New Concepts

Flexible Regions within the Membrane-Embedded Portions of Polytopic Membrane Proteins

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ABSTRACT: The conventional view of the structure of the membrane-embedded regions of integral membrane proteins is that they are in contact with lipids that interact with the hydrophobic surfaces of the polypeptide, and therefore have intrinsically rigid α-helical structures. Here, we briefly review the evidence that in the case of integral membrane proteins with many membrane spans (including membrane transporters and channels), some membrane peptide segments are more or less completely shielded from the lipid bilayer by other membrane polypeptide portions. These portions do not need to have α-helical structures and are likely to be much more flexible than typical membrane-spanning helices. The ability of the band 3 anion exchanger to accommodate anionic substrates of different sizes, geometries, and charge distributions suggests the presence of flexible regions in the active center of this protein. These flexible substructures may have important functional roles in membrane proteins, particularly in the mechanisms of membrane transporters and channels.

Band 3 protein is an obligatory exchange transporter that functions primarily to carry out the exchange of chloride and bicarbonate anions across the red cell membrane (*1*). Band 3 mediates the anion exchange of not only chloride and bicarbonate but also sulfate, phosphate, phosphoenolpyruvate (*2*), and pyridoxal 5-phosphate (*3*) by the same ping-pong mechanism (*1*). Krupka (*4*) has pointed out that although chloride and bicarbonate are equally good substrates for band 3 (form equally tight-binding transition state complexes), the spherical chloride ion and flat trigonal planar bicarbonate ion differ in both shape and charge distribution. Thus, the geometry of the sites in the protein that chelate the substrate in the transition state complexes of the two anions is also likely to differ. As illustrated in Figure 1, the van der Waals radii of band 3 substrate anions range from 3.6 to >10 Å, and they also have different charge distributions and charge geometries. An essential requirement for the anion exchange function of band 3 is that the active center should be able to adjust its structure to accommodate each of these anions, which implies that the protein has substantial conformational flexibility in the polypeptide portions which form the active center.

The consensus view of transmembrane peptide (TM) segments is that TM portions can stably reside in the membrane lipid bilayer by forming α-helices, since an α-helical secondary structure allows the main chain hydrogen-bonding potential to be satisfied internally within the polypeptide chain. However, α-helices are intrinsically rigid, rod-like structures. If the active center of band 3 protein consisted of rigid TM segments, the transporter transition state could not adapt to anions of different sizes, geometries, and charge distributions. The results of proteolytic cleavage studies on alkali-denatured band 3 are consistent with the presence of nonhelical, potentially flexible regions in the protein (*5*).

Only in the case when a TM span is exposed to the lipid bilayer do the energetics dictate that a TM segment must be folded into an α-helix. Integral peptide segments are usually regarded as being surrounded by lipids that interact with the hydrophobic surfaces of the polypeptide. While it is clear that the hydrophobic transmembrane peptide segments of single-spanning bitopic membrane proteins are always surrounded by lipids, is this also the case for the integral portions of multispanning polytopic membrane proteins? Some portions of the polypeptide in these latter proteins could reside within the membrane lipid bilayer without being associated with lipids; instead, they could be stabilized by interactions with other integral peptide regions of the molecule, and these interactions could be hydrophobic or polar in nature. It is well-known that typical hydrophilic proteins such as hemoglobin can contain a hydrophobic polypeptide core located within the protein interior that is shielded from the hydrophilic environment by other peptide segments of the molecule (*6*). However, discussions about the structure of membrane

