Reports

Infrared Spectrum of the Purple Membrane: Clue to a Proton Conduction Mechanism?

Abstract. *The infrared spectrum of the purple membrane of* Halobacterium halobium *has amide I and amide A frequencies that are anomalously high for standard α-helical structures. Normal mode calculations indicate that these and other unusual features of the spectrum can be attributed to α_{II}-helices. Such structures suggest that the helix backbone may provide the framework through which proton transport takes place.*

Studies of the infrared spectrum of the purple membrane of *Halobacterium halobium*, in both the dried (*1*) and wet (*2*) states, show anomalously high amide I (essentially C=O stretching) and amide A (N–H stretching) frequencies. It was suggested (*1, 2*) that this might be due to a distorted conformation of the α-helices in the membrane protein bacteriorhodopsin. An analysis of the normal vibrations of the α-helix (*3*) provides a structural basis for these unusual frequencies, and this in turn suggests a possible mechanism for the proton transport associated with the protein.

X-ray diffraction studies of the purple membrane (*4*) show spacings of 1.5 and 5 Å perpendicular and about 10 Å parallel to the membrane surface, indicative of the presence of α-helices with axes perpendicular to the membrane. Such a structure was confirmed by electron diffraction studies (*5*), which revealed that the molecule consists of seven rods, each about 40 Å long and essentially spanning the membrane. Three of these are almost perpendicular to the plane of the membrane, while the other four are tilted 10° to 20° from this orientation, consistent with a left-handed supercoil expected for a right-handed α-helix.

A dichroic infrared study of oriented dried purple membranes (*1*) shows the amide I and amide A bands polarized essentially perpendicular to the membrane, strongly supporting assignment of these modes to α-helical structures. It was, however, noted that the frequency of the observed amide I component parallel to the helix axis (A species), 1667 cm^{-1}, is higher than that typical for α-helical polypeptides and proteins ($\sim 1655 \pm 3$ cm^{-1}) (*6*). This was also the case for membranes in the wet state (*2*). Furthermore, the 1660 cm^{-1} frequency for the helical mode polarized perpendicular to the axis (E_1 species) is about 7 cm^{-1} lower than that for the A species mode, whereas normally the E_1 mode is a few wave numbers higher than the A mode (*6*). It was also observed (*1*) that the frequency of the amide A band is about 10 cm^{-1} higher than is typical. However, the amide II frequency (N–H in-plane bend plus C–N stretch) is essentially the same as that found in α-helical polypeptides (*6*).

It is of interest to know whether these results indicate a conformation different from that of the typical α-helix. The amide I frequency of the left-handed α-helix of poly(β-benzyl-L-aspartate), 1665 cm^{-1} (*7*), is higher than normal, but there is no reason to believe that the helices of bacteriorhodopsin are other than right-handed. It might be thought that supercoiling could affect the amide I frequency, but the dichroic infrared spectra of both porcupine quill and a helix-rich fragment of a low-sulfur wool protein have normal amide I frequencies although x-ray studies show that both exist as coiled-coil structures (*8*). Other structural variations must therefore be considered.

As part of a program to develop detailed vibrational analyses of peptides, polypeptides, and proteins (*9*), we calculated the normal modes of the α-helix of poly(L-alanine) (*3*). This was done not only for the standard structure (referred to as α_I), derived from a fiber x-ray diffraction pattern through a least-squares refinement (*10*), but also for a structure (referred to as α_{II}) whose NC^{α}, $C^{\alpha}C$ torsion angles (ϕ,ψ) are consistent with the same helical parameters n (residues per turn) and h (rise per residue) (*11*). The α_{II} type of structure has been observed in some proteins (*12*) and postulated for poly(L-alanine) in hexafluoroisopropanol (*13*). It occupies a region whose conformational energy is comparable to, or perhaps lower than (*14*), that of α_I. These two structures, which are illustrated in Fig. 1, differ in that the plane of the peptide group in α_I is essentially parallel to the helix axis, whereas in α_{II} it is tilted with the N–H bond pointing inward toward the axis. This difference has two effects on the normal modes. First, the C=O···H–N hydrogen

