PHOTO- AND THERMOCHROMISM OF CYTOCHROME OXIDASE: SIGNIFICANCE OF A NEW PHOTODISSOCIABLE SPECIES

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SUMMARY

A new photodissociable species of cytochrome oxidase occurred when reduced oxidase in a medium of pH 8.6 to 10.5 was cooled in liquid nitrogen. Its characteristic band at 575 nm (or 428 nm in the Soret region) diminished when it was illuminated at 26°K, and reappeared as the sample temperature was elevated, a half transition temperature being 81°K. This band did not appear when reduced oxidase had been complexed with CO or HCN. These results suggest that the formyl group of heme a is involved in the formation of this species and that a subtle conformational change induced by the ligands distorts a close spacial relationship of the heme a to its immediate environment.

The bond between the carbon atom of carbon monoxide and the heme iron in the carbon monoxide compounds of hemoproteins is known to be photodissociable (1). Contrary to this, we recently found that a new photodissociable species was formed when reduced cytochrome oxidase in a moderately alkaline medium was cooled in liquid nitrogen, and supposed that in this reaction the formyl group of heme a, instead of the heme iron, is involved. In this paper, we describe the conditions under which this new species is formed, its photo- and thermochromic changes, and its possible relation to the nature of the immediate environment of heme a in cytochrome oxidase.

MATERIALS AND METHODS

Cytochrome oxidase was prepared as described previously (2), and the final ammonium sulfate-precipitate was dissolved in 50 mM sodium phosphate buffer (pH 7.4) containing 0.25% (v/v) Emasol 1130 and 0.1% (w/v) cholate. For measurement of absorption spectra at low temperatures, the instrument constructed by Hagihara and Iizuka (3) was used. As a light source for illumination a flash bulb unit for photography was installed just outside of a Dewar's vessel near a twin cuvette assembly. As required, cytochrome oxidase solutions in test tubes were made alkaline with 1 to 6 N NaOH. Usually after 5 min standing the sample solution was transferred to a cuvette and it was dipped into liquid nitrogen. The sample temperature was monitored with a thermocouple of Au-Co alloy *vs.* Cu over the range from 20° to 300°K.

RESULTS

When reduced cytochrome oxidase was made moderately alkaline and cooled in liquid nitrogen it exhibited a unique absorption spectrum characterized by a sharp and symmetrical peak at 575 nm in addition to a main peak at 598 nm in the visible region, and by a spike-like peak at 428-9 nm in addition to a major one at 438 nm in the Soret region (Fig. 1). Figure 2 illustrates the relationship between the ratios of the absorbance at 575 nm to that at 600 nm and the pH's where the sample was incubated, and shows that the appearance of the 575-nm band is restricted to a narrow pH region between pH 8.6 to 10.5, corresponding to the activation of cytochrome oxidase (4). An increase in the absorbance ratio above pH 10.5, however, reflects the formation of a

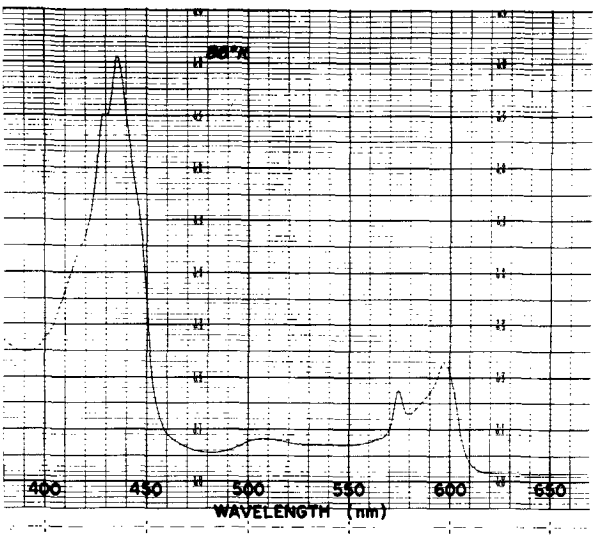

Fig. 1.

