EFFECTS OF THE OXIDATION STATE, LIGANDS, DETERGENTS AND AGING ON THE CONFORMATION OF CYTOCHROME OXIDASE

Yash P. Myer
Department of Chemistry, State University of New York at Albany, Albany, New York 12203

Tsoo E. King
The Laboratory for Respiratory Enzymology, Oregon State University, Corvallis, Oregon 97331

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Recent observations of oxidation state-dependent conformational alterations of respiratory enzymes by ORD[1] and CD measurements have given added support to the conformational mechanism of electron transfer in mitochondria. We have applied the CD technique to conformational studies of the terminal enzyme of the electron transport chain, viz. cytochrome oxidase. This note reports the effect of aging and detergents on the dichroic spectrum of ferri and ferro oxidases and of their complexes with carbon monoxide and cyanide. Both DOC, an ionic detergent, and aging of the preparation produce significant conformational alterations of the enzyme, especially in the reduced state. The change from Fe^{3+} to Fe^{2+} state of oxidation results in changes of the CD spectrum which, contrary to earlier reports (Urry et al., 1967; Urry and vanGelder, 1967), suggest an environmental change from rather asymmetric conformation to a fairly symmetric environment.

Experimental -- Cytochrome oxidase, two independent batches, was isolated from the Keilin-Hartree preparation of beef heart by our modification of the procedure developed by Okunuki et al. (1958). The

1. Abbreviations used, CD, circular dichroism; DOC, deoxycholate; ORD, optical rotatory dispersion.

modification permitted the isolation within 24 hours. Emasol-1130, a neutral non-ionic detergent, which is compatible with the natural lipid in the enzyme, was used as a dispersing medium. The preparations were kept in solution at 375 μM in terms of hematin a in 0.1 M phosphate buffer and 1% Emasol. The analytical parameters characterizing the preparation when determined according to the procedures reported by Takemori and King (1965) showed: heme a, 10.5 mμmole/mg; copper, 11.2 mμ atom/mg; lipid, 18-20%; turn-over number, 80-100 electron equivalents per sec per heme at pH 7.4; and specific activity in terms of the first order rate constant, 15 per sec per mg of protein in a 3 ml assay system at pH 5.75. Ultracentrifugation of the preparation showed a single symmetrical peak. The CD measurements were made on a modified dichrograph (Myer and MacDonald, 1967) using a thermostated cell of path length of 10 mm. The molar ellipticities were calculated by using a molecular weight of 100,000 per heme group. The stock solution was appropriately diluted with Emasol-phosphate buffer, and the aging was recorded 24 hours after preparation. The reduction of the preparation was made by addition of a slight excess of solid $Na_2S_2O_4$ after bubbling oxygen-free helium through the solution; the carbon monoxide complex by bubbling CO (generated by dehydration of formic acid); and oxidation of the reduced samples by adding solid potassium ferricyanide.

Results and Discussion -- As shown in Fig. 1, the CD spectrum of oxidized oxidase is more or less independent of the ionic nature of the detergent, but that of reduced oxidase exhibits significant dissimilarities dependent upon the nature of the detergent present. The complexity seen in the dichroic spectra in the presence of DOC (extrema at 445, 431 and about 415 mμ, consistant with those reported by Urry et al. (1967))is absent in the spectrum of oxidase in the presence of Emasol (a single positive peak at 445 mμ). An analogous inconsistancy has also been observed in the ORD spectra. Addition of DOC to the Emasol system, corresponding to about 50% decrease in enzymatic activity, however, yields ORD spectra which approach

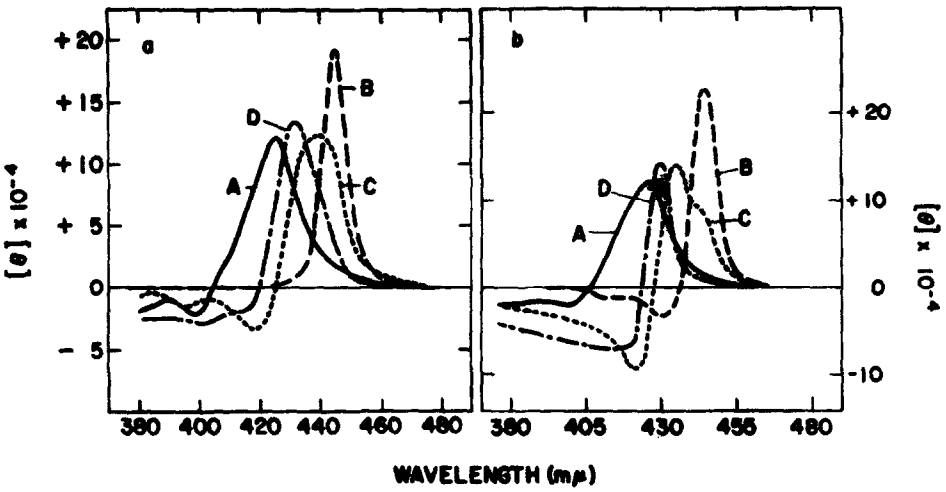

Fig. 1. Circular dichroism spectra of native and carbon monoxide complexes of oxidized and reduced cytochrome oxidase. 0.05 M potassium phosphate, pH 7.4; temperature 22°. a. 0.25% Emasol; b. 2% deoxycholate. A, oxidized; B. reduced (oxidized + sodium dithionite); C, carbon monoxide complex of reduced oxidase (reduced oxidase + carbon monoxide); and D, carbon monoxide complex of oxidized oxidase (carbon monoxide complex of reduced oxidase + potassium ferricyanide).

