

Dichroic Ratios in Polarized Fourier Transform Infrared for Nonaxial Symmetry of β -Sheet Structures

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ABSTRACT The transition moments for the amide bands from β -sheet peptide structures generally do not exhibit axial symmetry about the director in linearly polarized Fourier transform infrared (FTIR) measurements on oriented systems. The angular dependences of the dichroic ratios of the amide bands are derived for β -sheet structures in attenuated total reflection (ATR) and polarized transmission experiments on samples that are oriented with respect to the normal to the substrate and are randomly distributed with respect to the azimuthal angle in the plane of the orienting substrate. The orientational distributions of both the β -strands and the β -sheets are considered, and explicit expressions are given for the dichroic ratios of the amide I and amide II bands. The dichroic ratio of the amide II band, which is parallel polarized, can yield the orientation of the β -strands directly, but to specify the orientations of the β -sheets completely requires measurement of the dichroic ratios of both the amide I and amide II bands, or generally two bands with parallel and perpendicular polarizations. A random distribution in tilt of the planes of the β -sheets does not give rise to equal dichroic ratios for bands with perpendicular and parallel polarizations, such as the amide I and amide II bands. The results are applied to previous ATR and polarized transmission FTIR measurements on a potassium channel-associated peptide, the *Escherichia coli* outer membrane protein OmpA, and the *E. coli* OmpF porin protein in oriented membranes.

INTRODUCTION

Linear dichroism of the amide bands in the infrared spectra of membrane proteins has been used extensively to determine the orientation of the various secondary structural elements relative to the membrane normal. Both polarized attenuated total reflection (ATR) and transmission Fourier transform infrared (FTIR) spectroscopy have been used at nonzero angles of incidence with oriented membrane samples. Examples of the former include studies on melittin (Frey and Tamm, 1991), phospholamban (Arkin et al., 1995), and gramicidin (Axelsen et al., 1995); examples of the latter include bacteriorhodopsin (Rothschild and Clarke, 1979) and rhodopsin (Rothschild et al., 1980). Whereas the method is reasonably well developed for membrane-associated α -helical structures, which exhibit axial symmetry, there are far fewer applications to membrane proteins with β -sheet structures. A complication that arises in the analysis of infrared linear dichroism with the latter class of membrane proteins is that, in general, the transition moment does not exhibit axial symmetry (Rodionova et al., 1995). This is particularly the case for the amide I vibration in β -sheet proteins, and the result is that two angular distributions or order parameters are required to specify the dichroic ratios fully. Previous analyses for β -sheet proteins, however, have

usually made the approximation of axial symmetry with a single order parameter.

β -Sheet structures occur in intramembranous sections of integral proteins such as the porins and other *Escherichia coli* outer membrane proteins (Cowan et al., 1992; Rodionova et al., 1995), the acetylcholine receptor (Unwin, 1993; Görne-Tschelnokow et al., 1994), and certain membrane ATPases (Vigneron et al., 1995; Heimbürg et al., manuscript submitted for publication). It has also been suggested that the β -barrel or another β -sheet configuration may be a principal structural motif for the pore regions of voltage-gated ion channels (see Marsh, 1996, for review). For these reasons, analysis of the orientation of β -sheet proteins and peptides in membranes by infrared linear dichroism is particularly relevant. In the present paper, expressions are derived for the infrared dichroic ratios of the amide bands in terms of the orientational distributions that characterize the β -sheet structures. These results explicitly take into account the nonaxial symmetry for a general orientation of the transition moment. Consideration of the symmetries and relative strengths of the amide modes indicates that the resultant transition moment of the dominant mode is oriented perpendicular to the β -strand axis for the amide I band and parallel to this axis for the amide II band. Expressions are derived for the dichroic ratios of the amide I and amide II bands and are applied to the available data for membrane proteins with β -sheet structure. Combination of the dichroic ratios from both the amide I and amide II bands allows a full definition of the orientation of the β -sheet. Corresponding results for α -helical systems are given in the Appendix. In the latter case, unlike that for β -sheets, knowledge of the angular orientation of the individual amide transition moments is required.

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THEORETICAL RESULTS

The geometric arrangements for the incident beam in ATR and polarized transmission experiments are indicated in Fig. 1. The infrared linear dichroic ratio is defined by

$$R = \frac{A_{\parallel}}{A_{\perp}} = \frac{\langle M_x^2 \rangle E_x^2 + \langle M_z^2 \rangle E_z^2}{\langle M_y^2 \rangle E_y^2} \quad (1)$$

where A_{\parallel} and A_{\perp} are the absorbances for radiation polarized parallel and perpendicular, respectively, to the plane of incidence, and $\mathbf{M} = (M_x, M_y, M_z)$ and $\mathbf{E} = (E_x, E_y, E_z)$ are the transition dipole moment and the radiation electric field vectors, respectively. The laboratory coordinates are defined by z along the normal to the orienting substrate, x along the propagation direction, and y perpendicular to the plane of incidence. For a nonsingular orientation of the transition moment, integration of the squares of the elements of the transition moment over the orientational distribution must be performed. This is indicated by the angular brackets in Eq. 1.

For a polarized ATR experiment, the dichroic ratio, R^{ATR} , is given by Eq. 1, where the electric field intensities in the sample are determined by the refractive indices of the various media and the aperture angle of the ATR plate (Harrick, 1967). For polarized transmission measurements, the expressions for the electric field intensities are simpler and the dichroic ratio is given by

$$R^T = \frac{\langle M_x^2 \rangle}{\langle M_y^2 \rangle} + \frac{\langle M_z^2 \rangle - \langle M_x^2 \rangle}{\langle M_y^2 \rangle} \cdot \frac{\sin^2 i}{n^2} \quad (2)$$

where i is the angle that the incident beam makes with the substrate normal and n is the refractive index of the oriented sample. Here the dichroic ratio is designated by R^T to distinguish it from that which is obtained from ATR, i.e., R^{ATR} .

