CIRCULAR DICHROISM OF BIOLOGICAL MEMBRANES: PURPLE MEMBRANE OF HALOBACTERIUM HALOBIUM

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SUMMARY: Circular dichroism spectra of purple membrane suspensions exhibit distortions which are characteristic of particulate α-helical systems with ellipticities at 224 nm of the order of 10000. We show here that the pseudo reference state approach to the correction of CD data, when applied to the purple membrane system, results in a CD spectrum which, in its entirety, is typical of a protein with about 75% α-helix, e.g. the corrected ellipticity at 224 nm is 24000 and at 190 nm is 45000. This result is consistent with electron microscopy and diffraction data on the purple membrane which indicates 70-80% α-helix. Application to the purple membrane system validates the pseudo reference state approach. We also note that 80% trifluoroethanol solubilizes the membrane but results in a similar average secondary structure of the protein.

Circular dichroism (CD) spectra of biomembranes can provide insight into average membrane architecture and changes in this architecture, if, and only if, corrections are made for light scattering and flattening distortions which are common to all particulate systems (1). Typically the CD spectra of biomembrane suspensions show α-helical patterns but display shifts to longer wavelengths of the maximum, the two minima, and the cross over point when compared to the pattern of soluble α-helical proteins. In addition, and importantly for any attempt to estimate structure, the CD curves are significantly and variably dampened, depending on the particulate state of the membrane suspension. The anomalies are due to the suspension nature of the system and indeed are distortions, as has been well documented (1-10). A

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relatively simple and practical means of correcting these distorted CD spectra of membranes, called the pseudo reference state approach, has been developed (1). It is the purpose of this report to demonstrate the validity and applicability of this approach with a membrane whose protein structure has been independently described. Recently Henderson and Unwin (11-13) and Blaurock (14) published x-ray and electron microscopy studies of the purple membrane of Halobacterium halobium which indicated that bacteriorhodopsin, the single protein in this membrane (15), contains as much as 70-80% α-helix. The purple membrane, therefore, was chosen to corroborate the validity of the pseudo reference state method for the correction of CD data from particulate systems.

Materials and Methods

CD spectra were obtained at ambient temperature on a Cary 60 spectropolarimeter adapted with a model 6001 attachment for circular dichroism using the 100 mdeg range. UV spectra were recorded on a Cary 14 spectrophotometer with the sample cell placed close to the phototube in the cell compartment (16). The effective pathlengths of the cells were 0.233 mm for the suspensions and 1.7 mm for the membrane solutions. The final protein concentration, as determined by the Lowry method (17), was 0.53 mg/ml for the suspension and 0.053 mg/ml for the solution. Sonication was performed on ice with a Branson Sonic power sonifier at 7.5 amp for two 30 second intervals. Solubilization for the pseudo reference state was achieved by addition of stock sodium dodecyl sulfate, SDS (Eastman Organic Chemicals), to the membrane suspension first, followed by dilution to final volume with trifluoroethanol (TFE). The 80% TFE sample without SDS was filtered through a mitrex 5 μm Millipore filter giving a spurious high absorption due to absorbing material extracted by TFE from the filter itself. Trifluoroethanol, purchased from Halocarbon Products, Hackensack, N. J., was redistilled in glass with a Duflon column of glass beads (lower 3/4) and glass helices (upper 1/4). The first 10-15% of distillate and last 20-30% of pot residue were discarded. $NaHCO_3$ was added to the remainder to remove traces of acid. The purple membrane was prepared as outlined by Oesterhelt and Stoeckenius (15).

Results and Discussion

The spectrum of the purple membrane suspension (see Figure 1, Curve a) is characterized by red shifting and dampening. These features, common to all particulate systems, are due to light scattering and flattening distortions (1). Even when the suspension is sonicated (Figure 1, Curve b), the distortions are still apparent: the value of $[\Theta]^{224}$ is still only one-half the magnitude of a soluble protein which is 70-80% α-helical. Therefore it is apparent that corrections are required.

