DISTINCTION OF CYTOCHROMES a AND a_3 BY CHEMICAL REACTIVITY*

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Almost a quarter of a century after Keilin and Hartree (1939) first distinguished cytochrome a_3 from cytochrome a, there is still no uniform agreement that these two cytochromes are distinct. The evidence for two distinct cytochromes is based on the spectral properties of the cytochromes and on reactions with carbon monoxide and cyanide (Chance, 1953; Horie and Morrison, 1962; Lundegardh, 1957; Smith, 1955; Yonetoni, 1961). The validity of this evidence has been questioned (Okunuki, 1962; Wainio, 1961). It has been suggested that purified preparations give no evidence for two cytochromes, and that the spectra with carbon monoxide or cyanide does not establish two cytochrome components (Okunuki, 1962; Wainio, 1961). Further, physical and chemical methods have failed to separate "cytochrome a" into two cytochrome components. It is the object of this communication to present, in preliminary form, evidence that the carbon monoxide combining cytochrome component, cytochrome a_3, is chemically distinguishable from cytochrome a.

METHOD

The cytochrome c oxidase was prepared as previously described (Horie and Morrison, 1962). The preparation was then made 0.048 M with respect to cyanide. To this solution, 1 mg of solid sodium borohydride was added for each ml of

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enzyme preparation. The solution was allowed to stand for 30 minutes at zero degree and then stirred vigorously to insure destruction of excess borohydride. To remove excess cyanide the cytochrome c oxidase was passed through a Sephadex G75 column which was in equilibrium with the buffer solution in which the enzyme was dissolved. The material was then diluted approximately seven times with 0.1 M phosphate buffer, pH 7.4, containing one per cent Tween 80. To obtain the spectrum shown in Figure A and Figure B, cytochrome c oxidase was reduced with solid sodium dithionite. The spectrum was recorded on a DK-2 Beckman spectrophotometer. In order to obtain the carbon monoxide spectrum, the solution was saturated with carbon monoxide.

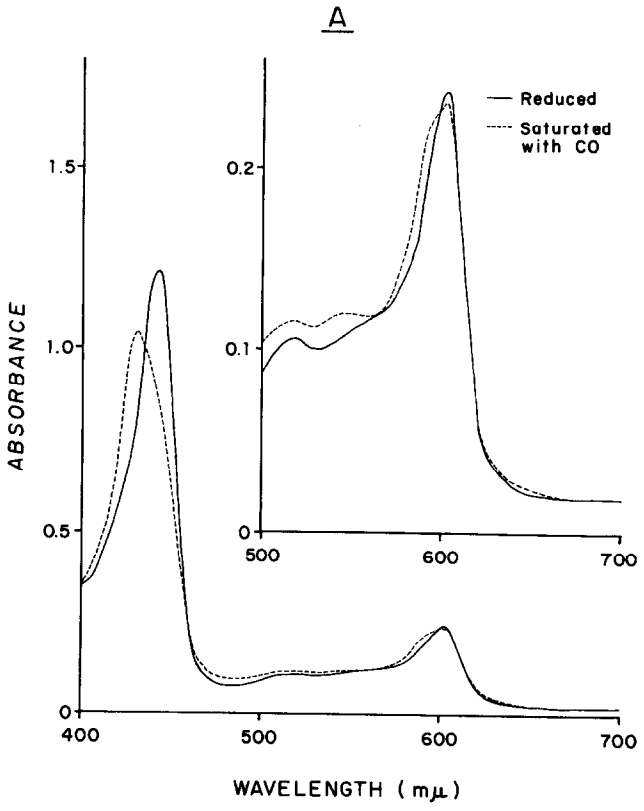

Figure A. Absorption spectra of cytochrome c oxidase preparation. ——— represents the preparation reduced with dithionite; ----- represents the same solution after saturation with carbon monoxide. The cytochrome c oxidase preparation contained 1.5 mg of protein per ml and was dissolved in 0.1 M phosphate buffer, pH 7.4, containing 1% Tween 80.