Nonaxial symmetry and β -strand orientation

Using the axis system defined in Fig. 2 and the transformation matrices given by Zbinden (1964), the components of the transition moment are given by

$$M_x = (\cos \vartheta \cos \gamma \cos \phi + \sin \vartheta \sin \phi) M \sin \Theta + \cos \phi \sin \gamma \cdot M \cos \Theta \quad (3)$$

FIGURE 1 Geometric relations of the incident radiation to the plane of the substrate on which the sample is oriented in ATR experiments (left), and a polarized transmission experiment (right). The z axis lies along the normal to the orienting substrate, the x axis is the orthogonal axis in the plane incidence, and the y axis is perpendicular to the plane of incidence.

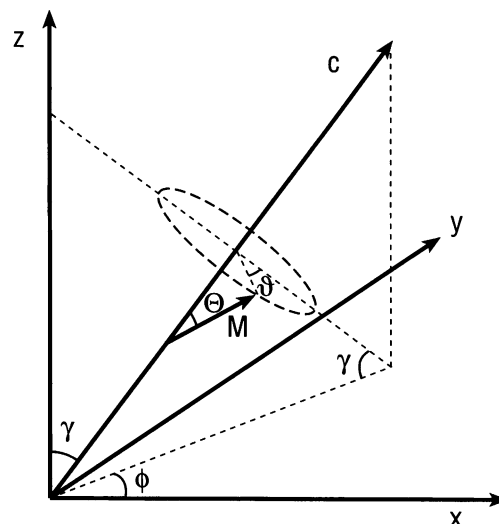
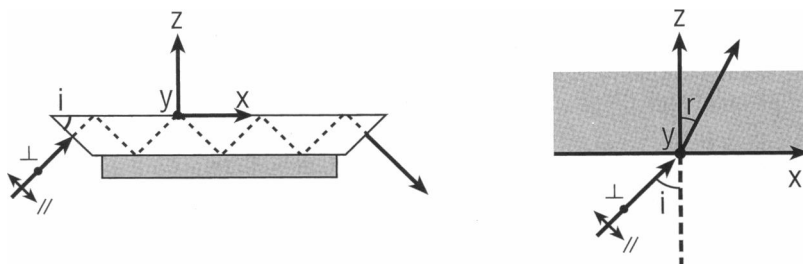


FIGURE 2 Orientation of the β -strand chain axis, c , and the resultant amide transition moment, \mathbf{M} , relative to the membrane-fixed axes, x, y, z . The chain axis is inclined at angle γ to the membrane normal, z , with azimuthal orientation ϕ , in the plane of the membrane. The transition moment is inclined at an angle Θ to the chain axis, with azimuthal orientation, ϑ , relative to the plane containing the z and c axes.

$$M_y = (\cos \vartheta \cos \gamma \sin \phi - \sin \vartheta \cos \phi) M \sin \Theta + \sin \phi \sin \gamma \cdot M \cos \Theta \quad (4)$$

$$M_z = -\sin \gamma \cos \vartheta \cdot M \sin \Theta + \cos \gamma \cdot M \cos \Theta \quad (5)$$

where M is the absolute magnitude of the transition moment. Here Θ is the orientation of the transition moment relative to the long molecular axis, γ is the orientation of the long molecular axis relative to the substrate normal (i.e., to z), ϑ is the azimuthal angle of the transition moment about the long molecular axis, and ϕ is the azimuthal angle of the long molecular axis about the substrate normal (see Fig. 2). For a random distribution of the azimuthal angle of the long molecular axis, i.e., a random distribution in the plane of the membrane, integration must be performed over the angle ϕ in Eqs. 1 and 2. From Eqs. 3–5, the resulting dichroic ratios are given by

$$R^{\text{ATR}} = \frac{E_x^2}{E_y^2} + \frac{\langle M_z^2 \rangle E_z^2}{\langle M_y^2 \rangle E_y^2} \quad (6)$$

and

$$R^T = 1 + \left(\frac{\langle M_z^2 \rangle}{\langle M_y^2 \rangle} - 1 \right) \frac{\sin^2 i}{n^2} \quad (7)$$

for ATR and polarized transmission experiments, respectively. In Eqs. 6 and 7, the value of $\langle M_z^2 \rangle / \langle M_y^2 \rangle$ is given by

$$\frac{\langle M_z^2 \rangle}{\langle M_y^2 \rangle} = 2 \frac{\langle \sin^2 \gamma \cos^2 \vartheta \rangle \sin^2 \Theta + \langle \cos^2 \gamma \rangle \cos^2 \Theta - 2 \langle \sin \gamma \cos \gamma \cos \vartheta \rangle \sin \Theta \cos \Theta}{(\langle \cos^2 \gamma \cos^2 \vartheta \rangle + \langle \sin^2 \vartheta \rangle \sin^2 \Theta + \langle \sin^2 \gamma \rangle \cos^2 \Theta + 2 \langle \sin \gamma \cos \gamma \cos \vartheta \rangle \sin \Theta \cos \Theta)} \quad (8)$$

where the angular brackets indicate integration additionally over any (normalized) distribution of the angles γ and ϑ . These distributions are not necessarily independent, but general trigonometrical identities such as $\langle \sin^2 \gamma \rangle = 1 - \langle \cos^2 \gamma \rangle$ must hold.