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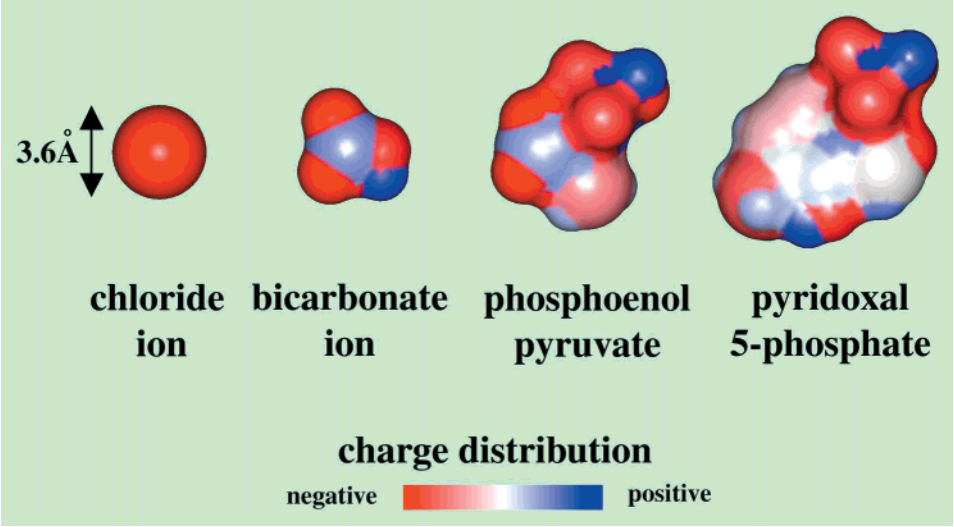

FIGURE 1: van der Waals radii of chloride, bicarbonate, phosphoenolpyruvate, and pyridoxal 5-phosphate, substrate anions for the anion exchanger. The surface charge distributions of individual anions are colored from red to blue as indicated.

