EVIDENCE FOR CONFORMATIONAL DIFFERENCES BETWEEN OXIDIZED AND REDUCED CYTOCHROME OXIDASE

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Previous studies on the optical rotatory dispersion of cytochrome c have shown this technique to be sensitive to different conformational states and to be particularly suited for determining changes in the heme environment (Ulmer, 1965; Urry and Doty, 1965; Urry, 1965, and Myer and Harbury,1965). This communication reports the optical rotatory dispersion (ORD) and circular dichroism (CD) of an enzymatically active cytochrome oxidase preparation* (Wainio, 1964).

While cytochrome oxidase presents additional difficulties in terms of purity, solubility, and stability, it provides an interesting example of a cytochrome which occupies the singular position at the oxygen terminus of the electron transport chain. One would like to know if the conformational changes observed upon reduction of cytochrome c are common to other cytochromes. Specifically, is there a change in the heme environment, and is there a detectable change in the protein structure? Uniquely in the case of cytochrome oxidase, information on the question of possible heme proximity can be sought.

In addition to heme protein the lyophilized material used in this study

* Preparations of cytochrome oxidase obtained by the procedure of Fowler, Richardson, and Hatefi (1962) have subsequently been studied. These give results (Urry and van Gelder) which are qualitatively similar to those presented here. The interpretations are independent of the preparation used.

contained 40-50% deoxycholate, 5-10% lipid, and 35-45% phosphate buffer salts. Using a molar extinction coefficient for the α band of 17.1×10^3 and a molecular weight of 72,000 per heme, cytochrome oxidase protein was estimated at 55%. Reconstitution of the lyophilized material with distilled water resulted in a solution at a pH of 8. The ORD curves were determined on a Cary Model 60 spectropolarimeter and the CD curves were obtained by using a prototype circular dichroism attachment built by Cary Instruments for the Model 60. As deoxycholate is optically active, exhibiting a positive plane curve in the 650-190 mμ range, a thermostatted differential ORD cell block was employed. The differential ORD technique is made possible by the beam configuration of the ORD instrument (the beam being reflected back through the cell compartment before striking the analyzer). Deoxycholate was added to a cell in the return beam bringing the rotation at 650 mμ to zero, that is, the contribution of the protein to the rotation at 650 mμ was assumed negligible. No attempt was made to compensate for the optical activity of lipid present in the preparation; however, the optical rotation of purified mitochondrial phospholipids in trifluoroethanol or in aqueous duponol solutions is minimal. The cytochrome oxidase samples were run at 15°C and reduction was achieved by addition of a small amount of dithionite.

The optical rotation results for cytochrome oxidase in the oxidized and reduced forms are presented in Figs. 1 through 4. Both in ORD (Fig. 1) and CD (Fig. 2) the Soret Cotton effects show marked dependence on the state of oxidation. Thus the heme environment is different in the two states. That marked changes in the shapes of the ORD and CD curves in the Soret region imply changes in the heme environment may be concluded from considerations of the sources of rotational strength of the transition and of changes in the complexity of the Soret Cotton effect. The direct experimental check on such an interpretation for heme systems is provided by myoglobin. The ORD results of Samejima and Yang (1964) show that the Soret Cotton effect of deoxy-

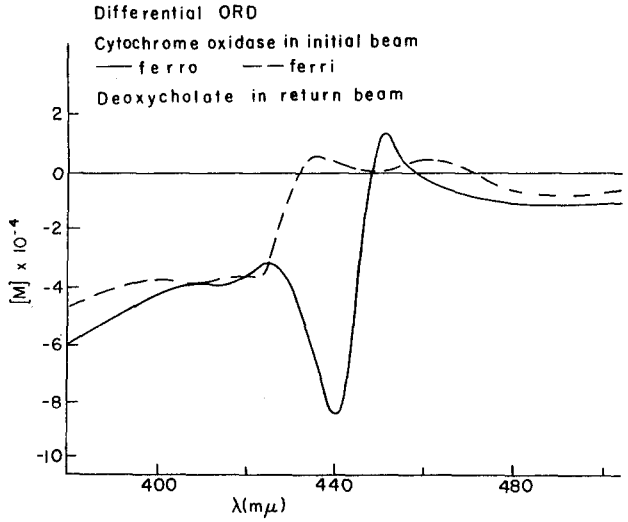

Fig. 1 Difference optical rotation of cytochrome oxidase. The molar rotation is plotted on a per heme basis.

