Infrared Dichroism of Amide I and Amide II Modes of α_I- and α_{II}-Helix Segments in Membrane Proteins

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Polarized infrared spectra provide a potentially powerful tool for determining the orientation of α-helical segments of membrane proteins. For example, this technique has been applied extensively to study such orientation in the bacteriorhodopsin (bR) protein of the purple membrane (Rothschild and Clark, 1979a; Nabedryk et al., 1985; Earnest et al., 1986; Nabedryk and Breton, 1986; Breton and Nabedryk, 1989; Fahmy et al., 1989).

It has now been unambiguously established by electron diffraction that bR contains seven α-helical segments and no β-sheet structure (Henderson et al., 1990). Attention has therefore been increasingly directed to trying to understand how unusual spectroscopic features of bR might be related to conformational details of the α-helices (Glaeser et al., 1991), which are not yet revealed by the diffraction data. The observation of anomalous amide I frequencies in the infrared spectrum (Rothschild and Clark, 1979a,b) led to the suggestion (Krimm and Dwivedi, 1982), made on the basis of normal mode analysis (Dwivedi and Krimm, 1984), that bR contains α_{II}-helices. (The α_{II}-helix differs from the α_I-helix in that the peptide group, whose plane is roughly parallel to the axis in the latter, is tilted such that the N-H bond points inward toward the axis with the C═O pointing away from the axis. Although the ϕ,ψ are different, the helical parameters are essentially the same for both.) This proposal has also received support from other studies (Vogel and Gärtner, 1987; Gibson and Cassin, 1989; Earnest et al., 1990).

It is therefore important to know the inherent dichroic properties of these helices if dichroism changes are to be correctly interpreted in terms of conformational and/or orientational changes. These properties cannot be reliably obtained from experimental peptide group transition moments applied to the optically active modes of infinite helices (Tsuboi, 1962; Rothschild and Clark, 1979a). Helices in membrane proteins are finite, and for such structures it is necessary to know the detailed form of the normal mode in the helical segment in order to calculate appropriate transition moments for each peptide group. Summation of the axial and radial components of such transition moments can then properly define the dichroic character of the helical segment.

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We have computed the normal modes of finite α-helices of poly(L-alanine), for which the side chain is represented by a point mass (Reisdorf, 1994; Reisdorf and Krimm, unpublished data), using an empirical force field that excellently reproduces experimental vibrational frequencies of synthetic polypeptides (Krimm and Bandekar, 1986). These calculations have the following characteristics: different hydrogen bond strengths are accounted for by adjusting C═O and O···H stretch force constants according to an algorithm based on spectra of α-helical and β-sheet poly(L-alanine); transition dipole coupling is incorporated on the basis of refined ab initio dipole derivatives (Cheam, 1992) of an *N*-methylacetamide/formamide$_2$ system (Cheam and Krimm, 1985); and infrared band intensity profiles are obtained from these dipole derivatives and the normal mode eigenvectors. The dichroic ratio, $R \equiv A_\parallel/A_\perp$ (A = absorbance), in the present study is obtained from the transition moment components parallel, $\mathbf{M}_\parallel$, and perpendicular, $\mathbf{M}_\perp$, to the axis:

$$R = \mathbf{M}_\parallel^2/\mathbf{M}_\perp^2 \tag{1}$$

where

$$\mathrm{A(total)} \propto \mathbf{M}_\parallel^2 + 2\,\mathbf{M}_\perp^2. \tag{2}$$

Overall band profiles are obtained by placing gaussian-lorentzian bands at the positions of the calculated modes and separately summing parallel and perpendicular components. This provides dichroic ratios based on peak height, R_h, as well as peak area, R_a, and permits determination of $\Delta\nu \equiv \nu_\parallel - \nu_\perp$.

In Table 1 we present the values of $\Delta\nu$, R_h, and R_a for the amide I, amide II, and amide I′ modes of various length α_I- and α_{II}-helices. (Amide I′ is the mode of the ND species.) Although calculated $\nu_\parallel$ and $\nu_\perp$ are sensitive functions of the force field, $\Delta\nu$ should be less so. Our $\Delta\nu = 4.9\ \mathrm{cm}^{-1}$ for the 100-mer of the α_I-helix compares favorably with the 2–5 cm^{-1} observed for esters of poly(L-glutamic acid) (Fraser and MacRae, 1973); our $\Delta\nu \sim 8\ \mathrm{cm}^{-1}$ for 20- and 25-mer α_{II}-helices is comparable to the 7 cm^{-1} observed for bR (Earnest et al., 1990). The values of R_h and R_a can differ because the resultant band shapes of parallel and perpendicular components are not necessarily the same, thus giving different measures of $A_\parallel$ and $A_\perp$.

