ZVM) is a membrane-channel forming polypeptide of 16 residues rich in Pro and Hyp residues (1). The crystal structure in methanol/water solution shows an assembly of amphiphilic helices that resembles a channel structure. We discuss the putative role of the Pro/Hyp residues in lengthening the helix to membrane spanning length, and in producing a gating mechanism for the channel.

HELIX LENGTH

Although many factors, such as lipid heterogeneity, could explain how a 16-aa helix may span the lipid bilayer, we suggest that intrinsic molecular interactions could favor an elongation of the helix to a span longer than that of an ideal α-helix. This is illustrated by a superposition of [L]-ZVM and an ideal $(Ala)_{16}$ α-helix according to minimal rms for the first nine residues. As shown in Fig. 1 *A* the ideal helix is clearly shorter. Moreover, alignment of an ideal $(Ala)_{25}$ α-helix next to the 16-aa long [L]-ZVM (Fig. 1 *B*) shows that the later is equivalent in span to a 18–19 amino acid (AA) long regular α-helix. This observation may have broader implications for methods predicting membrane spanning α-helices because they generally assume a requirement for ~20 AA. We have identified two major determinants, described below, for the larger span of the kinked helices: the intrinsic conformation of kink regions, and the extensive hydrogen bonding between backbone groups and water molecules located at the end of the transmembranal portion, e.g., at the "channel mouth."

Intrinsic conformation of Pro/Hyp kink-regions

There is a large diversity of *phi,psi* angles found in Pro-kink regions, without a pattern that is fully predictable as yet (2). However, it is clear from distance matrix analysis and superposition of several of these regions that a kink implies larger distances between backbone groups (3, 4). This effect agrees with the observed loss of the $C = O_{i-3} \ldots HN_{i+1}$ H-bond around the *i*th imide amino acids. Consequently, the kink can be considered to increase locally the helical pitch compared to an ideal α-helix. Whether the overall effect is to increase or decrease the helix length seems to depend on the Pro/Hyp position within the helix. In leu-zervamicin there are three Pro/Hyp in the COOH-terminal half and their effects add up. In citrate synthase the kink occurs in the middle of a 30-AA long helix and the long-range deviation induced on the helix axis dominates and slightly decreases the helix length.

H-bond pattern at a "channel mouth"

The extensive H-bonding between backbone groups and water molecules opens up the interior polar face of the helix, thus increasing the effect of the Hyp on the increased helix pitch, discussed above. In soluble proteins, the faces of α-helices exposed to water are usually exhibiting a convex bend due to double H-bonding (3): C = O . . HN/C = O . . . HOH. For [L]-ZVM this effect would be accompanied by the Hyp-kink in two ways: first, the direction of the bend is convex in both cases, thus increasing rather than balancing the trend. Second, Hyp-kinks induce unusually exposed $C = O_{i-3}$ and $C = O_{i-4}$ groups that facilitate H-bonding to water (4). Such a hydration scheme was observed recently in an analogue of zervamicin (5). There is a strong effect of these two factors on the H-bond pattern in the helical backbone: in the few cases where the backbone maintains H-bonding, it is more often $(i, i + 3)$ than $(i, i + 4)$. A two-dimensional representation of the structure in a linear distance plot (see reference 6 for definition) shows the combined effect of these factors in the increase of the Cα distance sum to 25.1 Å from the characteristic α-helix value of 20.7 Å.

GATING MECHANISM

The Hyp_{10}-kinks seem to close physically the channel formed by three of the molecules of [L]-ZVM (1). This

0006-3495/92/04/110/02 $2.00

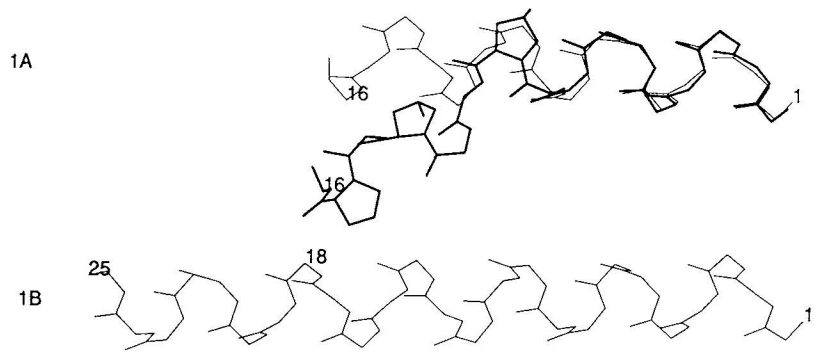

FIGURE 1 (*A*) Comparison of the [Leu]-zervamicin helix (*thicker line*) to an ideal $(Ala)_{16}$ α-helix; (*B*) and alignment of the [Leu]-zervamicin helix with an ideal $(Ala)_{25}$ α-helix. For clarity, only the Pro and Hyp residues are shown on the helix backbones.

arrangement suggests how they could function in the gating. A mechanism of structural rearrangement would involve the protonation, or cation binding, of one the $C = O_{i-3}$ or $C = O_{i-4}$ that are exposed in such a structure and do not participate in H-bonds. The structural rearrangement that would occur as a result of the electrostatic interaction could induce a bending of each helix, as well as a twisting of the helix faces before and after the kink region. Such a mechanism could be envisioned for the [L]-ZVM structure.

We are presenting elsewhere in this meeting the proposed role of transmembranal Pro-kinks in the signaling process of G-protein coupled receptors. Interestingly, the present inferences from the high resolution channel structure offer a model for the involvement of Pro-kink regions in the transmembrane helices of channels and G-protein coupled receptors in the activation mechanism or their biological functions. These processes can be simulated computationally on the strength of the structural data and can be used in theoretical analysis of the mechanisms.

The work was supported in part by grants from the National Institute on Drug Abuse (DA-06620), a Research Scientist Award (DA-00060) (H. Weinstein), and by a Fulbright/Ministry of Education and Science, Spain (MEC) scholarship (J. A. Ballesteros).

REFERENCES

1. Karle, I. L., J. L. Flippen-Anderson, S. Adarwalla, and P. Balaram. 1991. Crystal Structure of [leu]zervamicin, a membrane ion channel peptide: implications for gating mechanisms. *Proc. Natl. Acad. Sci. USA.* 88:5307–5311.
2. Piela, L., G. Nemethy, and H. A. Scheraga. 1987. Pro-induced constraints in α-helices. *Biopolymers.* 26:1587–1600.
3. Barlow, D. J., and J. M. Thornton. 1988. Helix geometry in proteins. *J. Mol. Biol.* 203:601–619.
4. Woolfson, D. N., and D. H. Williams. 1990. The influence of Pro residues on α-helical structure. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 277:185–188.
5. Karle, I. L., J. L. Flippen-Anderson, K. Uma, and P. Balaram. 1990. Hydration of helix backbones of hydrophobic peptides. *In* Peptides; Proc. of the 11th American Peptide Symposium. J. E. Riviero, and G. R. Marshall, editors. ESCOM Science Publishers B. V. Leiden, Netherlands. 544–547.
6. Liebman, M. N., C. A. Venanzi, and H. Weinstein. 1985. Structural analysis of carboxypeptidase A and its complexes with inhibitors as a basis for modeling enzyme recognition and specificity. *Biopolymers.* 24:1721–1758.