Resolution of Complex I (DPNH-coenzyme Q reductase) of the Mitochondrial Electron Transfer System*

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Complex I (1, 2) which catalyzes the Amytal- and rotenone-sensitive reduction of coenzymes Q by DPNH has been subdivided into three fractions (A, B and C) by treatment with urea (final concentration, 2.5 M) for 10 minutes at 37°. The precipitate formed after incubation (fraction C) was removed by centrifugation, and the supernatant fluid was fractionated with ammonium sulfate. Fraction B was collected by centrifugation at 27.5% ammonium sulfate saturation and fraction A was isolated between 36.4% and 52.9% salt saturation. Preparations of complex I were made from beef-heart mitochondria exactly as described earlier (1).

Fraction A

This is a flavoprotein (Fig. 1) containing per mg of protein 15 to 16 mμmoles of flavin (FMN), 60 to 65 mμatoms of iron and about 60 mμmoles of labile sulfide. The enzyme catalyzes the oxidation of DPNH by quinones, ferricyanide and cytochrome c (Table I). Comparable preparations extracted

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Abbreviations: pCMS, p-chloromercuriphenyl sulfonate; DCPIP, 2,6-dichlorophenol indophenol; Q, coenzyme Q; A, absorbance.

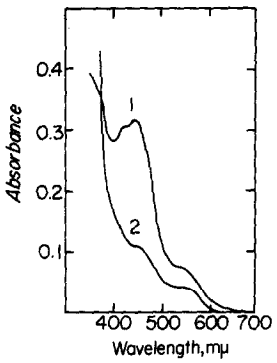

Fig. 1. Absoption Spectrum of fraction A. Curve 1, 1.13 mg/ml of fraction A protein in 50 mM Tris-Cl, pH 8.0. Curve 2, after addition of $Na_2S_2O_4$. Spectra were recorded by Beckman DK-2 spectrophotometer.

from mitochondrial particles by minor modifications of Mahler's original acid-ethanol-heat treatment (3) have been reported by others* (4-7). These preparations differ from fraction A in the following respects:

a) In general, they exhibit lower activities with the acceptors shown in Table I.

b) They have a lower concentration of flavin, iron and/or labile sulfide than fraction A.

c) Unlike fraction A which exhibits high activity toward all electron acceptors (Table I), the activity of the Mahler-type preparations fluctuates over a wide range with various acceptors. Thus, one preparation is much more active with ferricyanide than with quinones and cytochrome c (10); another has high quinone reductase activity and low ferricyanide and cytochrome c reductase activity (7).

* The DPNH dehydrogenase of Ringler et al (8, 9) is not comparable to these preparations as it contains much less flavin per mg of protein and has a significantly higher ratio of iron:flavin. In these respects the dehydrogenase of Ringler et al resembles complex I, except that it lacks Q reductase activity.

TABLE I

Activities of Fraction A

Assay	Electron Acceptor	Specific Activity*
1	Ferricyanide	215
2	Menadione	160
3	Coenzyme Q_1	150
4	Coenzyme Q_2	35-40
5	Coenzyme Q_6	30-33
6	Coenzyme Q_{10}	12-16
7	DCPIP	100
8	Cytochrome c	43**

Assay conditions:

1. 40 mM potassium phosphate, pH 7.5; 1.3 mM potassium ferricyanide; and 0.75 mM DPNH. Rates were calculated from mM $\Delta A_{410} = 1.0$ after correction for the nonenzymatic rate.

2 - 6. 50 mM Tris-sulfate, pH 8.0; 0.4 mM menadione in 1% final volume of ethanol, 0.4 mM coenzyme Q_1 (see ref. 1 for preparation of Q_1 solution), 0.15 mM Q_2, 0.15 mM Q_6 or 0.07 mM Q_{10} in 3% final volume of methanol; and 0.15 mM DPNH. Assay conditions for Q_2, Q_6 and Q_{10} were adapted from ref. 7, and the reaction mixtures containing these quinones were incubated 2.5 min. at 38° before addition of enzyme. Rates were calculated from mM $\Delta A_{340} = 6.22$.

