CONFORMATIONAL EVIDENCE FOR THE NON-IDENTITY OF CYTOCHROMES a AND a_3 IN CYTOCHROME OXIDASE

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In 1939 Keilin and Hartree[1] reported the functional and spectral resolution of cytochrome oxidase into two components, viz. cytochromes a and a_3. Cytochrome a_3 is the component which reacts with cyanide, carbon monoxide and oxygen with concomitant spectral changes, whereas the non-reactive moiety is designated as cytochrome a. This conclusion has been subsequently supported by evidence derived from spectroscopic, chemical, EPR*, and kinetic studies[2-6]. However, it has not been unanimously accepted by all schools of investigators[2-6]. The dissenting school recently explains the spectroscopic and chemical distinction of cytochromes a and a_3 as the manifestation of steric alteration[7], possibly through conformational changes by various chemical and physical methods used for these studies. The failure of attempts to separate the two cytochromes is actually a major reason for the dichotomy of opinion. We wish to report here evidence from CD spectroscopic measurements indicating the presence of two conformationally distinct and independent heme a linked moieties in cytochrome oxidase.

Experimental:-- Cytochrome oxidase was prepared from the Keilin-Hartree preparation of beef heart[8]. The final preparation contained 10.4

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*Abbreviations used: CD, circular dichroism, EPR, electron paramagnetic resonance.

μmoles of heme a per mg protein and showed an activity of 80 to 100 electron equivalents per second per heme, assayed by a method described previously[9]. Circular dichroism data were obtained with a modified JASCO-ORD/CD/UV-5 spectropolarimeter[10]. The instrument was also equipped for automatic repetitive scanning and had provision for the electronic averaging of CD spectra. The data acquisition capabilities in both direct and reversed polarity of the signal permitted (i) automatic compensation of background contributions or "base-line compensation"; (ii), direct determination of difference CD spectra (without manual manipulation of the recordings from the instrument); and (iii), algebraic permutation of the dichroic spectra in any sequence. The combination of averaging of multiple scans and electronic determination of the difference CD spectra was employed in these investigations. The results for the CD spectra of the CO complex were the averages of 16 consecutive scans, whereas those for the cyanide complex were from single scans. All final difference spectra were plotted on an X-Y recorder.

A thermostated cell of 1 cm light path was used. The oxidation or reduction of the samples was performed by addition of a slight excess of solid potassium ferricyanide or sodium dithionite directly to the cell in order to avoid volume change. Prior to reduction, oxygen-free helium was bubbled through the solution. The CO complex was formed by saturating the system with carbon monoxide, and the cyanide complex, by direct dilution of the stock solution into buffer containing 0.015 M neutralized potassium cyanide. The buffer system contained 0.05 M phosphate and 0.25% Emasol, No. 1130, pH 7.4. The CD measurements after addition of oxidizing and reducing agents were made after ensuring completion of the change as judged by the constantcy of the ellipticity.

Results and Discussion:-- Dichroic evidence for the presence of two conformationally independent and distinct species of cytochromes in cytochrome oxidase is derived on the bases of two well documented observations

(see e. g., 1-6): (i), ferricyanide cannot oxidize a hemoprotein which is coordinated with carbon monoxide and (ii), the cyanide-complexed portion of oxidase in the oxidized form is only slowly reduced (20-30 fold slower) by dithionite. The apparent change of oxidase upon addition of ferricyanide to the reduced CO complex, or upon addition of dithionite to the oxidized cyanide complex, is the change of valence state of the unreactive moiety only. The difference between the CD spectra for the two complexes before and after the addition of oxidizing or reducing agent therefore represents the difference CD spectrum for the change of the valence state of the unreactive component of oxidase. Since the CD spectrum is exceedingly sensitive to conformational differences, and since in the two complexes, the reactive heme a moiety differs not only in the nature of the central coordinated complex, but also in the valence state of the iron, a comparison of the difference CD spectra in the presence of cyanide and CO can unambiguously provide evidence for the conformational dependence of the two components.

The difference CD spectra from the cyanide and the CO complexes in the Soret absorption region are presented in Fig. 1. It is clear from the figure that the difference CD spectrum for the change of oxidation state of the unreactive component is independent of the chemical nature of the central coordinating complex of the reactive forms. Furthermore, since the two difference spectra are from systems which differ in the valence state of the extrinsically complexed heme a iron, the spectral change for the oxidation state of the unreactive component is also independent of the oxidation state of the reactive component. The identity of the two difference spectra could only be possible if the conformation of the unreactive component in both the ferric and ferrous forms of heme iron is independent of both the chemical nature of the central coordinated complex as well as the valence state of the reactive component. This observation is an expected occurrence only if oxidase contains two confor-

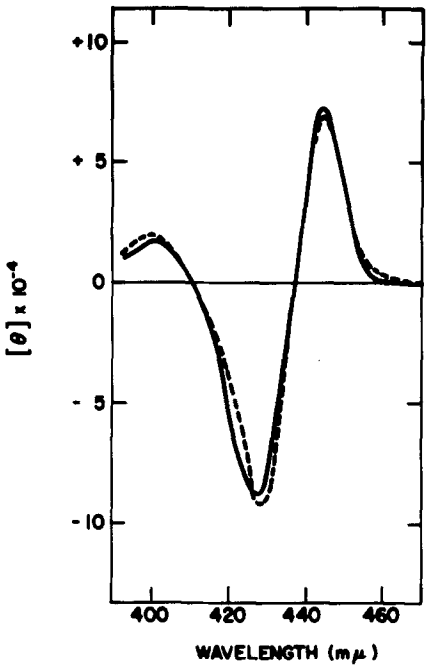

Fig. 1. Comparison of the oxidation-reduction linked difference CD spectra of cytochrome oxidase in the presence of carbon monoxide and of cyanide. Difference spectra: _ _ _ _ , [(CO reduced oxidase) - (CO reduced oxidase + ferricyanide)]; _____, [(oxidase + cyanide + dithionite) - (oxidase + cyanide)].