Because of the symmetry and phase relations of the coupled vibrations, the resultant transition moments in a β -strand must be oriented either parallel or perpendicular to the strand axis (Miyazawa, 1960; Fraser and MacRae, 1973). The orientations $\Theta = 0^\circ$ and $\Theta = 90^\circ$ are therefore of particular importance and correspond to the amide II and amide I bands, respectively, in β -sheet structures (see later section on orientation of the transition moments). For a transition moment parallel to the long molecular axis, the dichroic ratios are

$$R^{\text{ATR}}(\Theta = 0^\circ) = \frac{E_x^2}{E_y^2} + \frac{2\langle \cos^2 \gamma \rangle}{1 - \langle \cos^2 \gamma \rangle} \cdot \frac{E_z^2}{E_y^2} \quad (9)$$

$$R^T(\Theta = 0^\circ) = 1 + \frac{3\langle \cos^2 \gamma \rangle - 1}{1 - \langle \cos^2 \gamma \rangle} \cdot \frac{\sin^2 i}{n^2} \quad (10)$$

for ATR and polarized transmission, respectively. For the transition moment perpendicular to the long molecular axis, the corresponding dichroic ratios are

$$R^{\text{ATR}}(\Theta = 90^\circ) = \frac{E_x^2}{E_y^2} + \frac{2\langle \sin^2 \gamma \cos^2 \vartheta \rangle}{1 - \langle \sin^2 \gamma \cos^2 \vartheta \rangle} \cdot \frac{E_z^2}{E_y^2} \quad (11)$$

$$R^T(\Theta = 90^\circ) = 1 + \frac{3\langle \sin^2 \gamma \cos^2 \vartheta \rangle - 1}{1 - \langle \sin^2 \gamma \cos^2 \vartheta \rangle} \cdot \frac{\sin^2 i}{n^2} \quad (12)$$

The origin for the azimuthal angle ϑ is defined by the plane containing the long molecular axis and the normal to the orienting substrate (see Fig. 2). Equations 9, 10 and 11, 12 apply to the two unique orientations of the resultant transition moment in a β -sheet structure and are expressed in terms of the orientation of the peptide strands relative to the membrane normal. For amide bands for which the transition moment in the β -sheet is parallel to the strand axis (i.e., $\Theta = 0^\circ$), the azimuthal orientation ϑ does not enter (cf. Eqs. 9 and 10). The dichroic ratio of such amide bands therefore gives the orientation, γ , of the β -strands directly. For a tilted β -sheet structure, however, this is not identical with the strand orientation within the plane of the sheet.

Orientation of the β -sheets

For a β -sheet structure, the tilt angle γ of the β -strands relative to the membrane normal (i.e., to the z axis) is given by

$$\cos \gamma = \cos \alpha \cos \beta \quad (13)$$

where α is the angle by which the plane of the sheet is tilted to the z axis, and the strand axes are tilted at an angle β within the sheet (see Fig. 3). The individual amide transition moments in a β -sheet structure lie within the peptide planes, which are inclined to the plane of the sheet. Perpendicularly polarized modes have resultant transition moments that are perpendicular to the sheet or lie within the sheet. The latter situation, i.e., an in-plane orientation, is appropriate to antiparallel β -sheets and is that considered here (see later section on orientation of transition moments). The azimuthal orientation, ϑ , of the plane of the sheet relative to the plane containing the strand axis and the z axis is given by

$$\cos \vartheta = \tan \beta / \tan \gamma \quad (14)$$

The general expressions for the dichroic ratio in terms of the orientation of the β -sheet are then given by Eqs. 6 and 7, where

$$\frac{\langle M_z^2 \rangle}{\langle M_y^2 \rangle} = \frac{2[\langle \sin^2 \beta \rangle \sin^2 \Theta + \langle \cos^2 \beta \rangle \cos^2 \Theta - 2\langle \sin \beta \cos \beta \rangle \sin \Theta \cos \Theta] \langle \cos^2 \alpha \rangle}{(1 - \langle \sin^2 \beta \rangle \langle \cos^2 \alpha \rangle) \sin^2 \Theta + (1 - \langle \cos^2 \beta \rangle \langle \cos^2 \alpha \rangle) \cos^2 \Theta + 2\langle \sin \beta \cos \beta \rangle \langle \cos^2 \alpha \rangle \sin \Theta \cos \Theta} \quad (15)$$

for an arbitrary orientation, Θ , of the transition moment within the plane of the sheet. In this case, the distributions over the orientation, α , of the β -sheet and the orientation, β , of the β -strands within the sheet are independent and uncorrelated. On structural grounds it is possible that the β -strands might have a unique orientation within extended

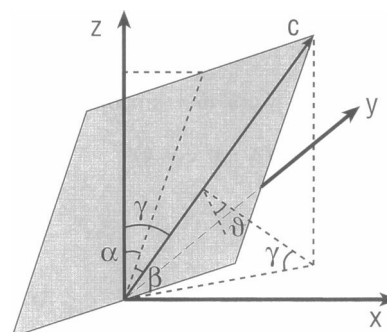


FIGURE 3 Orientation of the β -strand axis, c , and of the β -sheet relative to the membrane-fixed axes x , y , z . The β -sheet is inclined at an angle α to the membrane normal, z . The β -strand is tilted by the angle β in the plane of the sheet and inclined to the membrane normal at the angle γ . The azimuthal orientation of the plane of the sheet relative to the plane containing the z and c axes is given by ϑ .

sheets, but in general a distribution in the angle β is possible.

The orientations, α and β , of the β -sheet structure, relative to the membrane normal, can then be obtained from measurements of the dichroic ratios of two amide bands that have transition moments that are oriented parallel and perpendicular, respectively, to the strand axis by using Eqs. 6, 7, and 15. In terms of the orientation of the β -sheet, the dichroic ratios in the ATR experiment (cf. Eqs. 9 and 11) are given by

$$R^{\text{ATR}}(\Theta = 0^\circ) = \frac{E_x^2}{E_y^2} + \frac{2\langle\cos^2\alpha\rangle\langle\cos^2\beta\rangle}{1 - \langle\cos^2\alpha\rangle\langle\cos^2\beta\rangle} \cdot \frac{E_z^2}{E_y^2} \quad (16)$$

$$R^{\text{ATR}}(\Theta = 90^\circ) = \frac{E_x^2}{E_y^2} + \frac{2\langle\cos^2\alpha\rangle\langle\sin^2\beta\rangle}{1 - \langle\cos^2\alpha\rangle\langle\sin^2\beta\rangle} \cdot \frac{E_z^2}{E_y^2} \quad (17)$$

