EFFECT OF PHOSPHOLIPIDS ON THE PROTEIN CONFORMATION IN THE INNER MITOCHONDRIAL MEMBRANES

L. Masotti, G. Lenaz, A. Spisni
Institute di Chimica Biologica dell'Università di Bologna, Italy
and
D.W. Urry
Laboratory of Molecular Biophysics
University of Alabama in Birmingham
Birmingham, Alabama

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SUMMARY: Lipid extracted inner mitochondrial membranes, studied by circular dichroism, exhibit a mean protein conformation which differs from that of intact mitochondria. Reconstitution of the membranes by addition of different phospolipid preparations restores to varying extents the original mean protein conformation. The results demonstrate the effect of lipid in modulating protein conformation and of the lipids studied cardiolipin is the most effective in restoring ellipticity values to those of the intact mitochondrial membrane.

INTRODUCTION

Lipid requirements for the activity of several membrane bound enzymes have been well documented in the past few years. The results of these investigations point out the physiological significance of lipids in the lipoprotein enzymic complexes. A more complete role for lipids was suggested (1) introducing the concept of membranous subunits in which the lipids could also play a structural role of preventing the membrane subunits from polymerizing to give a tridimensional array. Indirect evidence that kinetic parameters of membrane bound enzymes can be modified by the composition of the membrane suggest the possibility that lipid evokes a conformational change in the proteins (2,3).

Thus the possibility that lipids act as modulators of protein conformation within the biological membranes deserves careful investigation. Use of CD allows evaluation of the secondary structure of soluble proteins and has the advantage of directly observing samples which have undergone limited structural modification and which, significantly, retain their biological activity.

When CD is applied to the study of insoluble membrane proteins, distortions arise from the particulate nature of the samples. The distortions, first recog-

nized by Urry and Ji in 1968 (4), can be largely corrected (5-10) thus making it possible to obtain meaningful data by means of this technique. Several biological membranes have been investigated (11-14) and a correlation found between magnitude of ellipticity and function of these systems: in particular, the higher and more complex the enzymatic activity, the larger the magnitude of the ellipticity of the membranes.

If lipids play a role in varying the conformation of proteins, CD provides a method with which to demonstrate this role. The present communication reports CD studies on beef heart mitochondria extracted of phospholipids and then re-associated with different phospholipids.

MATERIALS AND METHODS

Beef heart mitochondria, lipid-depleted membranes (LDM), phospholipid dispersions, as well as membranes reconstituted by reassociating lipids and LDM, were prepared as previously described (15).

Before running the CD spectra, 0.5 ml aliquots of the samples were sonicated at 4°C using the microtip of a Branson S 75 sonifier in order to decrease the particle size. The intensity was set at 5 and the sonication time was 7 minutes. The protein concentration, determined according to Lowry, was in the range of 1.0 to 2.0 mg/ml.

The CD spectra were run with a Cary 60 spectropolarimeter modified to obtain simultaneous recording of ellipticity and absorbance in order to correct the CD spectra for light scattering and absorption flattening distortions. The measurements were carried out using a 0.1° scale and a cell whose pathlength was 0.1 mm.

RESULTS AND DISCUSSION

The CD pattern of intact beef heart mitochondria, once corrected for the distortions arising from their particulate nature as previously reported (11), closely resembles that of polyaminoacids in the α-helical conformation.

The uncorrected molar ellipticities of beef heart mitochondria are -0.28×10^4 and -0.17×10^4 at 224 and 208 nm, respectively. LDM also show low ellipticities at these wavelengths as seen in Figure 1. The dampening of the 224 nm

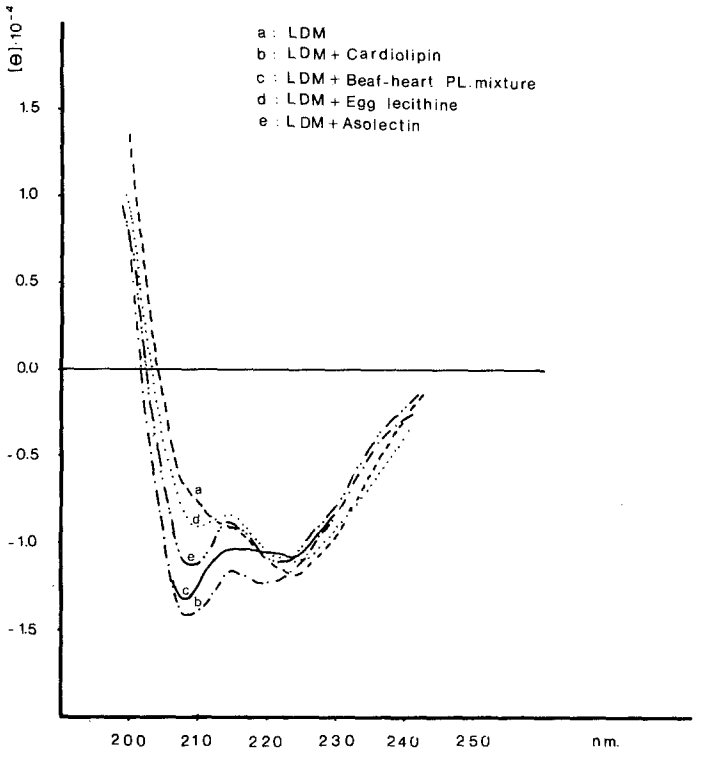

Fig. 1 Uncorrected circular dichroism spectra of lipid-depleted mitochondria (LDM) and membranes reconstituted by incubating LDM with different phospholipids. DL: dimyristoyl lecithin. EL: egg lecithin. BL: beef heart phospholipid mixture.

and the 192 nm bands, the loss of the 208 nm band, as well as the red shift of the extrema and the crossover point, can be recognized as a result of the particulate distortions. It is observed, however, that the 208 nm band is partially restored by sonicating the sample in the case of intact mitochondria, the band being restored to an ellipticity of 1×10^4, while it remains essentially lacking in the case of LDM.

