OPTICAL ROTATION OF MITOCHONDRIA AND ITS RELATION TO CONFORMATION

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Ultrastructural studies have recently established that macromolecular structural changes accompany transitions between metabolic states in mitochondria (Hackenbrock, 1966; Weinbach, Garbus and Sheffield, 1967; Deamer, Utsumi and Packer, 1967; Harris, Penniston, Asai and Green, 1968). We are interested in establishing if molecular conformational changes accompany the macromolecular structural changes that mitochondria manifest during changes in metabolic state. Optical rotatory dispersion (ORD) and circular dichroism (CD) studies have previously been used to investigate the conformation and interrelationships between membrane protein and lipids (Ke, 1965; Wallach and Zahler, 1966; Lenard and Singer, 1966; Urry, Mednieks and Bejnarowicz, 1967; Steim and Fleischer, 1967). The spectra of membrane preparations show a curve in the ultraviolet characteristic of an α-helix, but with a red shift of 2 mμ to 6 mμ depending upon the preparation. Urry, Mednieks and Bejnarowicz (1967) also report a dispersion curve of intact mitochondria similar in shape to membrane fragments but with much lower amplitude and greater variability. The oscillatory state provides a system particularly suited to the investigation of conformational changes accompanying metabolism since large changes in mitochondrial ultrastructure are associated with different metabolic states (Packer, Utsumi and Mustafa, 1966; Utsumi and Packer, 1967) and glutaraldehyde fixation provides a means for trapping a population synchronized in a uniform state for conformational study (Deamer, Utsumi and Packer, 1967).

METHODS

Rat liver mitochondria were isolated in sucrose-Tris-EDTA at pH 7.5 and put into the oscillatory state as previously described (Packer, Utsumi and Mustafa, 1966). Kinetics of structural changes during an oscillation were conveniently recorded using 90° light-scattering measurements at 546 mμ. Mitochondria were fixed at various phases of the oscillation by adding 0.2 ml of 13.6 % glutaraldehyde (in 10 mM sodium phosphate buffer, pH 7.8) to 3.3 ml

of reaction mixture. An aliquot of suspension was removed, stored for 1 hr at 0° and then centrifuged at 8000 x g for 10 min. The pellet was resuspended in water, recentrifuged as above, and then resuspended in water or 90 % glycerol. ORD measurements were made at room temperature in a Cary 60 spectropolarimeter using a 1 mm cell. CD spectra were obtained at 25° on a Jasco Model ORD/UV-5 apparatus. Protein concentrations were measured on unfixed samples by the Folin method (Lowry, Rosebrough, Farr and Randall, 1951). Poly-L-glutamic acid (MW 66,000) was obtained from Pilot Chemicals Inc. (Watertown, Mass.) and its spectrum measured at 0.05 % concentration. Submitochondrial fragments were prepared by sonicating isolated mitochondria in distilled water and isolating the fraction between 8,000 x g for 10 min and 100,000 x g for 60 min by centrifugation.

RESULTS

A typical light-scattering trace of mitochondria in the oscillatory state is shown in Fig. 1 (upper curve). Previous work (Deamer, Utsumi and Packer, 1967) has shown that a highly condensed inner-membrane system is present at high levels of light scattering, aerobic in state I and anaerobic in state IV. When the light scattering is minimal (state II) the inner membrane expands to give rise to well-defined cristae and a morphology typical

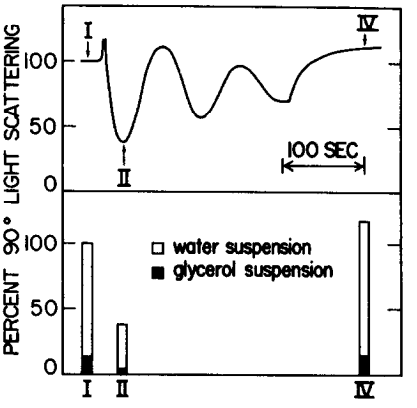

Fig. 1 - Light-Scattering Levels of Glycerol and Water Suspensions of Glutaraldehyde-fixed Mitochondria. The aerobic reaction mixture (3.3 ml) contained sucrose (0.1 M), EDTA (0.5 mM), Tris-HCl buffer (3 mM, pH 7.8), sodium succinate (1.5 mM) and rat liver mitochondria (ca. 2 mg protein/ml) at 25°. Oscillations were induced by the addition of sodium phosphate (30 mM, pH 7.8) and kinetics of structural changes followed by 90° light scattering (upper curve). An aliquot of redistilled glutaraldehyde (13.6 %) in 10 mM sodium phosphate buffer was added to a final concentration of 0.8 % for direct fixation in the cuvette. Samples were removed, and after 1 hr at 0°, centrifuged and resuspended in water or 90 % glycerol and their light-scattering level determined (lower figure).

