# IMPROVED ELLIPTICITY DATA FOR SEVERAL BIOLOGICAL MEMBRANES

D.W. Urry, L. Masotti and J. Krivacic
Section of Molecular Biophysics
Laboratory of Molecular Biology
University of Alabama Medical Center
Birmingham, Alabama

Received September 11, 1970

SUMMARY: Ellipticity data for mitochondria, red blood cell ghosts, plasma membranes and sarcotubular vesicles are presented. The data are corrected taking into account light scattering and absorption flattening distortions. Corrections are applied at 224 mμ and 192 mμ which are wavelengths where differential scatter effects are negligible. The approach utilizes absorption and ellipticity data on a molecularly dispersed pseudo-reference state. The corrections result in greatly enhanced ellipticity values.

When compared to those of model α-helical polypeptides and proteins, the optical rotation patterns of biological membranes are of low magnitude and the extrema are red-shifted. These differences have variously been interpreted to reflect associating α-helices within the membranes (α-helix-helix interaction), α-helices imbedded in a lipid matrix, β-structure, and contributions of membrane lipids to the optical rotation patterns. It is our contention that the red-shift and reduced magnitudes of optical rotation patterns of biological membranes are primarily artifacts resulting from the particulate nature of the systems. The ellipticity corrections reported here result in magnitudes that correspond to model polypeptide systems which are approximately 50% α-helical. Such correlation, however, must be viewed in light of several systems which are not helical yet which give optical rotation patterns similar but not identical to those of the α-helix.

Less than two years ago there existed a consensus that optical rotation of optically active particulate systems did not contain artifacts but rather contained only an annoying decrease in the signal to noise ratio. In contrast to this accord, we suggested that such optical rotation spectra are distorted by a wavelength dependent obscuring of chromophores, (Urry and Ji, 1968) this

decrease in effective concentration being due to light scattering and due to the absorption flattening effect of Duysens (1956). In addition it has been shown that circular dichroism spectra of optically active particles are further distorted by a differential scatter of left and right circularly polarized light (Urry and Krivacic, 1970). Distortions of the expected form were observed with suspensions of the model α-helical polypeptide, poly-L-glutamic acid (Ji and Urry, 1969). While the complete, distorted spectrum of particulate poly-L-glutamic acid can be closely calculated (Urry, Hinners and Masotti, 1970), a relatively simple expression can be used to relate distorted and correct ellipticities at wavelengths near 225 mμ and 190 mμ (Urry, 1970). (These are wavelengths where the molar rotation is zero and hence where there is no differential scatter.) At these wavelengths a useful relationship between suspension and corrected ellipticities is

$$[\theta]_{susp} = [\theta]_{corr} (Q_A - A_S)$$

where $A_S$ is an absorption measured by the phototube, but it is that part of the absorption which arises from beam intensity losses due to light scattering. $Q_A$ is the flattening quotient defined as the ratio of the absorption by chromophores in the suspension, $A'_{susp}$, to the absorption by chromophores in the molecularly dispersed solution $A_{soln}$, i.e.

$$Q_A = \frac{A'_{susp}}{A_{soln}}$$

Similar distortions in circular dichroism spectra have been observed with whole mitochondria and electron transport particles (Ji and Urry, 1968) and with aggregates of membrane protein (Steim and Fleischer, 1969). The present communication reports ellipticity data on four membrane systems which were prepared following methods of Crane, Glenn and Green (1956) for beef heart mitochondria, of Ray (1970) for plasma membranes, of Dodge et. al. (1963) for red blood cell ghosts and of Seraydarian and Mommaerts (1965), for sarcotubular vesicles.