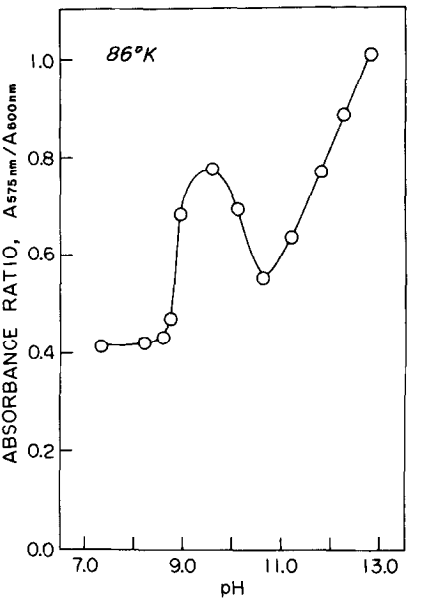

Fig. 2.

Fig. 1. A low temperature spectrum of reduced cytochrome oxidase incubated at pH 9.43. Cytochrome oxidase (11.2 μM) was reduced with a small amount of sodium dithionite, and then made to pH 9.43 with 6 N NaOH. After 5 min standing this solution was transferred to a sample compartment of a twin cuvette assembly, and a blank medium to a reference compartment. The cuvette assembly was dipped into liquid nitrogen, and both the sample and reference solutions were frozen in 2 min. The sample temperature was 86°K. The ordinate is for an arbitrary unit of absorbance.

Fig. 2. The A_{575nm}/A_{600nm} ratio *vs.* pH. The experimental conditions were the same as described in the legend for Fig. 1 except that the oxidase concentration was 29.8 μM and that pH's of the solutions adjusted were as appeared on the abscissa. The data were taken from absorption spectra as illustrated in Fig. 3.

Schiff's base (5 - 7) between the formyl group of heme a and an ε-NH_2 group of lysine residues in the alkali-denatured protein moiety of cytochrome oxidase, as shown in Fig. 3. When the Schiff's base formation was complete, a single α-peak appeared at 573 nm (6), whereas even by a prolonged incubation of reduced oxidase at pH 9.5 the 575-nm band of the low temperature-spectrum always accompanied the 598-nm band.

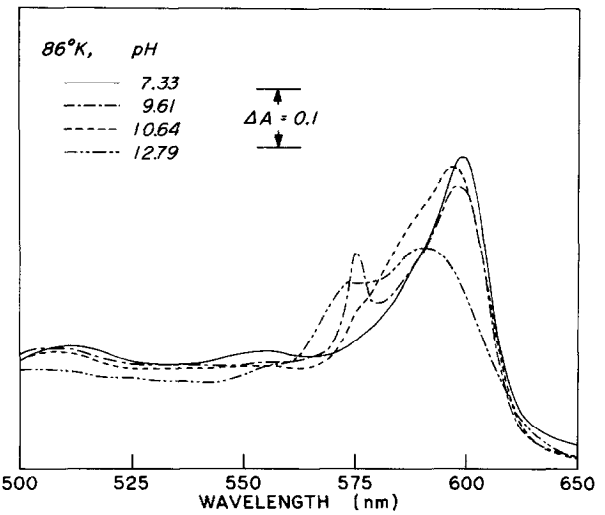

Fig. 3. Low temperature spectra of reduced cytochrome oxidase incubated at different pH's.