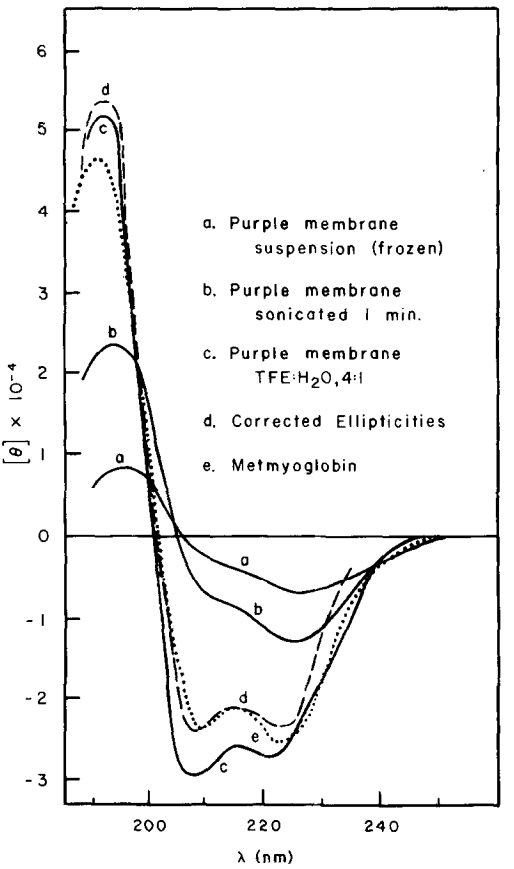

FIGURE 1: CD spectra of the purple membrane suspension which had been previously frozen (a) and after sonication (b) Curve d is the same corrected ellipticity as for Figure 2, Curve a. Note the marked correspondence between d and c, the membrane solubilized with 80% TFE and filtered to remove non-protein particles. Curve e is the spectrum of metmyoglobin which is 70% α-helix as determined by x-ray, reprinted with permission from Quadrifoglio, F., and Urry, D.W., J. Am. Chem. Soc. 90, 2755-2760, 1968. Copyright by the American Chemical Society.

With the pseudo reference state approach the corrected membrane spectrum was calculated, wavelength by wavelength, and is shown in Figure 1, Curve d and Figure 3, Curve d. In this calculation the membrane suspension and pseudo reference state (or solution) absorbances (see Figure 2), the former's CD

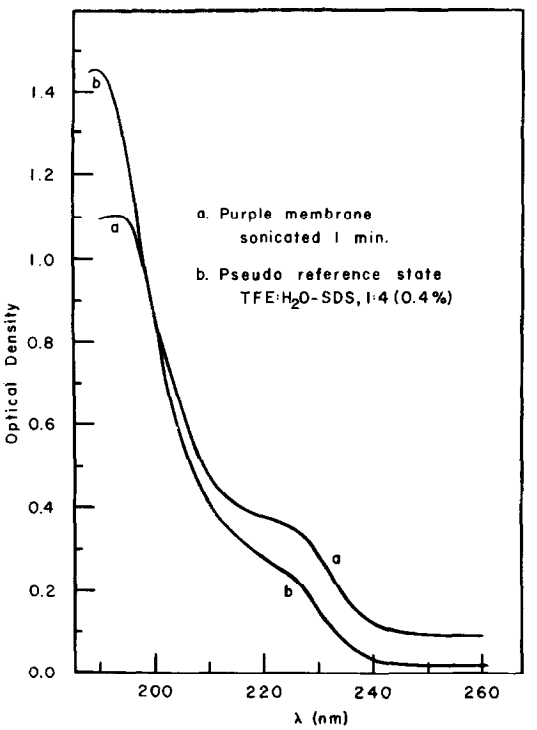

FIGURE 2: Absorbance data of the purple membrane suspension, (a): (0.53 mg protein/ml, 0.233 mm cell) and the purple membrane solution, (b): (0.053 mg protein/ml, 1.72 mm effective pathlength relative to a).

curve, the latter's ORD curve (Figure 3, Curve c), and Equation 1 were used.

$$[\theta]^{\lambda}_{corrected} = \frac{[\theta]^{\lambda}_{susp} - k\,[m]^{5/4}\,10^{-A_F}}{Q_A^2 \cdot Q_\sigma} \qquad (1)$$

$[\theta]^{\lambda}_{susp}$ is the suspension ellipticity at wavelength λ, k (=0.52) is a constant evaluated at one wavelength (1,16,18), [m] is the mean residue molar rotation of the pseudo reference state. It has been shown empirically that $[m]^{5/4}$ is a better fit than $[m]^{1}$ (19). A_F is the flattened absorbance at wavelength λ, Q_A is Duysens' correction factor for absorption flattening (20) and Q_σ is the correction factor for absorption obscuring due to light scattering.