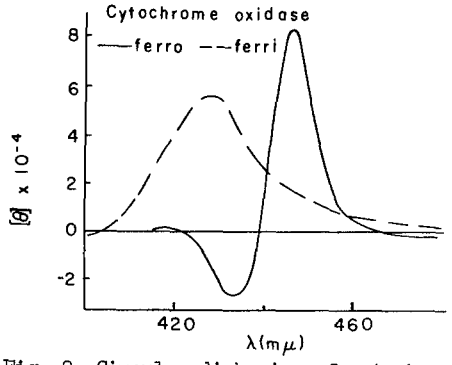

Fig. 2 Circular dichroism of cytochrome oxidase in the Soret region, plotted on a per heme basis.

myoglobin differs from that of metmyoglobin by little more than a long wavelength shift. There is, on the basis of these results, no detectable change in the heme environment of metmyoglobin upon reduction. This conclusion is in accord with the x-ray data of Kendrew, Watson and Nobbs (1966) in which the immediate heme environment of deoxymyoglobin is found to differ from that of metmyoglobin only in the absence of the coordinated water molecule. The relative orientation of protein groups and heme is unchanged.

The positive Cotton effect (Fig. 2) for the Soret band of ferricytochrome oxidase allows the interpretation that either the heme moieties are greater than 10-15Å apart or their relative orientation is such that the dipole interaction is negligible. The complex Cotton effect (Fig. 2) for the Soret band of ferrocytochrome oxidase with a positive extremum at 446 mμ and a negative extremum at 433 mμ raises the important possibility that the hemes may be juxtaposed in the reduced state. Such complexities are expected to arise from exciton resonance interaction of the Soret transition in proximally placed hemes (Urry, 1965, 1966, 1967a). As the Soret transition is doubly degenerate the complexity could also arise from a steric removal of the degeneracy which appears to be the case with oxidized and reduced cytochrome c. However, the heme octapeptide system, in which the complexity can be shown to be dependent on aggregation, exhibits circular dichroism curves which bear striking resemblance to those of ferrocytochrome oxidase (Urry and Pettegrew, in preparation). Thus the circular dichroism curves of ferrocytochrome oxidase support the possibility that the hemes may be juxtaposed, although additional data must be sought on this point.

While ferri- and ferrocytochrome c exhibit complex Cotton effects in the Soret region the complexity is of a different form for ferrocytochrome oxidase. More significantly, the ratio of the rotation to absorption is up to five times greater for reduced cytochrome oxidase than for oxidized and reduced cytochrome c. The ratio for ferricytochrome oxidase is similar to that for met and deoxymyoglobin and for freshly prepared oxy- and deoxyhemoglobin, and each CD band for these heme proteins approximates a simple Gaussian curve (unpublished data). The ratio of rotation to absorption is larger for ferrocytochrome oxidase (see also King and Schellman, 1966). In addition it may be noted that the ratio is considerably smaller for the Soret band of cytochrome c_4 (van Gelder, Urry and Beinert, 1967), catalase and peroxidase (Miles and Urry, unpublished data). As the concentration of heme is determined by the absorbance at the α band,