Some important points emerge from the results given in Table 1. With respect to amide I, 1) although $\Delta\nu$ is about the same for α_I and α_{II} for the 10- and 15-mers, it varies differently with longer lengths: for α_I, $\Delta\nu$ decreases and increases significantly before decreasing to 4.9 cm^{-1} for the 100-mer, whereas for α_{II}, $\Delta\nu$ remains nearly constant at

TABLE 1 Dichroic ratios of amide I and amide II modes of α_I- and α_{II}-helices

		α_I-Helix			α_{II}-Helix		
	n*	$\Delta\nu$‡	R_h§	R_a¶	$\Delta\nu$‡	R_h§	R_a¶
Amide I	10	11.4	7.46	6.12	11.6	5.66	4.24
	15	11.6	10.02	6.44	11.0	5.32	4.36
	20	7.3	8.16	6.20	8.0	4.86	4.18
	25	3.5	8.08	6.36	7.8	4.44	4.30
	30	6.3	8.22	6.36	7.6	4.26	4.26
	35	6.1	7.20	6.28	8.6	4.34	4.22
	40	7.1	7.42	6.40	8.6	4.40	4.30
	60	6.8	6.63	6.32	8.4	4.20	4.23
	80	5.7	5.97	6.38	8.0	4.18	4.26
	100	4.9	5.74	6.34	8.2	4.13	4.24
Amide II	10	−14.7	0.109	0.109	0.6	0.0458	0.0556
	15	−16.4	0.122	0.126	−21.4	0.0408	0.0590
	20	−16.9	0.119	0.122	−22.2	0.0364	0.0540
	25	−17.4	0.121	0.126	−22.4	0.0360	0.0540
	30	−17.6	0.123	0.128	−22.7	0.0368	0.0540
	35	−17.8	0.120	0.126	−23.0	0.0354	0.0514
	40	−18.1	0.122	0.130	−23.0	0.0360	0.0534
	60	−18.3	0.120	0.129	−23.4	0.0351	0.0511
	80	−18.5	0.120	0.131	−23.5	0.0356	0.0516
	100	−18.6	0.120	0.130	−23.5	0.0351	0.0506
Amide I′	10	0.3	5.06	4.62	9.4	5.64	3.64
	15	−1.7	7.42	4.88	6.8	4.76	3.80
	20	−0.5	6.98	4.70	5.2	4.16	3.66
	25	−1.6	5.94	4.80	4.8	4.00	3.76
	30	−2.1	6.50	4.82	4.0	3.94	3.74
	35	−4.8	7.10	4.76	3.6	3.94	3.70
	40	1.3	7.30	4.84	3.6	3.92	3.76
	60	0.9	6.54	4.78	3.7	3.85	3.72
	80	−0.3	5.86	4.83	3.7	3.98	3.75
	100	−3.2	7.16	4.80	3.8	3.96	3.73

*Number of residues in helix.
‡$\Delta\nu = \nu_{\parallel} - \nu_{\perp}$, in cm^{-1}.
§Dichroic ratio based on peak heights.
¶Dichroic ratio based on peak areas.

$\sim$8.2 cm^{-1} from the 20- to the 100-mer; 2) R depends on the helix length differently for the two structures: R_a is relatively constant and R_h generally decreases for α_I, whereas R_h is relatively constant and R_a decreases initially for α_{II}; 3) the magnitude of R is significantly different for α_I and α_{II}, being about 50% higher for α_I; 4) for amide I′, $\Delta\nu$ is generally negative for α_I and positive for α_{II}, and in both cases R is smaller than for amide I (the changes in R arise from differences in the eigenvectors). With respect to amide II, 5) $\Delta\nu$ becomes slightly more negative with increasing length for both α_I and α_{II}, being larger in magnitude for α_{II}; 6) although R increases initially with length for α_I, there is a corresponding decrease for α_{II}; 7) there is a very large difference between R for α_I and α_{II}; the consequence of this difference is that the ratio R(amide I)/R(amide II) may be a good diagnostic for the α-helix type. For example, for the 20-mer, R_h(amide I)/R_h(amide II) is 68.6 for the α_I-helix and 134 for the α_{II}-helix.

The two main messages conveyed by these results are that the amide I and amide II dichroic ratios can be functions of helix length and that they are quite sensitive to changes in internal helix conformation, even if the external helix parameters hardly change. This requires that caution be exercised, for example, in interpreting amide I dichroism changes during the bR photocycle in terms of simple changes in orientation of helical segments: changes in helix conformation could be involved.

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