7. 50 mM Tris-Cl, pH 8.0; 0.65 mM DCPIP; and 0.75 mM DPNH. Rates were calculated from mM $\Delta A_{600} = 21$.

8. 20 mM potassium phosphate, pH 8.5; 0.1% serum albumin; 0.75 mM DPNH and 0.15 mM cytochrome c. Rates were calculated from mM $\Delta A_{550} = 18.5$.

* Specific Activity is expressed as μmoles DPNH oxidized/min/mg of protein at 38°.

** V max $\simeq$ 220; apparent Km $\simeq$ 0.6 mM cytochrome c.

Although the Mahler-type dehydrogenases appear to represent the same segment of the respiratory chain as fraction A, it seems that variations in constituents and activities between these preparations are caused by differences

in the details of their isolation procedure.

All the activities shown in Table I are completely inhibited by pCMS, but the degree of inhibition at micromolar amounts of pCMS added to the reaction mixture varies with various electron acceptors. Some inhibition of quinone reductase activity is observed in the presence of Amytal and rotenone. However, by comparison to complex I, these effects occur at much higher concentrations of the inhibitors and appear to be mechanistically different from those observed with complex I. o-Phenanthroline is a potent inhibitor of the enzyme (see, however, ref. 11) and 3 mM 2-thenoyltrifluoroacetone inhibits the cytochrome c reductase activity of fraction A by about 65%, but has a feeble effect with other electron acceptors. The behavior of other inhibitors also suggests that the mechanism of cytochrome c reduction by fraction A is different from those involving the reduction of quinones and ferricyanide. DPNH above 0.3 mM concentration sharply inhibits the reduction of menadione and coenzyme Q; this effect is not observed in the reduction of ferricyanide, cytochrome c or DCPIP. In this and other respects the ferricyanide reductase activity of fraction A differs from that of complex I. In the latter system, ferricyanide reduction is sharply inhibited above 0.1 mM DPNH. Also per mole of flavin* the DPNH-ferricyanide reductase activity of fraction A (apparent $Km \simeq 5.9 \times 10^{-5}$ M DPNH) is 5 to 7 times lower than that of complex I (apparent $Km \simeq 7 \times 10^{-6}$ M DPNH). These observations suggest that the mechanism of ferricyanide reduction in the isolated flavoprotein is different from that in intact mitochondria and complex I (see also ref. 9 and 12). The studies of Huennekens, Mackler and their colleagues

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* Per mg of protein, the purified dehydrogenase contains 10 to 12 times as much flavin as complex I.

(13) on a Mahler-type dehydrogenase prepared from ETP indicate that the flavin-depleted enzyme retains full activity toward ferricyanide (30-40 μmoles DPNH oxidized per min per mg protein). Although in complex I the relationship of flavin and ferricyanide reductase activity is not known, the marked inhibition of this reaction in complex I by high concentrations of DPNH suggests the involvement of flavin in catalysis (14).

Fraction B

This is a brown protein fraction containing per mg of protein about 30 mμatoms of iron, 28 mμmoles of labile sulfide and no detectable flavin or cytochromes. The brown color is bleached by hydrosulfite and the spectral characteristics of fraction B are suggestive of an iron-protein (Fig. 2). An important property of fraction B is that it is reduced by DPNH plus DPNH dehydrogenase (fraction A) and that its reduced form is reoxidized by added coenzyme Q_1 or Q_2 (Table II). The oxidized form of fraction B is not reduced by addition of reduced coenzyme Q_2 (Table II). These properties suggest a redox potential for fraction B between those of the dehydrogenase flavoprotein and coenzyme Q.