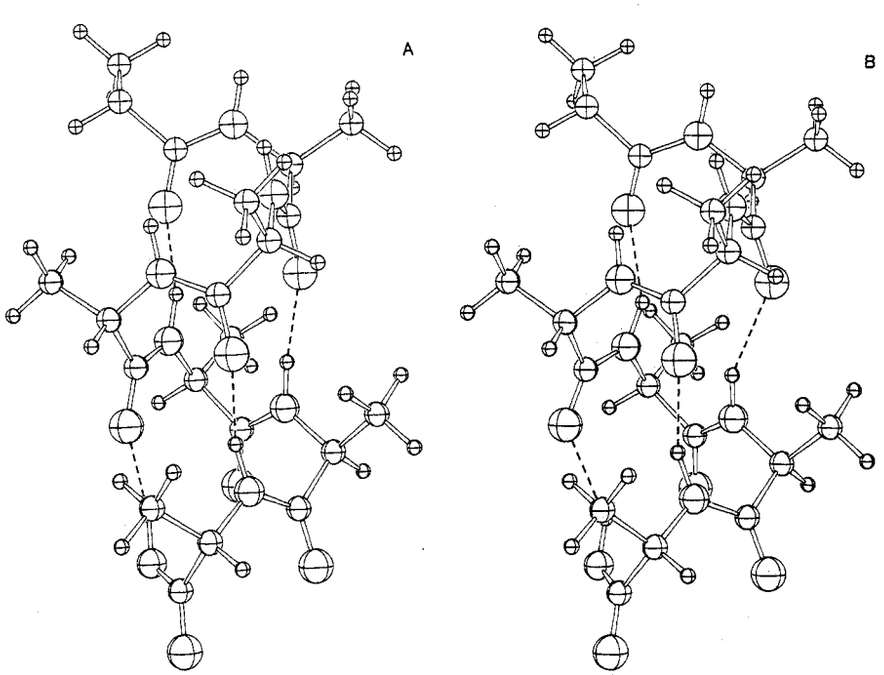

Fig. 1. α-Helical structures of poly(L-alanine): (A) α_I-helix and (B) α_{II}-helix.

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bond is slightly longer in α_{II} (N···O distance = 3.00 Å) than in α_I (N···O = 2.86 Å); this results in a slight weakening of the hydrogen bond and therefore should be accompanied by an increase in the C=O and N–H stretching force constants. The force constants for C=O, H···O, and N–H were altered in proportion to the change in the N···O distance from β-poly(L-alanine) (N···O = 2.73 Å) (*15*), and this led to increases of 4 cm^{-1} in the amide I and 10 cm^{-1} in the amide A frequencies (*3*). Second, and more important, the structural differences result in different transition dipole coupling effects (*16*) for the two helices. For α_I the calculated amide I frequencies (including transition dipole coupling) are 1659 (A) and 1656 (E_1) cm^{-1}; for α_{II} they are 1667 (A) and 1659 (E_1) cm^{-1} (*3*). For the amide II modes the values are α_I, 1517 (A) and 1537 (E_1) cm^{-1}; α_{II}, 1513 (A) and 1538 (E_1) cm^{-1} (*3*).