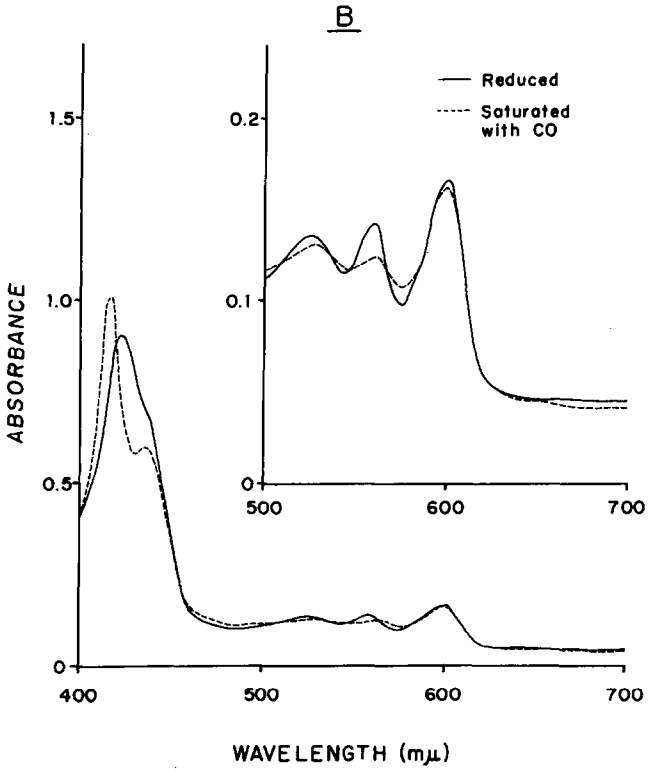

Figure B. The absorption spectra of the cytochrome c oxidase after treatment with borohydride as described in text. ——— represents the preparation reduced with dithionite; ----- represents the same solution after saturation with carbon monoxide. The cytochrome c oxidase preparation contained approximately 1.1 mg of protein per ml and was dissolved in 0.1 M phosphate buffer, pH 7.4, containing 1% Tween 80.

RESULTS AND DISCUSSION

The prosthetic group of the cytochromes a and a_3 is "heme a". In heme a, carbonyl groups are directly attached to the porphyrin nucleus. An electron withdrawing group, such as the carbonyl, has a marked effect on the spectrum of heme compounds.

Borohydride is a specific reagent for the reduction of carbonyl groups; thus, when heme a is treated with borohydride the carbonyl groups are converted to alcohol groups, and this results in a 30 mμ shift of the alpha peak of the pyridine hemochromogen (Morrison et al., 1960; Morrison and Stotz, 1961).

When a purified cytochrome c oxidase preparation is treated with borohydride, little, if any, effect on the spectrum can be noted (Okunuki, 1958; Yonetoni, 1961; Griffiths and Wharton, 1961). Upon treatment as described in Methods, however, there is a marked change in the spectrum as shown by comparison of Figures A and B. From the spectra shown in Figure B it is apparent that some of the heme prosthetic groups of the cytochrome c oxidase have been altered. The carbonyl groups of these hemes have been converted to hydroxyl groups. This has been established not only by the change in spectra of the hemoprotein, but by the actual isolation of the hemes.

The most significant aspect of the altered spectrum obtained by treatment with borohydride shown in Figure B is that obtained on saturation with carbon monoxide. The cytochrome whose prosthetic group has been altered by borohydride treatment combines with carbon monoxide as shown by the marked spectral shifts in both the visible and Soret regions of the spectra. This is in contrast to the cytochrome component whose prosthetic group remains unaltered, and which does not combine with carbon monoxide. Thus, it is shown that in a purified cytochrome c oxidase preparation, there are two cytochrome components. The prosthetic group of one of these components can be selectively altered by borohydride leaving the other group unaffected. These two components correspond to the cytochromes a_3 and a respectively.

These results do not establish that the cytochromes a and a_3 are separate proteins. It may well be that a single protein moiety is involved. The results do indicate that in a spectral, functional and chemical sense the two cytochromes a and a_3 do exist.

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