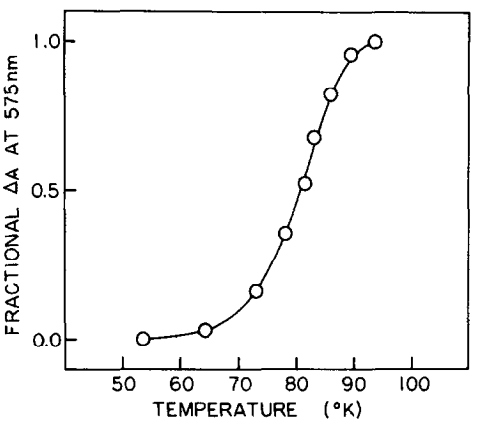

Fig. 4. Temperature-dependent restoration of the photoquenched band at 575 nm. A portion of a solution of sodium dithionite-reduced cytochrome oxidase (29.8 μM) was made to pH 9.55 with 1 N NaOH, and transferred to a sample compartment of the twin cuvette after 5 min standing at room temperature. Another portion was poured into a reference compartment. After pre-cooling with liquid nitrogen the cuvette assembly was dipped into liquid hydrogen. The 575-nm band of a difference spectrum was quenched by 50 flashes at 26°K, and then the sample temperature was elevated at a rate of 1.5°/min by using a heating system, while difference spectra in a range from 700 to 500 nm were recorded repeatedly.

This 575-nm band appeared when cytochrome oxidase had been reduced with either sodium dithionite or ascorbate plus cytochrome c, although it was less prominent in the latter case. With alkali-treated oxidase in the oxidized state no spectral change relevant to this band formation was observed.

When reduced cytochrome oxidase exhibiting the 575-nm band was illuminated at liquid nitrogen temperature the band intensity diminished instantly but reverted to its original level with time. Except for an earlier stage of the restoration process, it proceeded according to a first order kinetics, and its apparent rate constant was $8.3 \times 10^{-3} sec^{-1}$ at 90°K. This change was temperature-dependent, and at liquid hydrogen temperature no restoration of the 575-nm band, which had been quenched completely by more than twenty flashes, occurred. A difference spectrum for the photoquenching revealed two major peaks at 575 nm and 428 nm characterizing the photodissociable species of this kind. As the sample temperature was elevated slowly from that of liquid hyrogen, the 575-nm band reappeared as shown in Fig. 4, and a half transition temperature was determined apparently to be 80°K. Such a photo- and thermochromic species of cytochrome oxidase disappeared completely above 270°K.

When reduced cytochrome oxidase had been complexed with either CO or HCN, the alkali-treatment failed to produce the photodissociable species, instead the CO or HCN compound was formed. Once the CO compound was formed, the 575-nm band did not appear even when the CO was dissociated from the heme iron by illumination at liquid nitrogen temperature. Also this band appeared only to a small extent when 24 μM reduced cytochrome oxidase had been treated with 5 mM NaN_3 or 250 μM PCMB.

DISCUSSION

Although the peak positions of the photodissociable species are almost the same as those for an ordinary Schiff's base formed from alkali-denatured cytochrome oxidase (5 - 7), their absorption spectra at 86°K are quite different and usually the latter is formed in more alkaline pH regions, so that this species cannot be the Schiff's base. Rather, an intermediate which leads to the Schiff's base, or an addition compound between the formyl group and one of seven -SH groups in cytochrome oxidase (8) may have been formed.

The half transition temperature of 80°K for this photodissociable species is lower than that for the carbon monoxide compound (9) by 90°, thus suggesting that the formyl group and its counterpart in the reaction are in a fairly close spacial relationship in the reduced cytochrome. This is highly possible if the prosthetic group, heme a, is in a pocket surrounded by the polypeptide backbones, as suggested by the binding studies of alkylisonitriles with reduced cytochrome oxidase (10). These reactive groups, however, seemingly are dislocated once a conformational change around the heme a is induced by liganding of CO or HCN to the heme iron. This altered conformation would remain unchanged even when the CO is removed by illumination at liquid nitrogen temperature, and the dissociated CO may act as a wedge between the heme a and the wall of a pocket preventing their access.

A less potent effect of NaN_3 would relate to its unique behaviors in the inhibited cytochrome oxidase systems (11, 12). Contrary to these ligands, PCMB would induce a conformational change by blocking some -SH groups in the protein moiety, thus also preventing the interaction between the formyl side chain

with its immediate environment, the nature of which is now being investigated.

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