The agreement between these predictions and observations strongly suggest that the α-helices in bacteriorhodopsin are of the α_{II} type. (i) The amide I and amide A frequencies are predicted to be significantly higher for the α_{II}-helix, and the calculated values are in good agreement with the observed bands for the purple membrane. (ii) The difference between the amide I frequencies for the A and E_1 species is predicted to increase from α_I to α_{II}, consistent with the spectral observations (*1*). [The low frequency of the amide I perpendicular component is not likely to be due to the presence of unordered conformations (*1*), since the bacteriorhodopsin structure is 70 percent (*17*) to 80 percent (*5*) α-helical.] (iii) The strong component amide II mode, the E_1 species, is predicted to remain relatively constant in frequency from α_I to α_{II}, and this is what is found (*1, 2*). (iv) A consequence of the increased peptide group tilt in the α_{II} structure is that the intensity ratio of amide I (E_1) to amide II (E_1) is expected to increase from α_I to α_{II}; this is a result of the larger perpendicular component of the C=O stretching transition moment for the outward projecting C=O group, whereas the perpendicular component of amide II should hardly change. For α_I this ratio is about 0.7 (*18*); for the purple membrane it is about 1.0 (*1*). We believe these results indicate that the α-helical segments of bacteriorhodopsin have the α_{II} structure.

Such an assignment raises two questions: (i) what factors cause a preference for the relatively unusual α_{II} structure in bacteriorhodopsin, and (ii) could this structure be associated with the vectorial proton translocation properties of this protein (*19*)? With respect to (i), the high concentration of serine and threonine residues in the interior of the protein (*20*) may induce the small conformational change in which the C=O groups of the helix are tilted outward to permit the formation of (even weak) hydrogen bonds with the –OH groups of these side chains. With respect to (ii), it is necessary to recognize some additional structural characteristics of the α_{II} type of helix. First, for the same n and h a range of such structures is possible, depending on the (ϕ,ψ) of α_I (*3*). The α_{II} structures can vary from (ϕ,ψ) = (−96°,−16°) when α_I has a "standard" geometry (*14*) to (−70°,−36°) if α_I has the x-ray refined geometry (*10*). [Values of (ϕ,ψ) = (−65°, −40°) for the proposed structure of poly(L-alanine) in hexafluoroisopropanol (*13*) were based mainly on model building.] Second, C=O···H–N hydrogen bonds change from relatively linear and strong in α_I-helices, through bent bonds as ϕ decreases, to helices in which hydrogen bonds cannot be made when ϕ is at its lowest value (*12, 13*). Finally, the distance between adjacent NH hydrogens along the helix decreases from 2.79 Å (*10*) for α_I to 2.53 Å for α_{II}. This distance also depends on (ϕ,ψ) for α_{II}; for (−80°,−28°) it is 2.36 Å and for (−96°,−16°) it is 2.10 Å. Thus it is possible to have α_{II}-helices of the same helical parameters and comparable energies (*14*) in which NH hydrogens are almost in contact [the contact distance based on the observed mean van der Waals radius of H would be 2.4 Å (*6*)] and make bent or poor hydrogen bonds with CO oxygens.

These structural features of the α_{II}-helix raise the possibility that the helical array of NH hydrogens may constitute the framework through which proton transport takes place. Recent models for proton transport and "pumps" (*21*) are based on hydrogen-bonded chains consisting of side chain residues that are either in contact with one another or capable of moving so as to provide a proton hopping channel. Such molecular mechanisms have problems, and it may be worthwhile to consider the properties of the α-helix backbone itself as a proton conductor. This structure provides a relatively reproducible framework for transport, not subject to variations in side chain composition and conformation. In addition, it is somewhat flexible, since small changes in local structure are possible while the overall helical parameters remain constant. It is vectorial in nature and has a large static electric dipole moment (*22*), whose net value for bacteriorhodopsin points from the inside to the outside of the membrane. The electric field of the α-helix can lead to facilitated proton transfer (*23*), and it may be possible that the retinal on lysine 216 (*24*) acts as a "gate" that initiates the movement of a proton along helix G (*17*), aided by the dipole moment of this helix and the close contact of NH hydrogens in the α_{II} structure (*25*). The unusual structural characteristics of the bacteriorhodopsin α-helices suggest that this hypothesis is worth examining in greater depth.

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26. This is paper 14 in a series in which (*9*) is paper 13. We thank D. M. Engelman, R. Henderson, and B. Honig for helpful discussions. Supported by grants PCM79-21652 and DMR78-00753 from the National Science Foundation.

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