of mitochondria in vivo is assumed. Since high light-scattering levels can lead to artifacts in ORD spectra (Urnes and Doty, 1961), the effect of these high levels of light scattering on ORD parameters of mitochondria in different structural states was first investigated. To examine this, a comparison was made between water and 90 % glycerol suspensions where the light-scattering levels are considerably reduced (Fig. 1, lower portion). ORD spectra of glutaraldehyde-fixed mitochondria suspended in water gave a low signal-to-noise ratio and showed variation in specific rotation and wavelength characteristics as the samples increased in concentration (Fig. 2). At high light-scattering levels, the trough shifted to lower wavelengths. In contrast, samples in glycerol gave a much higher signal-to-noise ratio and when plotted as specific rotation the spectrum of any particular state showed no change with sample concentration until very high concentrations where light-scattering again began to affect the spectra. However, the mean residual rotation increased in all the glycerol samples compared to samples in water. This was confirmed by measuring the spectra of poly-L-glutamic acid in 90 % glycerol where the specific rotation increased by a factor of 1.6, but the spectrum was not altered in wavelength characteristics. An additional check on the effect of light scattering on ORD spectra was made using a differential technique with suspended mitochondria in both the initial and returning beam. Mitochondrial rotation was thus cancelled out but high light scattering remained. The spectrum of poly-L-glutamic acid, placed in the initial beam,

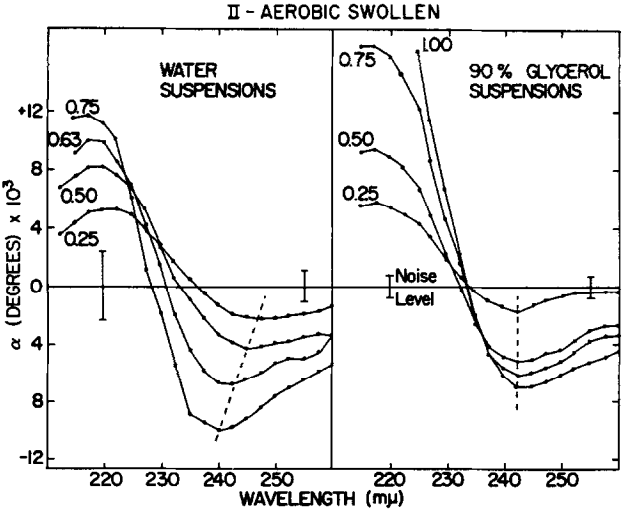

Fig. 2 - ORD of Glutaraldehyde-fixed Mitochondria Resuspended in Water and 90 % Glycerol. The samples were fixed in state II (aerobic swollen) as described in the legend to Fig. 1 and resuspended at different protein concentrations (1.00 = 1.76 mg protein/ml).

was not altered in this system although signal-to-noise ratios were reduced.

ORD spectra of glutaraldehyde-fixed mitochondria show significant differences in curve magnitude between state II and states I and IV (Fig. 3). In addition, states I and IV were consistantly shifted 2 mμ further to the red than state II. The spectral characteristics of the three states are given in Table I together with those for poly-L-glutamic acid and mitochondrial membrane fragments. The spectra of intact mitochondria all exhibit a pronounced red shift greater in magnitude than that reported for membrane preparations by other workers (Wallach and Zahler, 1966; Urry, Mednieks and Bejnarowicz, 1967) and greater than that showed by mitochondrial membrane fragments in the present experiments. This cannot be attributed to an artifact of fixation since the ORD curves of poly-L-glutamic acid and membrane fragments showed no detectable change under the conditions of glutaraldehyde fixation which we employ. A further feature apparent in mitochondrial spectra compared with poly-L-glutamic acid is that the peak of the curves are shifted to a greater extent than the troughs.

