CIRCULAR DICHROISM OF MAMMALIAN CYTOCHROME $\underline{c}_1$

by

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SUMMARY--The circular dichroic behavior of cardiac cytochrome $\underline{c}_1$ was examined between 190 and 600 nm. Both oxidized and reduced forms show a major positive Soret ellipticity band. In the visible region, the reduced cytochrome exhibits a split CD profile which becomes indistinct upon oxidation. These CD spectra are completely different from those of the mammalian cytochrome $\underline{c}$ despite the resemblance of the absorption spectra and the identity of the prosthetic group (mesoheme) of these two cytochromes.

Considerable information has been obtained from the ORD/CD* studies of several $\underline{c}$-type cytochromes (e.g. 1-6). Due mainly to the unsuitability of the preparations available, no such study has been conducted on cytochrome $\underline{c}_1$ which is an obligatory component of the mitochondrial respiratory chain. Recently we (7) have succeeded in the isolation of cytochrome $\underline{c}_1$ up to an essentially homogenous form suitable for spectropolarimetric work. This preparation retains enzymic activity, electron paramagnetic resonance behavior (collaboratively with H. Beinert, to be published) and the other properties of cytochrome $\underline{c}_1$ of submitochondrial particles and mitochondria.

Like all $\underline{c}$-type cytochromes, presumably cytochrome $\underline{c}_1$ contains a mesoheme covalently bound to the protein moiety through the cysteinyl linkages. Although this cytochrome exhibits absorption spectra very similar to those of mammalian cytochrome $\underline{c}$, the circular dichroic spectra are completely different, as can be seen from this communication. Such a difference may reflect structural characteristics especially around the heme site and may have a bearing on the function of the molecule as an electron donor to cytochrome $\underline{c}$ and as an acceptor most probably from cytochrome $\underline{b}$.

EXPERIMENTAL--Cytochrome $\underline{c}_1$ was prepared from the Keilin-Hartree

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*Abbreviations: CD, circular dichroism; ORD, optical rotatory dispersion

preparation of bovine heart muscle (7). The cytochrome thus isolated was in the reduced state, free from acid-extractable heme, and showed A_{417}/A_{280} of 2.5. It contained 24 nmoles of heme per mg of protein. Oxidation was accomplished by addition of solid ferricyanide crystals directly to the solution and excess ferricyanide was removed if necessary.

Absorption spectra were determined in a Cary spectrophotometer model 14. Circular dichroic measurements were conducted at 27° in a Cary spectropolarimeter, model 60 with a CD attachment, model 6002, calibrated with d-10 camphor-sulfonic acid. Ellipticities are expressed in degrees-cm^2 per decimole of heme. The minimum molecular weight of 42,000 and the mean residue weight of 115 were used in the computation.

RESULTS AND DISCUSSION--The absorption spectra of cytochrome $\underline{c}_1$ are shown in Fig. 1. In the visible region the reduced cytochrome exhibits a sharp absorption maximum at 552.5 nm and a β-band at 522.5 nm flanked by

