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FOR THE RECORD

Prediction of membrane-spanning β-strands and its application to maltoporin

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Integral membrane proteins often exhibit long hydrophobic stretches in their amino acid sequence that indicate membrane-spanning α-helices and therefore provide useful topological information. Accordingly, the algorithm of Kyte and Doolittle (1982), which determines the mean hydrophobicity within a sliding window, is routinely applied to new membrane protein sequences to identify transmembrane helices. There is, however, another class of membrane proteins, exemplified by bacterial porins, that are folded predominantly into β-pleated sheet structures and show an overall hydrophobicity similar to soluble proteins.

The recent crystal structure elucidations of matrix porin and phosphoporin from *Escherichia coli* (Cowan et al., 1992) and the unrelated porin from *R. capsulatus* (Weiss & Schulz, 1992) prompted us to reevaluate existing prediction methods for transmembrane β-strands. All of these porins are folded into 16-stranded antiparallel β-barrels (Kinemage 1) that are associated to homotrimers. The parts of the β-strands that are in contact with the hydrophobic core of the membrane are 7–9 residues in length with every second (i.e., external) residue being hydrophobic (Kinemage 2). The intervening, internal residues show no clear pattern and thus have no predictive value. These residues are found to be hydrophilic when being part of the pore lumen or hydrophobic when buried by the internal loop L3 (Cowan et al., 1992).

The mean hydrophobicity of one side of a putative β-strand (β-side-hydrophobicity, H_s) is obtained by averaging the hydrophobic indices h (Eisenberg et al., 1984) of every second residue within a sliding window (Vogel & Jähnig, 1986). A window width of 4 was used so that $H_s(i) = \frac{1}{4}[h(i-2) + h(i) + h(i+2) + h(i+4)]$. To improve the signal-to-noise ratio, the conspicuous accumulation of aromatic residues in flanking positions (Kinemage 3; Weiss et al., 1991; Cowan et al., 1992) was exploited. A modified β-side-hydrophobicity, H_s', was defined by assigning an arbitrarily increased "hydrophobicity" index of 1.6 to aromatic residues (instead of the consensus indices ranging from −0.4 to 1.19 for these residues) when encountered in positions $i-2$ or $i+4$. Significantly increased occurrence of aromatic residues in flanking positions has also been observed for transmembranous α-helices (Landolt-Marticorena et al., 1993; Sipos & van Heijne, 1993) and may turn out to be a general phenomenon.

The method was validated on the known porin structures. In each case, 15 of the ~19 highest peaks correctly identify membrane-spanning β-strands. Figure 1A shows the H_s' plot of phosphoporin. The pairwise appearance of many peaks is evident. Each pair corresponds to two β-strands connected by a short turn on the smooth side of the barrel. The method is remarkably precise: the X-ray structure shows that the segments corresponding to peaks 7–16 (i.e., the β-strands exposed to the membrane) are positioned at the same height along the membrane normal within ±1 Å.

Although the maltose-selective maltoporin (LamB) shows almost no sequence homology to the nonspecific porins from *E. coli* and is considerably larger, it also contains a high amount of β-structure (Vogel & Jähnig, 1986) and has a comparable shape at low resolution (Lepault et al., 1988). A similar gross structure has also been deduced from the analysis of X-ray diffraction patterns from single crystals (Pauptit et al., 1991). The H_s' plot of maltoporin (Fig. 1B) allows identification of 15 putative β-strands. Several overpredictions can be ruled out by negative inference (high sequence variability or high turn probability). The profile between peaks 3 and 6 is reminiscent of the corresponding region for phosphoporin

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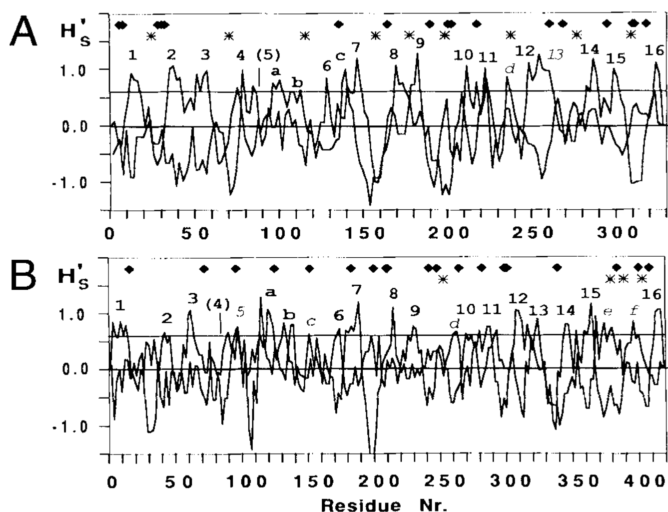

Fig. 1. Modified β-side-hydrophobicity plots, H'_s. Values for even and odd numbered residues are plotted as two separate curves. Above the graphs segments with the following properties are marked: ◆, turn probability above 1.8 (Gribskov et al., 1986); *, regions where gaps had to be introduced in the alignment of related sequences (Jeanteur et al., 1991; Werts et al., 1992). Peaks close to these regions are rejected by negative inference and are labeled with italic characters. **A:** Phosphoporin (peaks labeled by numbers represent transmembrane β-strands as revealed by the X-ray structure analysis). **B:** Maltoporin (numbered peaks represent putative transmembrane β-strands).