By simultaneously obtaining absorption and circular dichroism data from the

TABLE 1. Critical Values for Circular Dichroism for Several Membranes (ellipticities reported as $[\theta] \times 10^{-4}$)

| MEMBRANE SYSTEM | °224 mμ | | | 192 mμ | | | With .2% SDS sonication and TFE | | | Solvent system with maximal values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | As Prepared | Sonicated | With Correction | As Prepared | Sonicated | With Correction | °224 mμ | °208 mμ | 192 mμ | °224 mμ | °208 mμ | 192 mμ |
| $BHM_H$ Frozen | .28 | 1.07 | 1.65 | .26 | 1.82 | 3.22 | 1.53 | 1.62 | 2.97 | 1.53 | 1.62 | 2.97 |
| Plasma Membranes Frozen | .33 | .93 | 1.56 | .33 | 1.49 | 2.92 | 1.63 | 1.42 | 3.08 | 1.63 | 1.42 | 3.08 |
| Plasma Membranes Freshly Prepared | .58 | 1.00 | | .67 | 1.35 | | | | | 1.44 | 1.40 | 2.94* |
| Red Blood Cells Ghosts Frozen | .59 | 1.42 | 1.70 | .35 | 2.60 | 3.37 | 1.57 | 1.54 | 2.73 | 1.78 | 1.83 | 3.55 |
| Red Blood Cells Ghosts Freshly Prepared | 1.44 | 1.31 | | 2.18 | 2.53 | | | | | 1.90 | 1.93 | 3.91* |
| Sarcotubular Vesicles Freshly Prepared | .87 | .57 | 1.08 | .99 | 1.73 | 1.87 | 1.14 | 1.01 | 2.03 | 1.03 | 1.10 | 2.08** |

SDS: Sodium dodecyl sulfate
TFE: Trifluoroethanol
* in TFE; Membrane suspension/TFE = 1/4; ** in .2% SDS
$BHM_H$: Mitochondria ( beef heart)
o - : The values at 224mμ and 208mμ are negative

same phototube, it is possible to determine the relevant $A_S$. An initial $[\theta]^{224}$ can be achieved by taking $Q_A^{224}$ to be one and by using the absorption of the solublized membrane as $A^{224}_{soln}$. This is justified since there is little hyper- or hypochromism and since the absorption of the particle at this wavelength is so low that absorption flattening is small. Next a molecularly dispersed pseudo-reference state (PR) is experimentally found with an ellipticity at 224 mμ which approximates the initial corrected ellipticity, $[\theta]^{224}_{cl}$. Assuming an equivalence between absorption and ellipticity that is exhibited by α-helical-disordered systems, a set of relations can be used in an iterative manner to improve the corrected values at 224 mμ and 190 mμ. These values along with those of the freshly prepared membranes, sonicated membranes and solublized membranes are given in Table 1. The general conclusions are for a characteristic trend of enhanced ellipticities when the corrections are applied. That different membranes have significantly different ellipticities reflecting different average protein conformations is best demonstrated with the sarcotubular visicles which exhibit lower ellipticities at both 224 mμ and 192 mμ. Thus previous conclusions of similar membrane structures, based on the common features of damped and red-shifted optical rotation curves, are not substantiated. Also different behaviors of membranes with respect to dissolution by sodium dodecyl sulfate and/or trifluoro-ethanol imply different structural considerations (see Table 1).

## REFERENCES

Crane, A., Glenn, J.L., and Green, D.E., Biochem. Biophys. Acta, 22, 476, 1956.
Dodge, T.T., Mitchell, C., Hanahan, D.J., Arch. Biochem. Biophys. 100, 1963.
Duysens, L.N.M., Biochem. Biophys. Acta, 19, 1 (1956)
Ji, T.H. and Urry, D.W., Biochem. Biophys. Res. Commun., 34, 404 (1969)
Ray, T.K., Biochem. Biophys. Acta, 196, 1 (1970).
Seraydarian, K., Mommaerts, W.F.H.M., J. Cell Biology, 26, 641 (1965).
Steim, J.M., and Fleischer, S., Proc. Natl. Acad. Sci. U.S., 58, 1292 (1968).
Urry, D.W., Hinners, T.A. and Masotti, L., Arch. Biochem. Biophys., 137, 214 (1970)
Urry, D.W. and Ji, T.H., Arch. Biochem. Biophys., 128, 802 (1968)
Urry, D.W. and Krivacic, J., Proc. Natl. Acad. Sci. U.S., 65, 845 (1970).
Urry, D.W., in "Spectroscopic Approaches to Biomolecular Conformation" ed. by D.W. Urry, American Medical Association Press, Chicago, 1970, p. 33.