The corresponding expressions for the dichroic ratios in the polarized transmission experiment (cf. Eqs. 10 and 12) are

$$R^{\text{T}}(\Theta = 0^\circ) = 1 + \frac{3\langle\cos^2\alpha\rangle\langle\cos^2\beta\rangle - 1}{1 - \langle\cos^2\alpha\rangle\langle\cos^2\beta\rangle} \cdot \frac{\sin^2 i}{n^2} \quad (18)$$

$$R^{\text{T}}(\Theta = 90^\circ) = 1 + \frac{3\langle\cos^2\alpha\rangle\langle\sin^2\beta\rangle - 1}{1 - \langle\cos^2\alpha\rangle\langle\sin^2\beta\rangle} \cdot \frac{\sin^2 i}{n^2} \quad (19)$$

where $\langle\sin^2\beta\rangle = 1 - \langle\cos^2\beta\rangle$. Equations 16, 18 and 17, 19 apply to the two amide bands with transition moments parallel and perpendicular, respectively, to the strand axis. By combining the dichroic ratios for both transition moment orientations, the sheet orientation, specified by $\langle\cos^2\alpha\rangle$, and the strand orientation within the sheet, specified by $\langle\cos^2\beta\rangle$, may be determined independently.

In terms of changes in orientation for a given sheet structure, the tilt angle β could remain approximately constant because it is determined by the pattern of H-bonding within the sheet, and changes would then be expressed predominantly in the sheet orientation, α . For staggering of the H-bonds between adjacent strands by one or two residues, geometrical and dimensional relationships dictate that the values of the strand tilt within the sheet are $\beta = \tan^{-1}(3.47\Delta n_i/4.72) = 36^\circ$ or 56° , respectively. Here it is assumed that the distance between adjacent residues in a strand is 3.47 \AA and the separation between adjacent strands is 4.72 \AA ; Δn_i is the number of residues by which adjacent strands are staggered. Molecular modeling yields corresponding values of $\beta = 35^\circ$ and 60° , respectively (Manella et al., 1992), in reasonably good agreement with the simple geometric arguments.

Random orientation

The results for a random orientation of the tilt of the plane of the β -sheet with respect to the membrane normal are of interest. These are obtained by integrating over the tilt angle α of the sheet that gives $\langle\cos^2\alpha\rangle = 1/3$. The dichroic ratio, for a fixed orientation β of the peptide strands within the

plane of the β -sheet, is then given by Eq. 6 or Eq. 7, where

$$\frac{\langle M_z^2 \rangle}{\langle M_y^2 \rangle} = 2 \frac{\langle\sin^2\beta\rangle\sin^2\Theta + \langle\cos^2\beta\rangle\cos^2\Theta - 2\langle\sin\beta\cos\beta\rangle\sin\Theta\cos\Theta}{(3 - \langle\sin^2\beta\rangle)\sin^2\Theta + (3 - \langle\cos^2\beta\rangle)\cos^2\Theta + 2\langle\sin\beta\cos\beta\rangle\sin\Theta\cos\Theta} \quad (20)$$

The specific expressions for the dichroic ratios from randomly oriented β -sheets of amide bands with $\Theta = 0^\circ$ and $\Theta = 90^\circ$ orientations of the transition moment are given, respectively, by

$$R_{\text{rand}}^{\text{ATR}}(\Theta = 0^\circ) = \frac{E_x^2}{E_y^2} + \frac{2\langle\cos^2\beta\rangle}{3 - \langle\cos^2\beta\rangle} \frac{E_z^2}{E_y^2} \quad (21)$$

$$R_{\text{rand}}^{\text{ATR}}(\Theta = 90^\circ) = \frac{E_x^2}{E_y^2} + \frac{2\langle\sin^2\beta\rangle}{3 - \langle\sin^2\beta\rangle} \frac{E_z^2}{E_y^2} \quad (22)$$

for the ATR experiment. The corresponding expressions for the polarized transmission experiment are

$$R_{\text{rand}}^{\text{T}}(\Theta = 0^\circ) = 1 - 3 \frac{1 - \langle\cos^2\beta\rangle}{3 - \langle\cos^2\beta\rangle} \cdot \frac{\sin^2 i}{n^2} \quad (23)$$

$$R_{\text{rand}}^{\text{T}}(\Theta = 90^\circ) = 1 - 3 \frac{1 - \langle\sin^2\beta\rangle}{3 - \langle\sin^2\beta\rangle} \cdot \frac{\sin^2 i}{n^2} \quad (24)$$

It will be seen that, in general, the dichroic ratios of the amide bands with different orientations of the transition moment are not equal for a random tilt of the planes of the β -sheets. This is unlike the situation that would obtain for a completely random distribution of the β -strand axes (which is specified by $\langle\cos^2\gamma\rangle = 1/3$, $\langle\cos^2\vartheta\rangle = 1/2$) and arises because the β -strands have a fixed orientation, β , within the sheet.