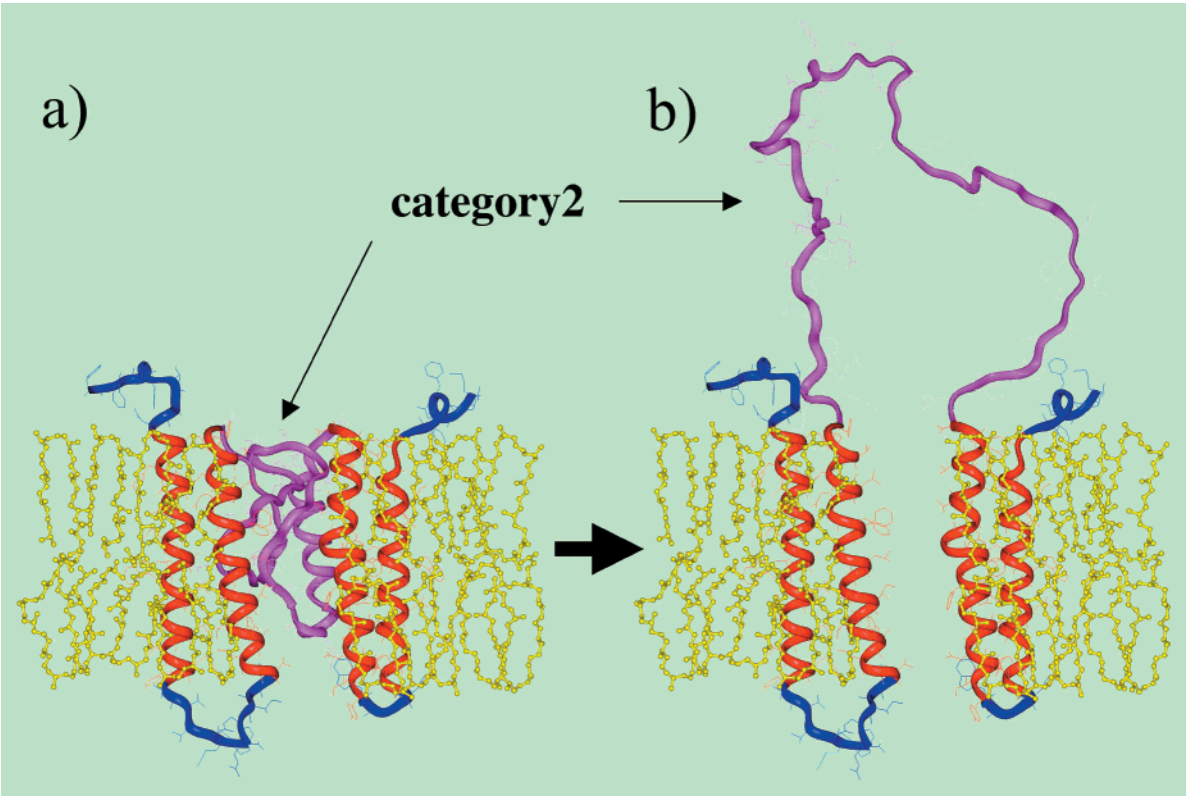

FIGURE 2: Schematic drawing of an integral membrane polypeptide region shielded from boundary lipids. Some integral membrane peptide regions can form substructures that are surrounded by other membrane polypeptide segments, and are more or less completely shielded from the bilayer lipids. One possible substructure is shown in purple in cartoon a. These substructures are susceptible to alkali denaturation of membranes as indicated in panel b, and have been described as "category 2" structures (*16*). Once exposed, these regions become susceptible to protease treatment of the membranes.

proteins do not usually consider the analogous situation of integral polypeptide regions shielded from the membrane lipid bilayer. These regions do not need to have α-helical structure or other ordered secondary structure; they could have hydrophobic and/or polar elements, and their structure would be determined by the amino acid sequence characteristics of the neighboring peptide segments, rather than the lipid bilayer.

Several recent lines of evidence support the existence of membrane peptide segments shielded from the membrane lipid bilayer by the other membrane polypeptide portions in polytopic membrane proteins. Namely, (i) some polypeptide portions anticipated to have an integral membrane disposition are extruded from the membrane bilayer upon partial denaturation of the red cell band 3 protein (*5*) (as indicated schematically in Figure 2). These extruded regions are susceptible to proteolytic cleavage (*5*). (ii) During biosynthesis in the endoplasmic reticulum, the proper assembly of some integral membrane peptide segments requires interactions with other integral membrane polypeptide regions, and

these segments only insert correctly in the membrane when the appropriate peptide−peptide interactions occur (*7*−*10*). (iii) The high-resolution structures of aquaporin (*11*) and the KcsA K^+ channel (*12*) show regions located within the interior of the lipid bilayer that are completely surrounded by other polypeptide segments and are not exposed to lipids. (iv) The three-dimensional structure of NhaA indicates a bundle of three transmembrane helices that includes one helix for which density is not continuous through the lipid bilayer (*13*). Similar supportive evidence has been observed in the cases of GlpF (*14*). (v) The recently reported X-ray structure of the ClC chloride channel (*15*) also shows several integral polypeptide regions that are not exposed to lipids. Helix−loop−helix motifs and other nonhelical regions that do not penetrate through the membrane serve to position loops originating from different regions of the polypeptide chain in the center of the membrane, where they form the selectivity filter for chloride. Interestingly, the structure showed that the aqueous channel leading from the extracellular surface to the selectivity filter was blocked by a glutamate residue. The authors suggest that movement of the glutamate residue, and perhaps other regions of the molecule, would have to occur to allow chloride conduction to take place, implying that the structure of the polypeptide in this region is probably flexible.

The above evidence indicates that hydrophobic membrane peptide portions are not necessarily in contact with lipids in the membrane lipid bilayer. Instead, some integral membrane peptide portions form substructures that are surrounded by other membrane polypeptide segments (illustrated in Figure 2) and are more or less completely shielded from the bilayer lipids. These substructures, which have been described as "category 2" regions (*16*), are susceptible to alkali denaturation as shown schematically in Figure 2. They need not take up a helical structure and have the potential to be much more flexible than typical membrane-spanning helices, which are rigid and highly constrained by the boundaries of the phospholipid bilayer. Although these regions are not necessarily always flexible (there is no evidence indicating their flexibility in the case of the KcsA, aquaporin, or GlpF channels), the alkali denaturation studies on band 3 protein (*5*, *16*) and the structure of the ClC channel (*15*) suggest flexible regions can be present within multispanning membrane proteins. Located within assemblies of rigid transmembrane helices, these flexible substructures are likely to have important functional roles in membrane proteins and, in particular, in the mechanisms of membrane transporters and channels. We suggest that it is the presence of these flexible regions that gives the active center of the band 3 anion exchanger the structural plasticity that allows it to carry out the obligatory exchange transport of a wide variety of anionic substrates.

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