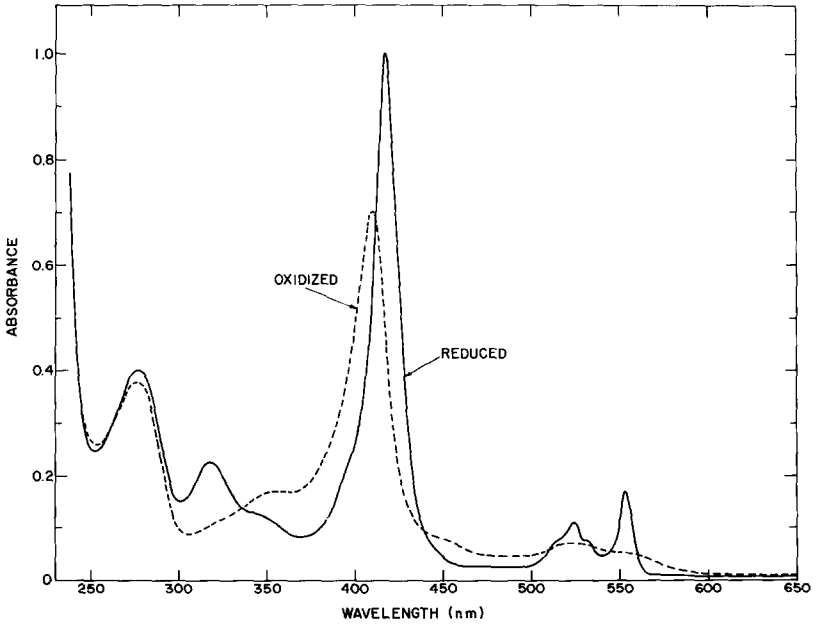

Fig. 1. Absorption spectra of cytochrome $\underline{c}_1$ in 0.1 M phosphate, pH 7.4. Reduced ———; oxidized -----.

distinct shoulders at 530 and 512 nm respectively. Upon oxidation these bands broaden and become barely discernible at 558 and 525 nm respectively. The Soret absorption peak of the reduced cytochrome appears at 417 nm. Upon oxidation this maximum is shifted to 411 nm with concomitant hypochromicity. A distinct δ-band at 317 nm can be observed in the reduced but not in the oxidized form. Both forms of the cytochrome show similar light absorption behavior in the UV region.

The dichroic spectra of cytochrome $\underline{c}_1$ are given in Fig. 2. The reduced cytochrome shows in the visible region a split CD profile with a positive extremum at 556 nm ($[\theta] = 1.0 \times 10^4$) and a negative extremum at 549 nm ($[\theta] = -0.7 \times 10^4$). The iso-elliptical point (the crossing-over) at 552 nm corresponds closely to the α-absorption maximum at 552.5 nm. The fact that the molar ellipticities at these two extrema are not exactly equal is due evidently to the compensation effect of adjacent positive ellipticity bands in the 530 nm region. In the envelope of the β-absorption band (cf. Fig. 1)

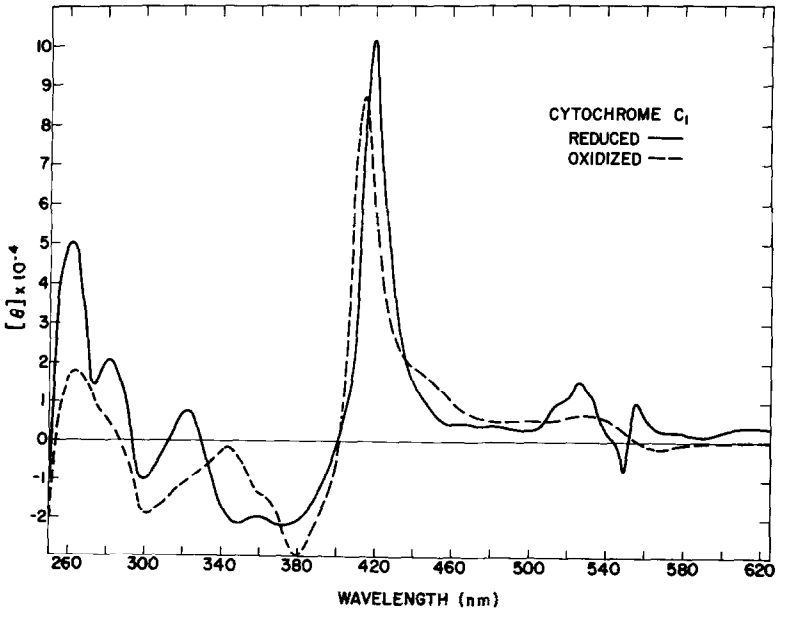

Fig. 2. Circular dichroic spectra of cytochrome $\underline{c}_1$ in the 625-250 nm region. Reduced ———; oxidized -----.

there appear at least 4 positive CD bands. The ellipticity for the main band at 525 nm is about 1.6×10^4. Oxidation of the cytochrome abolishes all these characteristics; instead two broad, unstructured extrema at (-)558 and (+)530 nm become apparent.

The Soret ellipticity band at 417 nm for the reduced cytochrome $\underline{c}_1$ is relatively simple. Upon oxidation the extremum shifts to shorter wavelength (414 nm) and decreases in amplitude in direct correspondence to the changes in absorption. Both CD bands are slightly skewed on the longer wavelength side; in fact, in the oxidized form, an additional band at approximately 440 nm is also discernible. In the wavelength region from 340 to 400 nm, both the oxidized and the reduced cytochrome exhibit negative ellipticity bands.