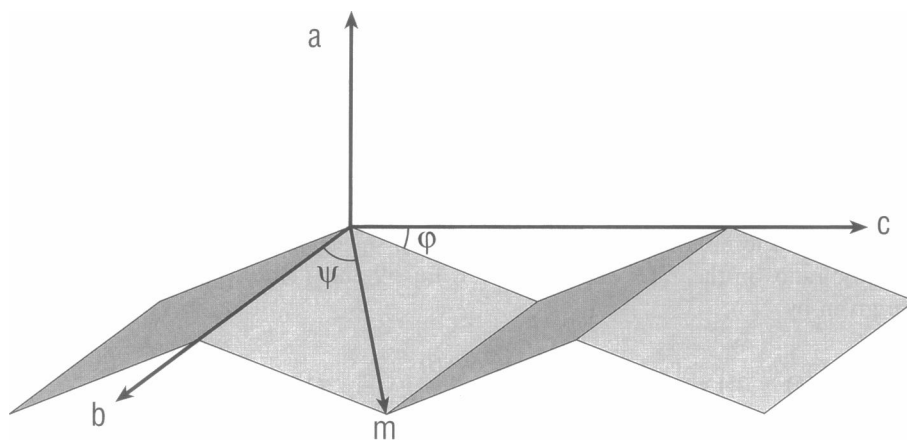
Orientation of the amide transition moments

The transition moment, \mathbf{m} , of an individual amide group lies in the peptide plane. For a β -pleated sheet structure, the peptide planes are inclined at an angle $\varphi \approx 30^\circ$ relative to the strand axis, c (see Fig. 4). The individual transition moments are assumed to be inclined at an angle ψ to the perpendicular axis, b , that lies within the plane of the sheet. The relative intensities of the various parallel and perpendicular polarized modes are specified by the projections of the transition moment \mathbf{m} on the orthogonal a , b , and c axes, which are determined by the angles ψ and φ .

In general, the vibrational modes of a β -strand in pleated sheet structures are determined by the coupling between amide units (Fraser and MacRea, 1973). For a parallel-chain β -sheet structure, the frequencies of the modes with resultant transition moment oriented parallel (\parallel) and perpendicular (\perp) to the strand axis are, respectively (Miyazawa, 1960),

$$\begin{aligned} \nu_{\parallel}(0, 0) &= \nu_0 + D_{10} + D_{01} \\ \nu_{\perp}(\pi, 0) &= \nu_0 - D_{10} + D_{01} \end{aligned} \quad (25)$$

FIGURE 4 Orientation of the transition moment of an individual peptide group in a β -pleated sheet structure. The transition moment, \underline{m} , lies in the peptide plane, which is inclined at an angle φ to the strand axis, c . The latter is defined by the line joining successive α -carbon atoms. The b axis is defined by the intersection of adjacent peptide planes, and the transition moment makes an angle ψ with this axis. The b, c plane corresponds to the plane of the sheet.



where ν_0 is the unperturbed peptide group frequency and D_{10} and D_{01} are interaction terms representing the intra-chain and interchain couplings, respectively. The transition moment of the parallel polarized mode, $\nu_{\parallel}(0, 0)$, is directed along the c axis, and its magnitude is proportional to $\sin \psi \cos \varphi$. That of the perpendicular polarized mode has a and b components proportional to $\sin \psi \sin \varphi$ and $\cos \varphi$, respectively. For an antiparallel-chain β -sheet structure, the frequencies of the corresponding infrared-active modes are (Miyazawa, 1960)

$$\begin{aligned}\nu_{\parallel}(0, \pi) &= \nu_0 + D_{10} + D_{01} - D_{11} \\ \nu_{\perp}(\pi, 0) &= \nu_0 - D_{10} + D_{01} - D_{11} \\ \nu_{\perp}(\pi, \pi) &= \nu_0 - D_{10} - D_{01} + D_{11}\end{aligned}\quad (26)$$

where the additional interaction term D_{11} represents the transition dipole coupling between adjacent strands (Krimm and Abe, 1972). The transition moment of the parallel polarized mode, $\nu_{\parallel}(0, \pi)$, is directed along the c axis and has a magnitude proportional to $\sin \psi \cos \varphi$. Those of the perpendicularly polarized modes, $\nu_{\perp}(\pi, 0)$ and $\nu_{\perp}(\pi, \pi)$, are directed along the b and a axes, respectively, and have magnitudes proportional to $\cos \varphi$ and to $\sin \psi \sin \varphi$, respectively.

Normal mode analyses and dipole coupling calculations have been performed for the parallel-chain β -pleated sheet (Chirgadze and Nevskaya, 1976a; Bandekar and Krimm, 1988). In the amide I region, the strong infrared absorption arises from the mode of B_2 symmetry class in C_2 , which has perpendicular polarization, $\nu_{\perp}(\pi, 0)$, and is predicted to lie in the region of 1640 cm^{-1} . In general, the transition moment for this mode is expected to be inclined to the plane of the β -sheet, but the exact orientation in the a, b plane is not known. Equations 11 and 12 (or Eqs. 17 and 19) are therefore appropriate for the dichroic ratios of the amide I band for the parallel β -sheet. The strong infrared absorption in the amide II region arises from the mode of the A symmetry class, which has parallel polarization, $\nu_{\parallel}(0, 0)$, and is predicted to occur in the region around 1550 cm^{-1} . Equations 9 and 10 (or Eqs. 16 and 18) give the dichroic ratio of this band.

Band assignments for the antiparallel-chain β -pleated sheet have been established experimentally from measurements of the infrared dichroism in oriented fibers (Suzuki, 1967; Fraser and Suzuki, 1970; Krimm and Bandekar, 1986). The very strong absorption at low frequency around 1635 cm^{-1} in the amide I region has perpendicular polarization and corresponds to the $\nu_{\perp}(0, \pi)$ mode for which the resultant transition moment lies in the plane of the sheet. The orientation of the transition moment of the principal band in the amide I region of the antiparallel β -sheet therefore corresponds to that for which Eqs. 17 and 19 for the dichroic ratio were derived. The weak absorption at high frequency around $1680\text{--}1690 \text{ cm}^{-1}$ in the amide I region has parallel polarization and corresponds to the $\nu_{\parallel}(\pi, 0)$ mode. These assignments have been confirmed by normal-mode analysis and dipole coupling calculations (Chirgadze and Nevskaya, 1976b; Krimm and Bandekar, 1986). The strong low-frequency amide I mode belongs to the B_2 symmetry class with y polarization in D_2 and the weak high-frequency mode to the B_1 symmetry class with z polarization. The strong amide II absorption from the antiparallel sheet is parallel polarized and therefore corresponds to the $\nu_{\parallel}(\pi, 0)$ mode (Suzuki, 1967; Fraser and Suzuki, 1970). Normal-mode analysis predicts that the mode belonging to the B_1 symmetry class arises at around 1530 cm^{-1} in the amide II region (Krimm and Bandekar, 1986). Equations 16 and 18 (or Eqs. 9 and 10) are therefore appropriate for the dichroic ratio of the principal amide II absorption of the antiparallel β -sheet.