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those reported by Urry (King, Yong and Bailey to be published). Complexity similar to that in the presence of DOC is also obtained upon aging of the enzyme (Fig. 2). The effect of DOC is almost instantaneous, whereas the effect of aging is relatively slow and extends up to 14 days at 2° and to about 4 days at room temperature. It therefore indicates that both the presence of DOC and aging of the preparation cause similar, if not identical, changes in the environment of the enzyme. Small but significant differences are also evident (Fig. 1) in the CD spectrum of the reduced CO-complex, as well as in the "oxidized CO-complex", in the presence of the two types of detergents. The alteration of the simple CD spectrum of oxidized oxidase to a rather complex reduced spectrum in the presence of DOC (Fig. 1b) has been interpreted by Urry et al. (1967) and by Urry and vanGelder (1967) as due to the removal of the double-degeneracy of the heme Soret transition in the reduced state, possibly due to the juxtaposition of the two heme groups, thus suggesting the nature of the conformational alterations associated with the

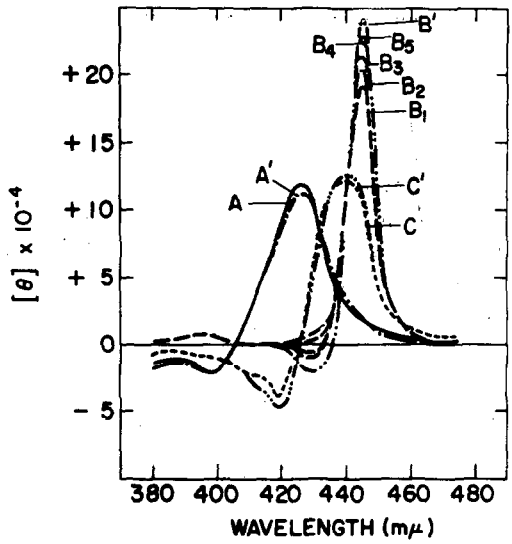

Fig. 2. Effect of aging on the CD spectra of oxidized, reduced, and carbon monoxide reduced complex of cytochrome oxidase. 0.05 M phosphate + 0.25% Emasol; pH 7.5; temperature 22°. Cytochrome oxidase was stored in solution at 375 μM in terms of hematin a in 0.1 M phosphate + 1% Emasol, pH 7.4. The program of the aging experiment is as follows:

Sample	C. D. spectrum	Temp. in storage	Storage time (days)
A	oxidized	2°	14
A'	oxidized	room temp.	4
B_1	reduced	2°	2
B_2	reduced	2°	5
B_3	reduced	2°	6
B_4	reduced	2°	7
B_5	reduced	2°	14
B'	reduced	room temp.	4
C	CO-reduced	2°	14
C'	CO-reduced	room temp.	4

change of the valence state of the heme irons. Since dichroic features similar to those observed in DOC are absent in biologically-active preparations containing Emasol (a natural lipid-like detergent) (Fig. 1a and 1b), since the ionic detergents are known to inactivate the enzyme (Yonetani, 1959) and furthermore, since the addition of Emasol partially regenerates the activity (Lemberg et al., 1964), we therefore interpret the behavior of oxidase in the presence of DOC as not that of the native molecule, but of a

system whose conformation has been significantly altered by the detergent. Prolonged contact of the preparation with Emasol also causes alterations comparable to those seen in systems containing DOC (Figs. 1a and 2). This is consistant with the observation that the enzymatic activity of oxidase decreases with increase of the storage time (cf. Gibson and Greenwood, 1963). The conclusions on the oxidation state-dependent conformational phenomenon based on investigations in the presence of ionic detergents, therefore, must be treated with caution.

The dichroic spectra of oxidized and reduced oxidase in the presence of Emasol (Fig. 1a) seem to be rather simple, each exhibiting a single positive peak in the Soret absorption region. However, the Gaussian analyses of the native protein (Myer, 1968), as well as those of the cyanide and carbon monoxide complexes (Myer and King, unpublished data) show that the positive limb of the oxidized CD spectrum is a summation of at least four Gaussian bands and that of reduced oxidase is a composite of two. Since the heme Soret transition is double-degenerate, the presence of four Gaussian bands in the dichroic spectrum of oxidized oxidase is consistant with the ideas that (a) there exist contributions from two chromophores, namely the two heme a groups, and the chromophores are asymmetrically located so that the double-degeneracy is non-operational, and (b) the two moieties are conformationally distinct so that the magnitude of splitting of the transition is different for the two chromophores.

The presence of only two Gaussian bands in the dichroic spectrum of reduced oxidase, on the other hand, suggests (a) the two moieties are symmetrically oriented so that the degeneracy is operational and (b) the moieties are still conformationally distinct so as to exhibit a single positive band at slightly different wavelengths. The absence of ellipticity bands with opposite signs in the Soret region (an expected occurrence due to splitting produced by the interaction of identical chromophores (Moffitt et al. 1957; Tinoco, 1962)) further indicates that the symmetry-perturbing

factors, such as the heme-heme interactions, are absent. The change of valence state of heme iron therefore induces alteration of the symmetry degeneracy of the Soret transition, thus suggesting the environmental differences of the heme moiety in the two oxidation states of iron, symmetrical in the reduced state and rather asymmetric in the oxidized state. The alteration of the asymmetric heme environment may possibly be due to alteration of induced conformational factors, such as the conformation of the polypeptide chain surrounding the heme group and/or the conformation of the orientation of the group, or it may be due to alteration of the intrinsic asymmetry of the chromophore, i. e., involvement of changes of the chemical nature of the central coordinated complex and/or the geometry of the heme iron corresponding to the heme plane. Dichroic investigations in the intrinsic absorption region of the native and of the CO- and CN-complexes, however, suggest the latter as t possible explanation. This and other aspects are being presently investigated.

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