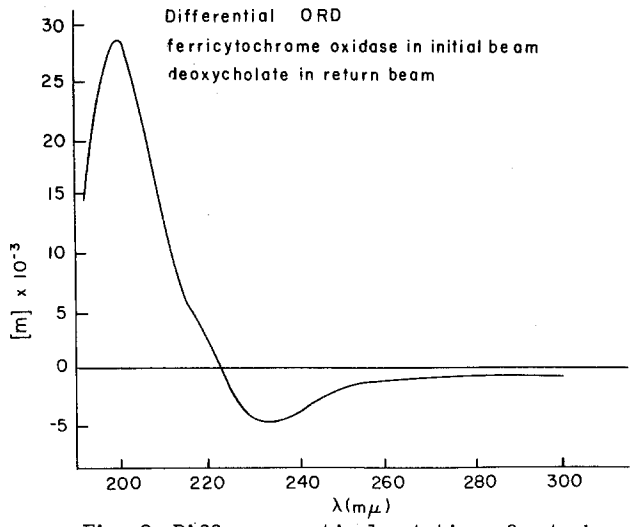

Fig. 3 Difference optical rotation of cytochrome oxidase. The ordinate is plotted as the mean residue rotation using an average residue molecular weight of 115.

scattering of light would lead to a higher concentration. To the extent that scattering occurs, the calculated values of rotation and ellipticity are low making comparisons of ferrocytochrome oxidase with other hemeproteins even more unique. Thus the magnitude and complexity of the Soret Cotton effect set ferrocytochrome oxidase apart from the heme proteins so far studied and provide additional evidence for proximally placed heme moieties.

The presence of 45% noncytochrome protein (likely mitochondrial structural protein) limits the direct application of the short wavelength data to cytochrome oxidase. However, the curves (Figs. 3 and 4) are sufficiently characteristic of α type helices as to exclude the presence of large amounts of other regular structures and to place limits on the helical content of cytochrome oxidase. Using Riddiford's (1966) ORD parameters for paramyosin, an approximate value of 30% α-helix is obtained. The circular dichroism data gives a similar figure. The 222 mμ CD extremum is shifted to 220 mμ which may reflect a small amount of β-structure. An additional point of interest is that cytochrome c, hemoglobin and myoglobin exhibit pronounced Cotton effects in the aromatic region (Urry,

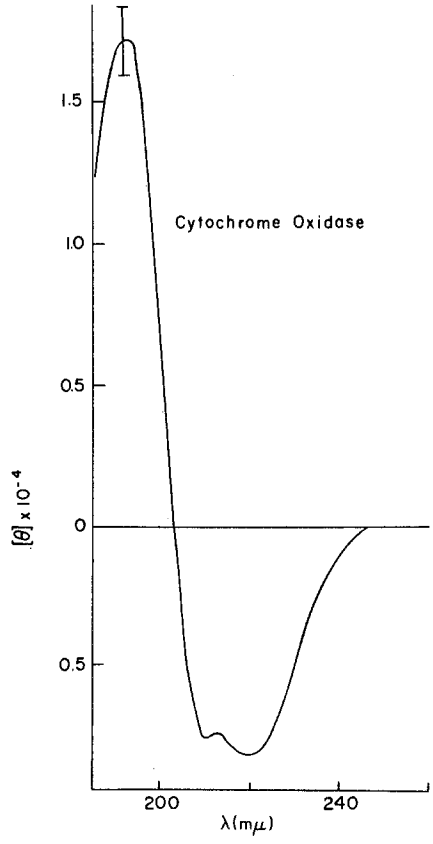

Fig. 4 Circular dichroism of cytochrome oxidase. The ordinate is plotted as the mean residue ellipticity using an average residue molecular weight of 115.

1967b) while none is detected in cytochrome oxidase. This is because the ratio of heme to protein is lower in the latter case.

In summary there is a change in heme environment upon reduction. Considerations of the complexity and magnitude of the Soret Cotton effect for ferrocytochrome oxidase raises the important possibility that in this oxidation state there may be a juxtaposition of heme moieties. The conformation of the protein is of the α-helix type.

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