It should be noted that variations from ideal geometry, produced either by end effects or by the fact that the structures are not homopolymers, may produce some deviation from the above selection rules. This may lead to transition moments whose net orientation is not exactly in accord with the symmetry arguments that are given above. However, measurements on silk fibroin and other oriented fibres (Suzuki, 1967; Fraser and Suzuki, 1970; Fraser and MacRae, 1973) suggest that the effects of a nonhomogeneous residue composition may be relatively unimportant. In addition, the effects of finite size of the β -sheets have

been investigated by Chirgadze and Nevskaya (1976a,b), using dipole-coupling calculations. It was found that the symmetry classes of the major amide bands were preserved and that the predicted IR spectra were relatively insensitive to strand length and to the number of strands when the latter was approximately eight or more.

APPLICATIONS

IsK-channel peptide

FTIR-ATR studies have been performed on a 27-residue peptide that corresponds to the single putative transmembrane section of a 130-residue protein, IsK, which when expressed in oocytes gives rise to slowly activating voltage-gated potassium channels (cf. Takumi et al., 1988). The peptide incorporated in phosphatidylcholine membranes by dialysis from 2-chloroethanol at lipid/peptide ratios of 20–25:1 mol/mol is predominantly in a β -sheet conformation (Horváth et al., 1995; Aggeli et al., 1996). Data obtained from dried membranes for which the dichroic ratios of both the amide I and amide II bands could be measured are given in Table 1 (Aggeli et al., 1996). The frequencies of the major amide I and amide II bands at 1627 cm^{-1} and 1538 cm^{-1} , respectively, together with the weak high-frequency band in the amide I region at $\sim 1695\text{ cm}^{-1}$, indicate that the β -chains are in an antiparallel configuration.

The dichroic ratios for the amide II band can be used directly to determine the orientation, $\langle \cos^2\gamma \rangle$, of the β -strands relative to the membrane director by using Eq. 9. These values are given in Table 1 for membranes of both dimyristoyl and dipalmitoyl phosphatidylcholine. Assuming a single orientation of the strands with a very sharp distribution, the values of $\langle \cos^2\gamma \rangle$ imply that the β -strands are tilted by an angle $\gamma \approx 56^\circ$ relative to the bilayer normal, as reported originally. This large tilt is expected because in the extended conformation the hydrophobic span of the peptide, 23 residues, is much larger than the hydrophobic thickness of the lipid bilayer (Horváth et al., 1995; Aggeli et al., 1996). Combining the dichroic ratios of both the amide I and amide II bands allows determination of the orientational parameters, $\langle \cos^2\alpha \rangle$ and $\langle \cos^2\beta \rangle$, of the β -sheet by using Eqs. 17 and 16, respectively. The former equation is that appropriate to the principal perpendicularly polarized amide I band of an antiparallel sheet. These values are given in Table 1; they imply that not only are the β -strands tilted within the plane of the sheet, but also the sheets themselves are tilted with respect to the membrane normal. Assuming a

sharp, singular distribution of orientations, the values of $\langle \cos^2\beta \rangle$ and $\langle \cos^2\alpha \rangle$ imply that the strands are tilted by an angle $\beta \approx 35\text{--}36^\circ$ within the sheets and that the sheets are tilted at an angle $\alpha \approx 46\text{--}48^\circ$ relative to the bilayer normal. The value of the tilt angle β is that expected for a stagger by one residue in the H-bonding between adjacent strands (see above) and therefore is consistent with the β -sheet geometry. The large value of α suggests that the sheets may also be twisted, in which case there could be a broad distribution in the effective values of α .

The dichroic ratio of the amide I band from the peptide in hydrated dimyristoylphosphatidylcholine (DMPC) membranes at temperatures in the gel phase ($R^{\text{ATR}} = 1.07 \pm 0.04$) was found not to be significantly different from that for the dried membranes, but increased to a value of $R^{\text{ATR}} = 1.17 \pm 0.04$ at temperatures in the fluid phase (Aggeli et al., 1996). The dichroic ratio of the amide II band could not be determined because of spectral overlap problems. If it is assumed that the orientation of the strand within the sheet is not changed on hydration, or on fluidization of the lipid membranes, the changes in orientation of the sheets may be determined from the dichroic ratios of the amide I band alone. Taking $\langle \cos^2\beta \rangle = 0.67$ from Table 1 in each case, the orientation of the sheets in hydrated DMPC membranes is then given by $\langle \cos^2\alpha \rangle = 0.49 \pm 0.05$ and 0.56 ± 0.05 in the gel and fluid phases, respectively (corresponding values of $\langle \cos^2\gamma \rangle$ are 0.33 and 0.38, respectively). This suggests that the tilt of the sheets relaxes somewhat on going from the gel to the fluid phase, although the effect is not particularly large.

OmpA protein

The dichroism of the amide I' band from the *E. coli* outer membrane protein OmpA reconstituted in bilayers of various phosphatidylcholines has been determined using ATR-FTIR by Rodionova et al. (1995). By analogy with porins for which the structure is known, OmpA is predicted to form an eight-stranded antiparallel β -barrel. The ATR dichroic ratios for the amide I' band are given in Table 2. In the absence of additional data from the amide II band, individual angular orientations cannot be determined. Values for the compound average orientational parameter $\langle \sin^2\gamma \cos^2\theta \rangle$ deduced from Eq. 11 are given in Table 2.