The CD pattern of LDM is noticeably changed to various extents by re-addition of phospholipids. Even if not corrected for distortions, the amplitudes of the 224 and 192 nm bands are improved and the 208 nm band partially restored.

In order to achieve a more meaningful interpretation of these data, however, corrections for light scattering and absorption dampening can be applied.

A suitable solubilized state (10) was chosen and the corrected ellipticities calculated by means of the following equation:

$$[\theta]_{corr.} = \frac{[\theta]_{suspension}}{Q_A^2 \, Q_\sigma} \qquad (1)$$

where Q_A is the absorption flattening quotient and Q_σ is the absorption obscuring quotient derived from light scattering effects, Eqn 1 neglects differential light scattering.

A solubilized state was achieved by treating the membranes with sodium dodecyl sulfatate (SDS) and trifluoroethanol (TFE). However, the absorption data in the spectral region between 200 and 185 nm so obtained proved to be unsuitable for carrying out complete spectral corrections. The solubilized state did not satisfy the requirements of a pseudo reference state (10) but was adequate for improving the negative bands.

Particularly when dealing with reconstituted membranes, their heterogeneous composition should be taken into account. The corrections applied so far to the CD spectra have been derived studying homogeneous polypeptides; therefore in such systems the light scattering distortions in the absorption and CD spectra are strictly related. When studying membranes, one deals with systems comprised of lipids and proteins and the relation between light scattering in CD and that observed in absorption will depend on their lipid/protein ratio. In view of these considerations, volume fraction corrections have been proposed (16); the relation between X_{sp}, the fraction of light scattered from the protein, and X_s, the total amount of scattered light is

$$X_{sp} = f(V_{fp}) \, X_s \qquad (2)$$

where V_{fp} is the volume fraction of protein. While details of $f(V_{fp})$ are under study, a first power factor V_{fp}, which surely will improve the corrections, has been used, the factor being

$$V_{fp} = \frac{W_p/d_p}{W_p/d_p + W_\ell/d_\ell} \qquad (3)$$

where W_p and W_ℓ are the weights per cent of protein and lipid and d_p and d_ℓ the densities of protein and lipid, respectively. Mean values of 1.35 and 0.97 for d_p and d_ℓ, respectively, have been used (17).

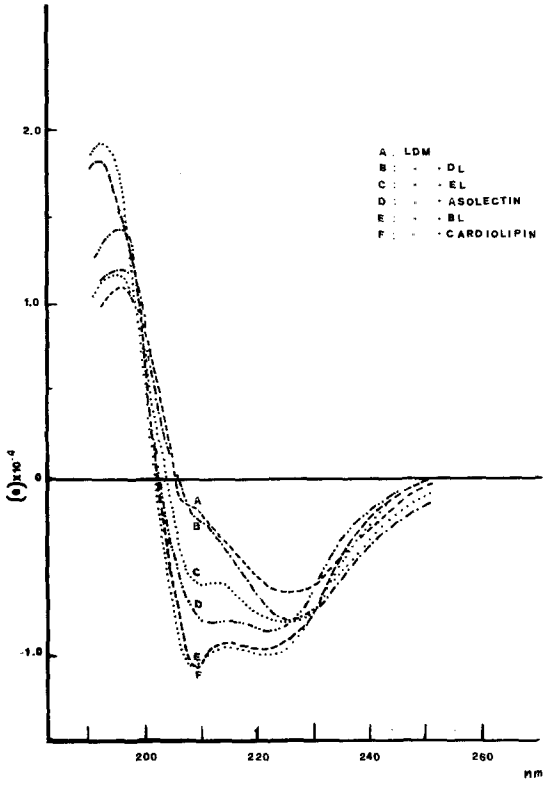

Fig. 2 Corrected circular dichroism spectra of the systems shown in Figure 1.

The corrected curves are shown in Figure 2. All the membrane systems show CD patterns whose ellipticities are greatly improved; the extrema of the troughs are blue shifted to 224 nm for LDM and to 220-222 nm for all the other samples.

It is worthwhile to note that although LDM, corrected also for differential light scattering, show an improvement of the ellipticity at 224 nm, they still exhibit virtually no 208 nm band suggesting that aggregation alone does not explain the CD spectrum. The results indicate that lipid extraction causes an extensive modification of the protein conformation within the membrane. Addition of phospholipids restores, to different extents, a CD pattern which resembles that of intact mitochondria. The ellipticity values of LDM reassociated with cardiolipin are similar to those shown by intact mitochondria; particularly evident is the restoration of the 208 nm band to large ellipticities. This

finding is of particular interest since cardiolipin is a major phospholipid component of the inner mitochondrial membrane. Furthermore, recent studies (18) have shown that cardiolipin is tightly bound to cytochrome oxidase and is required for its activity. ESR studies (19) also show that cardiolipin is strongly immobilized in the membrane as a consequence of its interaction with the oxidase.

It can be anticipated that when full spectral corrections are obtained in the region below 200 nm even larger ellipticity values will be obtained.

The results reported in this paper indicate that when particulate distortions are properly considered, lipid interactions alter the CD pattern of the proteins of the inner mitochondrial membrane, thus demonstrating a role for lipids in modulating the secondary structure of the membrane proteins with cardiolipin being the most effective lipid investigated.

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