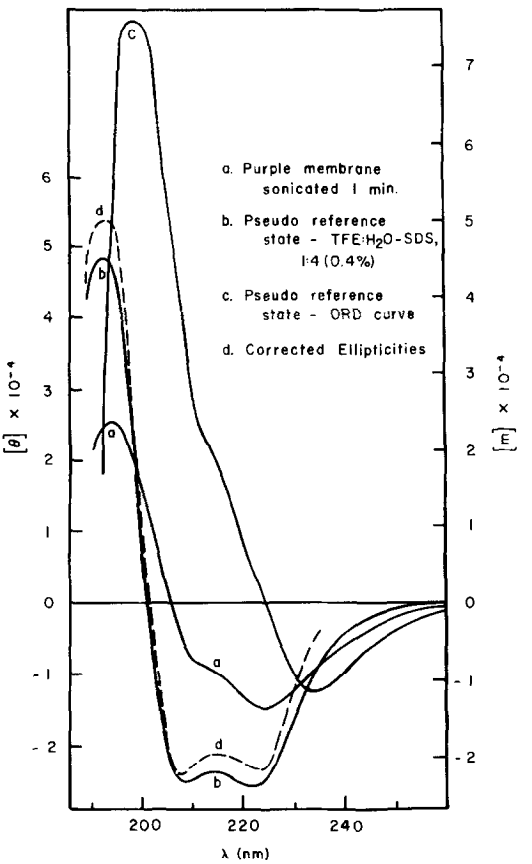

FIGURE 3: CD spectra of the purple membrane sonicated suspension (this is a different sample from Figure 1, Curve b), the purple membrane solution (a), and pseudo reference state (b), the pseudo reference state ORD (c), and the corrected ellipticity (d). The calculated percent of α-helix using Curve d is 73% for the purple membrane - in good agreement with the x-ray data indicating between 70 and 80% α-helix.

The TFE - SDS molecularly dispersed state (mds) of the purple membrane meets the criteria for an appropriate pseudo reference state by obeying Equations 2 and 3.

$$\left| [\Theta]^{224}_{mds} \right| > \left| [\Theta]^{224}_{C_1} \right| = \left| \frac{[\Theta]^{224}_{susp}}{(Q_A^{224})^2 \cdot Q_\sigma^{224}} \right| \qquad (2)$$

In the initial approximation indicated by the subscript, C_1, to $[\Theta]^{224}$, Q_A is

taken as 1, making $\left|[\theta]^{224}_{C_1}\right|$ a conservative value, which generally should be about 10 to 20% less than $\left|[\theta]^{224}_{mds}\right|$. In this case it was 9% less.

$$[\theta]^{190}_{mds} > [\theta]^{190}_{C_1} = \frac{[\theta]^{190}_{susp}}{(Q_A^{190})^2} \qquad (3)$$

In this approximation at 190 nm, Q_σ is taken as 1, making $[\theta]^{190}_{C_1}$ also a conservative value; here it was within in 25% of $[\theta]^{190}_{mds}$. Usually it should be within 10-30%. With the CD requirements met at critical wavelengths (224 and 190 nm) the pseudo reference state and membrane suspension absorption curves (Figure 2) and the pseudo reference state ORD curve (Figure 3c) were used to obtain the complete corrected ellipticity curve (Figures 1 and 3, Curve d). In Figure 3, Curve a is the membrane suspension spectrum; Curve d is its corrected spectrum which is in good agreement with the ellipticity of the pseudo reference state (Curve b). The characteristic ellipticity of an α-helix at the 224 nm extremum of the CD spectrum is -3.3×10^4. This gives a calculated value of 73% α-helix. X-ray and electron microscopy studies indicated bacteriorhodopsin in situ is between 70 and 80% α-helix (11-13). The CD value of 73% α-helix which was calculated from the purple membrane's corrected CD spectrum agrees well. This is to be compared with the estimate of 45% calculated from the uncorrected CD spectrum of the purple membrane by Becher and Cassim (22), which underscores the importance of correcting raw CD data of membranes. Not only do the corrected absolute magnitudes at the extrema match a protein with 73% α-helix, but also the shape of the corrected spectrum is appropriate. This is evident by comparing the metmyoglobin spectrum (Figure 1, Curve e) with the calculated curve for bacteriorhodopsin. The correspondence is striking showing that the corrections result in a similar calculated quantity of α-helix for the membrane protein bacteriorhodopsin and the soluble protein metmyoglobin.

TFE alone and in combination with detergents has proven especially effective in solubilizing membranes and other particulate systems (1,23-27).

It is interesting to note that the membrane suspension solubilized with 80% TFE without SDS and filtered (Figure 1, Curve c) has a CD spectrum close to the corrected spectrum. This suggests that, in a membrane TFE solution, the average secondary structural properties of the proteins are similar to those in the membrane. This does not necessarily mean that polypeptides retain the details of their membrane conformation in TFE.

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