If it is assumed that on average the plane of the sheet is untilted relative to the membrane normal, i.e. $\langle \cos^2\alpha \rangle = 1$, in the β -barrel structure, then the orientation of the

TABLE 1 Dichroic ratios, R^{ATR} , for the amide bands and derived orientational parameters for the IsK channel peptide in dry phosphatidylcholine bilayers at 20°C

Lipid*	R^{ATR} (amide II)	$\langle \cos^2\gamma \rangle$	R^{ATR} (amide I)	$\langle \cos^2\beta \rangle$	$\langle \cos^2\alpha \rangle$
DPPC	1.7 ± 0.3	0.28 ± 0.05	1.05 ± 0.04	0.64 ± 0.05	0.44 ± 0.06
DMPC	1.9 ± 0.3	0.32 ± 0.05	1.05 ± 0.04	0.67 ± 0.04	0.48 ± 0.05

Experimental data from Aggeli et al. (1996). Orientational parameters are derived from Eq. 9 and Eqs. 16 and 17.

*DPPC, dipalmitoyl phosphatidylcholine; DMPC, dimyristoyl phosphatidylcholine.

TABLE 2 Dichroic ratios, R^{ATR} , for the amide I' band and derived orientational parameters for OmpA in hydrated phosphatidylcholine bilayers at 21°C

Lipid	R^{ATR} (amide I')	$\langle \sin^2 \gamma \cos^2 \vartheta \rangle$	$\langle \cos^2 \beta \rangle^*$
DPPC	2.07 ± 0.17	0.42 ± 0.03	0.58 ± 0.03
DMPC	1.81 ± 0.05	0.36 ± 0.01	0.64 ± 0.01
POPC [#]	1.94 ± 0.04	0.39 ± 0.01	0.61 ± 0.01

Experimental data from Rodionova et al. (1995). Orientational parameters are derived from Eqs. 11 and 17.

*Calculated assuming that $\langle \cos^2 \alpha \rangle = 1$.

[#]POPC, palmitoyl-oleoyl-phosphatidylcholine.

β -strands can be determined from Eq. 17. These values for $\langle \cos^2 \beta \rangle$ deduced solely from the dichroic ratios of the amide I' band are given in Table 2. For a unique orientation, this corresponds to tilt angles in the range $\beta = 37$ – 40° for DMPC and (DPPC) dipalmitoylphosphatidylcholine, respectively. This particular axial approximation corresponds to a perfectly oriented β -barrel. The values for the tilt differ slightly from those found originally for this model (Rodionova et al., 1995), only because an unnecessary correction for the orientation of the individual transition moments was attempted in the latter case. The assumption that $\langle \cos^2 \alpha \rangle \approx 1$ is supported to some extent by the results from the analogous OmpF porin that are given in the following section. If the same value of $\langle \cos^2 \alpha \rangle = 0.9$ as found for OmpF (see below) is taken for OmpA, then the effective tilt angles, β , are increased by $\sim 6.5^\circ$ for OmpA.

OmpF porin

Polarized transmission measurements have been performed on the *E. coli* matrix porin OmpF reconstituted in dimyristoyl phosphatidylcholine vesicles that were oriented by air-drying (Nabedryk et al., 1988). The dichroic ratios determined with an angle of incidence $i = 40^\circ$ were $R^T = 1.10$ and $R^T = 1.13$ for the amide I and amide II bands, respectively. From the dichroic ratio of the amide II band, the orientation of the antiparallel β -strands obtained using Eq. 10 with $n = 1.5$ is $\langle \cos^2 \gamma \rangle = 0.46$. For a homogeneous orientation, this corresponds to a tilt angle $\gamma = 47^\circ$ of the β -strands relative to the membrane normal. By combining the dichroic ratios of both the amide I and amide II bands, the orientation of the planes of the β -sheets is specified by $\langle \cos^2 \alpha \rangle = 0.90$, and the tilt of the β -strands within the sheet by $\langle \cos^2 \beta \rangle = 0.51$. These values are obtained by using Eqs. 18 and 19 with $n = 1.5$ for the refractive index of the layer of lipids. Again for a homogeneous orientation, these orientations correspond to effective angles $\alpha = 19^\circ$ and $\beta = 44^\circ$, respectively. The tilt of the β -sheets is therefore relatively small, which is reasonably consistent with the assumption made in determining the orientation, β , of the peptide strands from the ATR dichroic ratio of the amide I band of OmpA in the previous section. The effective tilt angle of the β -strands in OmpF is somewhat larger than that estimated for OmpA and does not correspond with one of

the canonical values expected for a fixed difference in register between adjacent strands. The latter suggests that there is a distribution of tilt angles for the β -strands of OmpF, as is found in the x-ray crystal structures of the porins (Weiss et al., 1990; Cowan et al., 1992). For the three-dimensional structure of OmpF, the tilt angle of the strands was found to vary between 35° and 50° around the β -barrel structure (Cowan et al., 1992).

ATR-FTIR experiments also have been performed for *E. coli* porin reconstituted in a thin film of DMPC with air as the upper phase (Goormaghtigh et al., 1990). A dichroic ratio of $R^{ATR} = 1.8$ was determined for the amide I' band. Using Eq. 11, this implies a value of $\langle \sin^2 \gamma \cos^2 \vartheta \rangle = 0.67$ for the orientational parameter. Assuming that the sheets are aligned perpendicular to the plane of the membrane (i.e., $\langle \cos^2 \alpha \rangle = 1$) results in a value of $\langle \cos^2 \beta \rangle = 0.33$ for the orientation of the β -strands within the sheets. For a unique orientation of the strands, this implies an effective tilt angle of $\beta = 55^\circ$. The latter is consistent with the orientation derived originally within this approximation of axial symmetry. It again implies that the β -strands have a very pronounced tilt relative to the membrane normal, possibly somewhat greater than for the samples investigated by polarized transmission FTIR. If the same value of $\langle \cos^2 \alpha \rangle = 0.9$ is assumed, as from the transmission experiments above, then $\langle \cos^2 \beta \rangle = 0.22$ for the ATR results corresponding to an effective tilt angle of $\beta = 62^\circ$.