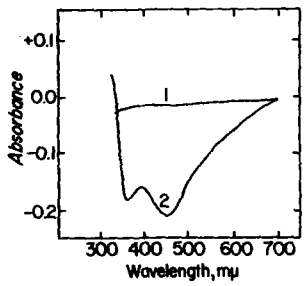

Fig. 2. Difference Spectrum of fraction B. Curve 1, 4.29 mg/ml of fraction B protein in 50 mM Tris-Cl, pH 8.0, in the experimental and reference cells. Curve 2, after addition of $Na_2S_2O_4$ to the experimental cell. Specta were recorded by Beckman DK-2 spectrophotometer.

TABLE II

Reduction and Oxidation of Fraction B

Exp.	Protein Fractions	Additions	ΔA_{450}
1	A	DPNH, 0.2 μmole	-0.025
2	A	DPNH, 0.4 μmole	-0.092
3	B	DPNH, 0.4 μmole	-0.007
4	A + B	DPNH, 0.2 μmole	-0.065
5	A + B	DPNH, 0.4 μmole	-0.135
6	reduced B	Q_2, 15 mμmoles	+0.065
7	reduced B	Q_2, 30 mμmoles	+0.155*
8	B	Q_2H_2, 40 mμmoles	-0.012

Assay conditions:

The experiments were carried out in the DK-2 Beckman spectrophotometer with the experimental and reference cells each containing the protein solutions given below in 50 mM Tris-Cl, pH 8.0. At zero time, additions were made to the experimental cell as indicated in the Table and the difference spectrum of the experimental versus the reference cell was recorded between 700 mμ and 300 mμ.

Exp. 1 & 2 - 0.5 mg fraction A protein in 0.5 ml buffer.
3 - 0.75 mg fraction B protein in 0.5 ml buffer.
4 & 5 - 0.5 mg fraction A protein plus 0.75 mg fraction B protein in 0.5 ml buffer.
6 & 7 - 2.5 mg fraction B protein in 0.6 ml buffer. The material in the experimental cell was carefully reduced by addition of graded amounts of hydrosulfite. The solution was then aerated until excess hydrosulfite was oxidized and the diff erence spectrum of reduced minus oxidized fraction B could be recorded without interference due to hydrosulfite absorption at about 320 mμ. Then both cells were deaerated before addition of coenzyme Q.
8 - 2.75 mg fraction B protein in 0.65 ml buffer under anaerobic conditions.
* A cyclohexane extract of the solution in the experimental cell showed that at least 16 mμmoles of the added Q_2 had been converted to Q_2H_2.

Rieske et al (15) have isolated an iron-rich protein from complex III (reduced coenzyme Q-cytochrome c reductase) (16) by succinylation of complex III proteins. The succinylated protein retains its characteristic ESR signal at

g = 1.90. However, no reactivity with other components of complex III or of the electron transfer system has yet been shown with this modified protein.

Fraction C

This fraction consists of about 85% of complex I protein and, in addition to the residual cytochrome impurities of complex I,* it contains per mg of protein 10 to 12 mμatoms of iron and 10 to 12 mμmoles of labile sulfide, which might be in part due to incomplete extraction of fraction B. It is insoluble in pH 8.0 buffer or sucrose solutions, but dissolves in 0.1 N NaOH containing 4 M urea. The solubility properties of fraction C are reminiscent of the mitochondrial structural protein (17).

Spectrophotometric, analytical and ESR studies have shown that three different species of nonheme iron are present and undergo reduction and oxidation in complexes I, II and III, respectively (2). The isolation from complex I of an iron-rich protein fraction capable of reduction by DPNH plus DPNH dehydrogenase and reoxidation by coenzyme Q indicates that in the mitochondrial electron transfer system nonheme irons are present in the form of discrete iron-proteins distinct from succinic and DPNH dehydrogenase flavoproteins which themselves contain iron.

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* 0.1 mμmoles cytochrome b plus c_1 per mg protein.

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