The 222 mμ minimum in the CD of the α-helix curve was also found to be

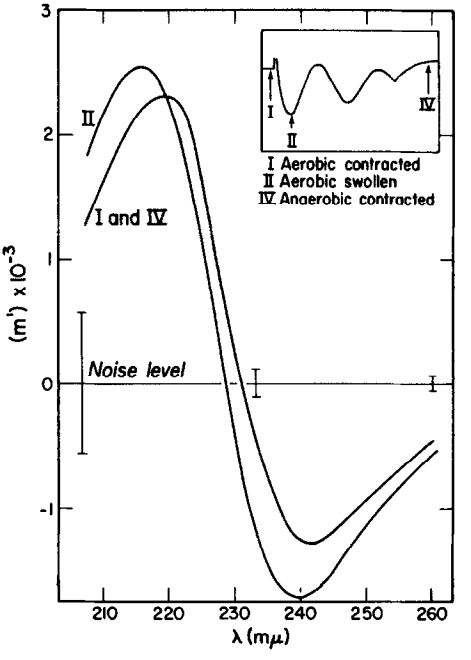

Fig. 3 - ORD of Mitochondria Fixed with Glutaraldehyde at Different Phases of the Oscillatory State. The position of fixation in the oscillatory cycle is shown in the insert. Oscillatory state conditions and glutaraldehyde fixation as in Fig. 1. Samples were resuspended in 90 % glycerol to the same protein concentration (0.8 mg/ml).

TABLE I

ORD AND CD PARAMETERS OF MITOCHONDRIAL STATES

Sample	ORD					CD
	[m][b] (degrees cm^2 gm^{-1})	λ_{min} (mμ)	$\lambda_{crossover}$ (mμ)	$\lambda_{inflection}$[c] (mμ)	λ_{max} (mμ)	λ_{min} (mμ)
Mitochondria[a]						
I Aerobic contracted	3,540 ± 130	241 ± 1	231 ± 1		218 ± 3	232 ± 1
II Aerobic swollen	4,320 ± 160	239 ± 1	227 ± 1		215 ± 3	
IV Anaerobic contracted	3,650 ± 260	241 ± 1	231 ± 1		218 ± 3	
Submitochondrial fragments	20,900 ± 2,000	237 ± 1	226 ± 1	214 - 217	201 ± 3	224 ± 1
Poly-L-glutamic acid	74,000 ± 2,000	233 ± 1	223 ± 1	212 - 217	198 ± 1	222.5[d]

[a] Measured in 90 % glycerol.

[b] Calculated from peak to trough values assuming mean residual weight = 115, n_{235} mμ glycerol = 1.69; n_{265} mμ water = 1.37.

[c] Inflections were not detectable in mitochondrial samples.

[d] Cassim and Yang (1967).

shifted in the mitochondrial spectrum by an amount equivalent to the shift in the ORD trough (Table I) further confirming the absence of a wavelength-dependent light-scattering artifact. Because of low signal-to-noise levels, however, it was not possible to check the movement of the 208 mμ and the 191 mμ bands in the CD spectrum of intact mitochondria.

DISCUSSION

This investigation shows that the ORD and CD spectra of mitochondria reveal differences which depend on the functional and structural state of the organelle when the mitochondrial population is uniform with respect to ultrastructure. The long wavelength shift in the ORD and CD spectra of mitochondria and submitochondrial fragments is in agreement with the work of Urry, Mednieks and Bejnarowicz (1967). However, in our experiments a larger red shift is shown with mitochondria than with fragments. In this respect, Steim and Fleischer (1967) obtained evidence for a protein-protein interaction, accompanying aggregation in mitochondrial structural protein which induced a shift of the conventional α-helical Cotton effect to higher wavelengths. Aggregation was also accompanied by decrease in rotation. Both the red shift and decrease in rotation are seen in the present work when mitochondria are compared to fragments. Of these two parameters the magnitude of the Cotton effect would seem to be a more sensitive indicator of conformational change. ORD and CD parameters would therefore appear to depend closely on the structural organization of the organelle.

A comparison of aerobic contracted and anaerobic contracted mitochondria (states I and IV) shows no detectable difference in ORD despite the difference in oxidation-reduction state of respiratory enzymes at the time of fixation. Therefore, the large difference in rotary strength, and the red shift observed in aerobic swollen (state II) as compared with aerobic (and anaerobic) contracted mitochondria would not appear to result from oxidation-reduction changes. It is evident from electron microscopy (cf. Hackenbrock, 1966; Deamer, Utsumi and Packer, 1967) that the main ultrastructural changes between states are an unfolding of the inner membranes and an expansion of the inner membrane compartment causing an apparent deaggregation of matrix material. Unfolding (or unmasking) of the membrane would be expected to increase the OR but not to give rise to a change in wavelength characteristics. In view of this, it is tentatively suggested that changes in the aggregation state of mitochondrial protein at both macromolecular and molecular levels occur during mitochondrial swelling.

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