CONCLUSIONS

The resultant amide transition moments for β -sheet structures are oriented either parallel or perpendicular to the polypeptide chain axis. In general, one of the modes in the amide band will be intense because the individual transition moments are oriented either close to the C=O bond, or closely perpendicular to it (Fraser and MacRae, 1973). The dichroic ratio of bands polarized parallel to the β -strand axis (e.g., the amide II band) allow determination of the orientation of the strands directly. Determination of the orientation of the sheets requires combined measurement of the dichroic ratios of two modes polarized parallel and perpendicular to the chain axis, respectively. The major components of the amide I and amide II bands are suitable for this. In principle, the low- and high-frequency components in the amide I region could be used, but in practice the latter is of too low an intensity. Dichroic measurements on the amide I band alone therefore yield the orientation neither of the β -strands nor of the β -sheets directly.

Dichroic ratios determined by integrating over the parallel and perpendicular polarized components of the entire amide band are dependent on the orientation of the individual amide transition moments. This is unavoidable for the amide I band of α -helical structures because the parallel and perpendicular polarized components are very close in frequency (see Appendix). The situation for β -sheets is simpler than for α -helices in this respect, but is complicated by the nonaxial symmetry.

APPENDIX: DICHOIC RATIO FOR α -HELICAL STRUCTURES

To establish a connection with other formalisms for the dichroic ratio, the corresponding expressions for an α -helical structure are given in the present notation. These are obtained by integrating over the azimuthal angle, ϑ , about the helical axis. The dichroic ratios are then given by Eqs. 6 and 7 for ATR and polarized transmission, respectively, where Eq. 8 is replaced by

$$\frac{\langle M_z^2 \rangle}{\langle M_y^2 \rangle} = 2 \frac{\langle \sin^2 \gamma \rangle \sin^2 \Theta + 2 \langle \cos^2 \gamma \rangle \cos^2 \Theta}{(1 + \langle \cos^2 \gamma \rangle) \sin^2 \Theta + 2 \langle \sin^2 \gamma \rangle \cos^2 \Theta} \quad (\text{A.1})$$

This result is consistent with the expression normally used for the dichroic ratio in ATR experiments (Frey and Tamm, 1991; Arkin et al., 1995; Axelsen et al., 1995), where the order parameter of the helix axis is given conventionally by $\langle P_2(\cos \gamma) \rangle = \frac{1}{2}(3\langle \cos^2 \gamma \rangle - 1)$. By rearrangement of Eq. A.1 together with Eq. 6, the helix order parameter is given by

$$\langle P_2(\cos \gamma) \rangle = \frac{E_x^2 - E_y^2 R^{\text{ATR}}(\Theta) + E_z^2}{P_2(\cos \Theta) [E_x^2 - E_y^2 R^{\text{ATR}}(\Theta) - 2E_z^2]} \quad (\text{A.2})$$

where $R^{\text{ATR}}(\Theta)$ is the ATR dichroic ratio and the orientation of the transition moment is given in terms of the second-order Legendre polynomial. The corresponding expression for the order parameter of an α -helical structure in terms of the dichroic ratio, $R^{\text{T}}(\Theta)$, in a polarized transmission FTIR experiment is

$$\langle P_2(\cos \gamma) \rangle = \frac{R^{\text{T}}(\Theta) - 1}{P_2(\cos \Theta) [3 \sin^2 i / n^2 + R^{\text{T}}(\Theta) - 1]} \quad (\text{A.3})$$

which is obtained from Eqs. 7 and A.1. This latter expression is in agreement with previous results (Rothschild and Clark, 1979; Nabadryk and Breton, 1981).

For an α -helix, the frequencies of the modes with resultant transition moment oriented parallel and perpendicular to the helix axis are given by, respectively (Miyazawa, 1960),

$$\nu_{\parallel}(0) = \nu_0 + D_{10} + D_{30} \quad (\text{A.4})$$

$$\nu_{\perp}(\pm 2\pi/p) = \nu_0 + D_{10} \cos(\pm 2\pi/p) + D_{30} \cos(\pm 6\pi/p) \quad (\text{A.5})$$

where $p = 3.6$ is the number of residues per helix turn, and the phase relations are indicated explicitly. Unlike the case for the β -sheet, the parallel and perpendicular polarized modes for the α -helix are close in frequency (around 1650–1655 cm^{-1}) and comparable in intensity (Miyazawa and Blout, 1961; Nevskaya and Chirgadze, 1976; Krimm and Bandekar, 1986). The dichroic ratio then may be obtained only for the total integrated intensity of the amide band and not for the independently polarized modes. Because the intensities of the parallel and perpendicular polarized modes are determined by the projections parallel and perpendicular to the helix axis of the individual amide transition moments, integration over the entire amide band is equivalent to integration over the individual transition moments, and not just over their projections. Therefore, the general form of Eq. A.1 is necessary, which requires knowledge of the orientation, Θ , of the transition moment of an individual peptide group vibration, as is also indicated explicitly in the standard Eq. A.2. For the amide I band of α -helices, this value has been determined variously as $\Theta = 39\text{--}40^\circ$ (Tsuboi, 1962; Bradbury et al., 1962), $\Theta = 29\text{--}34^\circ$ (Miyazawa and Blout, 1961), or $\Theta = 24\text{--}28^\circ$ (Rothschild and Clark, 1979). This range of values contributes to a considerable uncertainty in determination of transmembrane helix orientations by FTIR-ATR spectroscopy (Axelsen et al., 1995). In this respect, the situation with regard to the

highly dichroic bands from β -sheet structures is somewhat more advantageous.

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