DEPOLYMERIZATION OF CYTOCHROME OXIDASE TO A WATER-SOLUBLE MONOMERIC PROTEIN

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Cytochrome oxidase has been isolated in particulate form by cholate-salt fractionation of mitochondrial fragments (Eichel, Wainio, Person and Cooperstein, 1950; Hatefi, 1958; Mackler and Penn, 1957; Smith and Stotz, 1954). Although such particulate preparations contained essentially only one cytochrome, namely cytochrome a, the cytochrome oxidase activity was lower than would have been anticipated from the degree of concentration of cytochrome a. It has recently been discovered that the cytochrome oxidase activity of particulate preparations can be greatly augmented (a) by fine dispersion of the particles with deoxycholate or other detergents (Takemori, Sekuzu, Yonetani and Okunuki, 1958), and(b) by carrying out the enzymatic assay in the presence of lipide extracts rich in phospholipides (Hatefi, 1958; Wainio and Greenless, 1958).

Criddle and Bock (1959) have succeeded in depolymerizing polymolecular cytochrome c_1 (5 hemes per m. wt. unit of 371,000) to the unimolecular form (1 heme per m. wt. unit of 70,000). Thioglycolate was the depolymerizing agent. In the present communication the depolymerization of the polymolecular particulate form of cytochrome oxidase to a soluble unimolecular form is described.

Cytochrome oxidase is prepared from beef heart mitochondria essentially according to the method of Hatefi (1958). Further removal of red hemoproteins is achieved by refractionating with ammonium sulfate and selecting the

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fraction precipitating between 20 and 30 per cent saturation of ammonium sulfate. The precipitate is dissolved in water, and the solution is dialyzed for 16 to 20 hours against 1 X 10^{-2} M Tris buffer of pH 7.5. The precipitate which forms is collected by centrifugation and solubilized in water with deoxycholate (1.5 to 2.0 mg/mg protein) or a minimal amount of Triton X-100. This "solution" is made 0.3 M with respect to sodium thioglycolate (pH 8.5), kept for 2 to 4 hours at 0° and then dialyzed against 1 X 10^{-3} M Tris buffer of pH 8.0 for 72 to 96 hours with frequent changes of buffer. The dialyzed solution is centrifuged for 30 min. in the Spinco preparative ultracentrifuge. The enzyme remains in solution.

In an accompanying communication Criddle and Bock (1959) present the evidence for the homogeneity of the "monomeric" cytochrome oxidase. They estimate the molecular weight to be 72,000. According to the a heme** content of the soluble preparations (10.7 mμmoles per mg protein), the minimum molecular weight would be 93,000, which is in close agreement with the estimate based on equilibrium sedimentation data.

The ratio of iron to a heme is 1:1 and that of copper to a heme is also unity. The presence of copper in an amount stoichiometric with a heme in a homogeneous preparation of cytochrome oxidase confirms that copper as well as cytochrome a is an integral part of the cytochrome oxidase (Okunuki, Sekuzu, Yonetani, and Takemori, 1958).

The soluble cytochrome oxidase at the highest purity level is essentially inactive in catalyzing the oxidation of reduced cytochrome c by oxygen unless supplemented with any of several phospholipides of which phosphatidyl inositol is the most active. The activity in absence of phosphatide is one twentieth of the maximum. These phosphatides can react with cytochrome c to form the corresponding lipide cytochrome c complexes. Under maximal conditions 19 μmoles of reduced cytochrome c are oxidized per min. per mg. protein at 38°

** The a heme was converted to the pyridine hemochromogen and determined spectrophotometrically. The Δ-extinction value (peak of α-band minus short wavelength trough of α-band) was assumed to be 15.6 (Connelly, 1958).

as determined by manometric and spectrophotometric methods (Schneider and Potter, 1943; Hatefi, 1958) and by the oxygen electrode (Hagihara and Kuby, 1959). The specific activity of the soluble enzyme is usually only 50 per cent of that of the parent particulate preparation. Cyanide (3×10^{-4} M) and azide (3×10^{-3} M) completely inhibit the activity of the enzyme. Unlike the particulate cytochrome oxidase, the soluble enzyme is maximally active in absence of detergent.

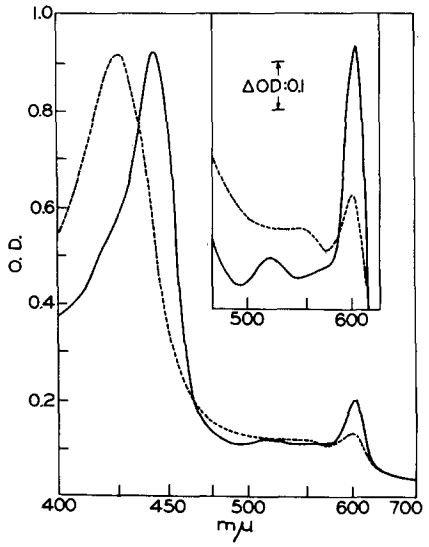

Figure 1. Direct spectrum of the cytochrome oxidase recorded with a Beckman DK-2 spectrophotometer. The dotted curve shows the spectrum of the oxidized and the solid curve the spectrum of the cytochrome oxidase reduced with dithionite.

The absorption spectrum of the soluble enzyme is shown in Figure 1. In agreement with the spectrum published by Connelly, Morrison and Stotz (1959) the oxidized form of the hemoprotein has an α band at 600 mμ which shifts to 605 mμ upon reduction. The reduced cytochrome has a distinct β band at 518 mμ. Table I summarizes the spectral shifts induced by KCN, CO and $K_3Fe(CN)_6$. It is of interest that cyanide and CO affect both the positions and heights of the α- and Soret bands of the reduced enzyme. The twin facts that soluble cytochrome oxidase in homogeneous state contains only one molecule of heme per molecule of protein and that the spectrum of

Table I

Absorption Band Positions of Soluble Cytochrome Oxidase in Oxidized and Reduced States

	Peaks of bands (mμ)		
	α	β	γ
Oxidized form			
(a) as prepared	600		427
(b) treated with $K_3Fe(CN)_6$	600		424
(c) in presence of KCN	600		428
Reduced form			
(a) reduced with $Na_2S_2O_4$	605	518	444
(b) as above + KCN	603	516	441-442
(c) as in(a) + CO	603	512-514	431

this heme is modified by reagents such as cyanide and CO which were presumed to react only with cytochrome a_3 and not with cytochrome a weaken the experimental foundation for the cytochrome a_3 hypothesis. The available evidence is unambiguous that the cytochrome oxidase contains only one hemoprotein, namely, cytochrome a, and there appears to be no necessity for postulating a second cytochrome to account for the spectral shifts induced by cyanide and CO.

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