The δ-absorption band of the reduced cytochrome has a corresponding positive CD band at 324 nm which is not apparent in the oxidized form (cf. Fig. 2). On the other hand, both forms show a negative extremum at 300 nm. The CD profile of the cytochrome in the 250 to 300 nm region lacks the fine structure so richly displayed in the CD spectra of mammalian cytochrome $\underline{c}$ (8). In fact, only two major ellipticity bands, at 262 and 282 nm respectively, can be detected in reduced cytochrome $\underline{c}_1$ and the magnitude of these bands decreases considerably upon oxidation.

The ellipticity band at 282 nm, which has also been observed in mammalian cytochrome $\underline{c}$, is due most probably to asymmetric interactions of the side chain of an aromatic amino acid(s) with the polypeptide chain (1, 2). The disappearance of this band upon oxidation suggests a change in the environment of this chromophore. Also, in the oxidized form the amplitude of the 262 nm ellipticity band is approximately 60% less than that of the reduced. This band has been claimed to be derived from linkages of the type such as histidyl-heme iron-porphyrin (6). If the magnitude of the 262 nm band is an indication of the extent of non-planarity of the heme-iron relative to the porphyrin ring, then the decrease of this band reflects an

increase in the relative planarity of the iron-center with respect to the prophyrin ring. The changes observed in the visible region (cf. Fig. 2) most probably arise from the same event since the circular dichroic and light absorption spectra in this region reflect perturbations of the heme-iron by groups or ligands situated within the co-ordination sphere of the metal atom.

The cytochrome exhibits a typical far UV dichroic spectrum with negative extema at 222 and 208 nm and a positive extremum at 193 nm (cf. Fig. 3). All these suggest the existence of a right-handed α-helix (9) in the cytochrome. There seems to be no significant change in the magnitude of the ellipticities upon oxidation despite the dramatic difference between the oxidized and the reduced cytochrome in the visible and Soret regions. The effective helicity calculated from the value at 208 nm is about 20-25% for both forms. The estimated β-structure (10) is approximately 25%. The remainder is presumably in the so-called random form. The resolution of the

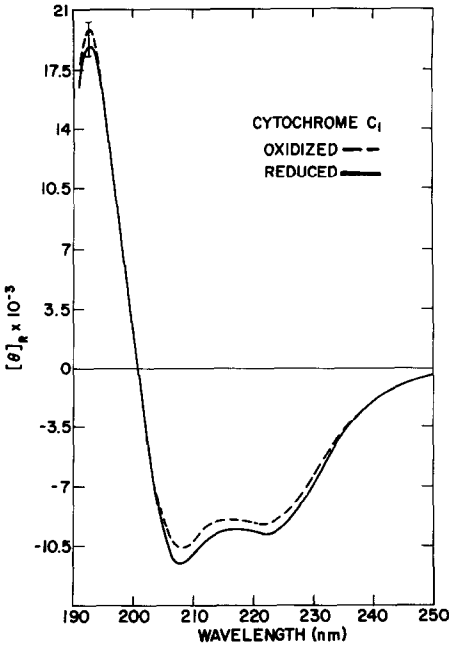

Fig. 3. Circular dichroic spectra of cytochrome c_1 in the 250-190 nm region. Reduced ———; oxidized -----.

individual transitions from the overall CD spectra is being pursued in an attempt to obtain a unique answer.

Here it is perhaps safe to conclude that the CD data of cytochrome $\underline{c}_1$ reveal quite clearly a completely different set of the heme environment from that of the mammalian cytochrome $\underline{c}$, although superficially both cytochromes perform a comparable physiological role in electron transfer in the nearby region of the respiratory chain. These structural variations are undoubtedly a prerequisite of a highly integrated three-dimensional structural organization of the mitochondrial membrane so as to facilitate an efficient transfer of electrons from one component to another. Thus it seems dangerous to approximate the structure of cytochrome $\underline{c}_1$ by that of $\underline{c}$, deduced from crystallographic results, in an attempt to formulate the electron transport mechanisms in mitochondria.

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