

# Infrared Dichroism of Amide I and Amide II Modes of $\alpha_I$ - and $\alpha_{II}$ -Helix Segments in Membrane Proteins

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Polarized infrared spectra provide a potentially powerful tool for determining the orientation of  $\alpha$ -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; Nabadryk et al., 1985; Earnest et al., 1986; Nabadryk and Breton, 1986; Breton and Nabadryk, 1989; Fahmy et al., 1989).

It has now been unambiguously established by electron diffraction that bR contains seven  $\alpha$ -helical segments and no  $\beta$ -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  $\alpha$ -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  $\alpha_{II}$ -helices. (The  $\alpha_{II}$ -helix differs from the  $\alpha_I$ -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  $\phi, \psi$  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.

We have computed the normal modes of finite  $\alpha$ -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  $\alpha$ -helical and  $\beta$ -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<sub>2</sub> 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,  $M_{\parallel}$ , and perpendicular,  $M_{\perp}$ , to the axis:

$$R = M_{\parallel}^2/M_{\perp}^2 \quad (1)$$

where

$$A(\text{total}) \propto M_{\parallel}^2 + 2 M_{\perp}^2. \quad (2)$$

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  $\alpha_I$ - and  $\alpha_{II}$ -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  $\Delta\nu = 4.9 \text{ cm}^{-1}$  for the 100-mer of the  $\alpha_I$ -helix compares favorably with the  $2\text{--}5 \text{ cm}^{-1}$  observed for esters of poly(L-glutamic acid) (Fraser and MacRae, 1973); our  $\Delta\nu \sim 8 \text{ cm}^{-1}$  for 20- and 25-mer  $\alpha_{II}$ -helices is comparable to the  $7 \text{ cm}^{-1}$  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  $\alpha_I$  and  $\alpha_{II}$  for the 10- and 15-mers, it varies differently with longer lengths: for  $\alpha_I$ ,  $\Delta\nu$  decreases and increases significantly before decreasing to  $4.9 \text{ cm}^{-1}$  for the 100-mer, whereas for  $\alpha_{II}$ ,  $\Delta\nu$  remains nearly constant at

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TABLE 1 Dichroic ratios of amide I and amide II modes of  $\alpha_1$ - and  $\alpha_{II}$ -helices

	n*	$\alpha_1$ -Helix			$\alpha_{II}$ -Helix		
		$\Delta\nu^\ddagger$	$R_h^\S$	$R_a^\P$	$\Delta\nu^\ddagger$	$R_h^\S$	$R_a^\P$
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
Amide II	100	4.9	5.74	6.34	8.2	4.13	4.24
	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
Amide I'	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
	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.

 $^\ddagger\Delta\nu = \nu_{||} - \nu_{\perp}$ , in  $\text{cm}^{-1}$ . $^\S$ Dichroic ratio based on peak heights. $^\P$ Dichroic ratio based on peak areas.

$\sim 8.2 \text{ cm}^{-1}$  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  $\alpha_1$ , whereas  $R_h$  is relatively constant and  $R_a$  decreases initially for  $\alpha_{II}$ ; 3) the magnitude of  $R$  is significantly different for  $\alpha_1$  and  $\alpha_{II}$ , being about 50% higher for  $\alpha_1$ ; 4) for amide I',  $\Delta\nu$  is generally negative for  $\alpha_1$  and positive for  $\alpha_{II}$ , 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  $\alpha_1$  and  $\alpha_{II}$ , being larger in magnitude for  $\alpha_{II}$ ; 6) although  $R$  increases initially with length for  $\alpha_1$ , there is a corresponding decrease for  $\alpha_{II}$ ; 7) there is a very large difference between  $R$  for  $\alpha_1$  and  $\alpha_{II}$ ; the consequence of this difference is that the ratio  $R(\text{amide I})/R(\text{amide II})$  may be a good diagnostic for the  $\alpha$ -helix type. For example, for the 20-mer,  $R_h(\text{amide I})/R_h(\text{amide II})$  is 68.6 for the  $\alpha_1$ -helix and 134 for the  $\alpha_{II}$ -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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