(Fig. 1A), and therefore a similar organization (a pair of β-strands followed by a long internal loop) is assumed and the presence of an additional strand (β4) is inferred.

It is reasonable to assume that the 16 strands form an antiparallel β-barrel with a strand tilt similar to the non-specific porins. The corresponding topology is depicted in Figure 2, where transmembrane β-strands have been positioned according to the H'_s plot and are extended at both ends as long as they obey the characteristic pattern. The length of the segments varies from 5 to 13 with all of

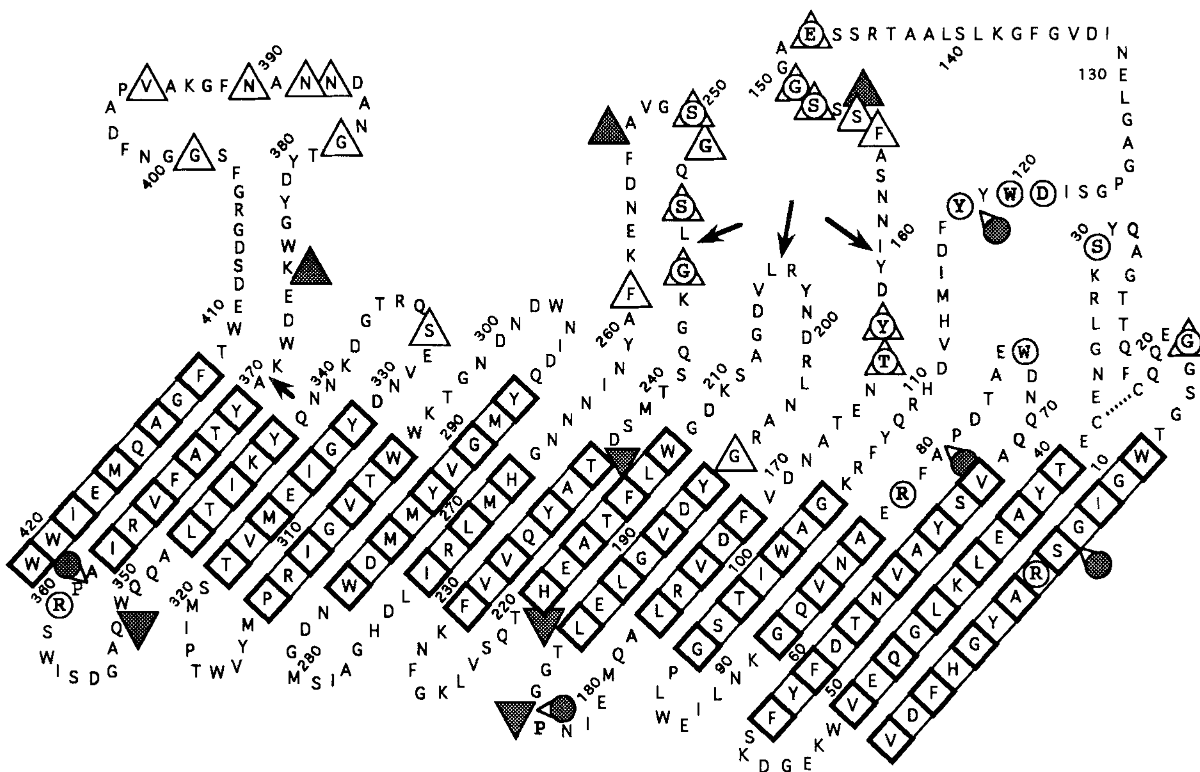

Fig. 2. Predicted topology of maltoporin. The view is onto the outside of the 16-stranded antiparallel β-barrel. Symbols are as follows: □, residues in transmembrane β-strands, with a bold outline if their side chains are external; →, proteolytic cleavage sites (Schenkman et al., 1984); △, sites of point mutations that result in impairment of λ-phage or antibody binding (Gehring et al. [1987] and references therein); ▲, a foreign epitope inserted at these sites is detected in vivo by appropriate antibodies (Charbit et al., 1986, 1991); ▼, epitope inserted at these sites is detected only on solubilized protein and not in intact cells (Molla et al., 1989). Circles indicate mutations that affect sugar binding or translocation (see Francis et al. [1991] and references therein; Benz et al., 1992): ○, sites of point mutations; ●, sites of small peptide insertions.

the external residues (except Q48) being hydrophobic (including threonine) or aromatic. Flanking aromatic residues are observed in many cases.

Comparison of the topology schemes of maltoporin and the nonspecific *E. coli* porins reveals that the local homology detected by Nikaido and Wu (1984) is reproduced although the overall sequence identity is very low (15%). The topology model is consistent with the vast body of relevant biochemical and genetic data but is significantly different from the current topology scheme (Charbit et al., 1991). Figure 2 shows that sites found experimentally to be exposed at the cell surface are all located at the same side of the predicted barrel, whereas almost all sites exposed to the periplasm are found at the opposite side. Mutations that affect starch binding cluster in adjacent long loops at the extracellular side and it is conceivable that they are part of an extended maltodextrin binding site at the mouth of the pore. The imminent X-ray structure (Stauffer et al., 1990) will put the proposed topology to a stringent test.

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