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mationally distinct and independent heme a moieties.

It is possible, however, that the identity of the two difference CD spectra can be justified on the basis that in the event oxidase contains two distinct but conformationally dependent components, the formation of the cyanide or the CO complex of the reactive component causes conformational alterations such that the conformation of the unreactive moiety is identical in the two complexes. Also, in the case in which the moieties are initially indistinguishable, the identity of the difference CD spectra could arise if the coordination of the extrinsic ligands causes identical changes. The binding of cyanide causes a directionally differ-

ent type of alteration of the CD spectrum in the intrinsic region (which reflects predominantly the conformation of the protein moiety) when compared to those changes induced upon formation of the CO complex (see Fig. 2). This suggests that if long-range conformational dependence between the moieties were prevalent, the coordination with two extrinsic ligands would cause different types of conformational changes of the prosthetic group. A similar conclusion is also unavoidable in the case of short-range conformational dependence (i. e., shared proximal conformation) from the fact that the dichroic alteration in the Soret region differs not only in the nature, but also in complexity (Myer and King,

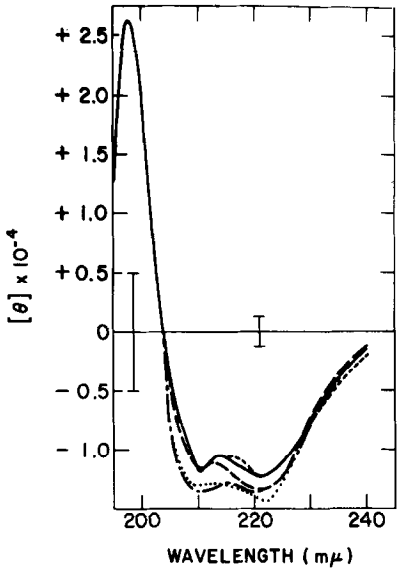

Fig. 2. Intrinsic CD spectra of oxidized and reduced cytochrome oxidase and of the corresponding cyanide and carbon monoxide complexes. Medium: 0.05 M phosphate buffer, pH 7.4, 0.25% Emasol. ————, oxidized; —— —— ——, reduced; — — — — — —, reduced + carbon monoxide (reduced CO complex); —— — —— — ——, oxidase + 0.015 M cyanide (oxidase cyanide complex); ·····, oxidase cyanide complex + dithionite (reduced cyanide complex; CD spectrum measured within six minutes after the addition of dithionite).

unpublished data). The possibility of generation of identical conformation of the unreactive moiety upon formation of the cyanide and of the CO complex of the reactive portion of oxidase, if constituted of two distinct but conformationally dependent components, is, however, highly unlikely. In addition to these observations, the indication of the absence of heme-heme interaction in native oxidase[11] (Myer and King, unpublished results) further eliminates the possibility of the presence of indistinguishable heme a moieties.

From these considerations, it is thus concluded that the identity of the two difference CD spectra could arise only if oxidase contains two distinct and independent heme a linked moieties. They may be two molecularly distinct species, i. e., two cytochromes, or may represent a single cytochrome containing two heme a moieties (Siamese twins, see Ref. 3, Vol. 2, p. 539) significantly separated from one another such that the alteration of the chemical nature of the central coordinated complex of one does not alter the conformation of the other. Further studies are being pursued to delineate these two possibilities.

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REFERENCES

1. Keilin, D., and Hartree, E. F., Proc. Roy. Soc. (London), B127, 167 (1939).
2. Falk, J. E., Lemberg, R., and Morton, R. K. (Eds.), Hematin enzymes, Vols. 1 and 2, Pergamon Press, London, 1961.
3. King, T. E., Mason, H., and Morrison, M. (Eds.), Oxidases and related redox systems, Vols. 1 and 2, Wiley, New York, 1965.
4. Chance, B., Estabrook, R., and Yonetani, T. (Eds.), Hemes and hemoproteins, Academic Press, New York, 1967.
5. Okunuki, K., Kamen, M. D., and Sekuzu, I. (Eds.), Structure and function of cytochromes, University of Tokyo Press, Tokyo, 1968.
6. Gibson, Q. H., Palmer, G., and Wharton, D. C., J. Biol. Chem., 240, 888 (1965).
7. Green, D. E., and Wharton, D. C., Biochem. Z., 333, 335 (1963); Wald, G., and Allen, D. W., J. Gen. Physiol., 40, 593 (1957).
8. King, T. E., in Methods of Enzymology, 10, 202 (1967).
9. Takemori, S., and King, T. E., J. Biol. Chem., 240, 504 (1965).
10. Myer, Y. P., and MacDonald, L. H., J. Am. Chem. Soc., 89, 7142 (1967).