RECONSTITUTION OF THE ELECTRON TRANSPORT SYSTEM
I. Preparation and Properties of the Interacting Enzyme Complexes

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The electron transport system, operative in succinoxidase and DPNH-oxidase, can be divided into four enzyme complexes:

(a) DPNH-coenzyme Q reductase

(b) Succinic-coenzyme Q reductase

(c) Reduced coenzyme Q (QH_2) - cytochrome c reductase

(d) Cytochrome c oxidase

Each complex has now been obtained in our laboratory in relatively pure form with little or no contamination by the others.

Recently, we have been able to combine the first three complexes and reconstitute the DPNH - cytochrome c reductase system, the succinic-cytochrome c reductase system as well as the DPNH, succinic-cytochrome c reductase system. Functionally the reconstituted particles are indistinguishable from the original systems, which exist in the mitochondrion and derivative particles.

Coupling one enzyme system to another is quite common in biochemical research and reconstruction in the sense that an enzyme system can be functionally coupled to another is very easily achieved with soluble as well as with particulate enzymes. The only requirement in such cases is that the two systems should share a water-soluble intermediate which must be present during assay at relatively high concentration. Thus the alcohol dehydrogenase system can be linked to the respiratory chain of mitochondria provided that DPN, the common intermediate, is present in high concentration. Two particulate

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enzymes, such as mitochondrial cytochrome c reductase and cytochrome c oxidase can also be coupled when sufficient amounts of cytochrome c (cyt. c) are added to the assay medium. Reconstitution of the electron transport system in the sense that will be described in this and the accompanying communication is of an entirely different nature. In these instances reconstitution is achieved by interaction between two particles to reform the larger unit from which they were derived; and no water soluble, common intermediate is required during assay to establish electronic communication between the daughter particles. Reconstitution occurs when relatively concentrated solutions of the daughter particles are mixed together (Hatefi et al. 1961a). If dilute solutions of the two particles are mixed, reconstitution does not occur to any appreciable extent. However, once reconstitution has been achieved by mixing together of concentrated solutions of the daughter particles, then the solution can be diluted and the reconstituted system acts as a functional entity showing no tendency for dissociation. Reconstruction of the original enzyme unit such that structural and functional integrity are optimally attained suggests that a specific recombination of the daughter particles takes place.

In the present communication the preparation and general properties of the above complexes are discussed. In the accompanying communication (Hatefi et al. 1961a) the conditions for reconstitution and general properties of the reconstituted succinic-cyt. c reductase, DPNH-cyt. c reductase and succinic, DPNH-cyt. c reductase systems will be presented.

DPNH-Coenzyme Q Reductase

This enzyme complex constitutes the first half of the DPNH-cyt. c reductase system of mitochondria, which has been isolated in purified form and its properties extensively studied (Hatefi et al. 1961 b, c). Preparations of DPNH-cyt. c reductase contain DPNH flavoprotein, non-heme iron, coenzyme Q (Q), cyt. b and cyt. c_1. The enzyme catalyzes the reduction by DPNH of about 50-60 μmoles cyt. c/min/mg protein at 38°. The overall activity of the system is inhibited by p-chloromercuriphenyl sulfonate (p-CMS), Na-Amytal, antimycin

A, SN-5949 and 2-nonyl-4-hydroxyquinoline-N-oxide. The point of inhibition by Amytal is after the flavoprotein and before Q and the cytochromes, while antimycin A blocks electron flow below cyt. c_1 and after Q and cyt. b. The Q-reductase activity of the preparation and the conditions for assay of this activity have been reported (Hatefi et al. 1960).

The DPNH-Q reductase complex has been isolated from DPNH-cyt. c reductase by fractionation with cholate and $(NH_4)_2SO_4$. This unit contains DPNH flavoprotein, non-heme iron and Q. The spectrum of the preparation is given in Fig. 1 and its enzymic properties are summarized in Table I. The preparation is essentially free of other activities. A residual amount of cytochromes b and c_1 (less than 0.10 mμmoles/mg protein) is still present in the enzyme; consequently a small degree of cyt. c reductase activity remains. DPNH-Q

TABLE I

Enzymic Properties of DPNH-Coenzyme Q Reductase

Electron Acceptor	Inhibitor	Specific Activity
Q_1	--	27.2
Q_1	p-CMS (6 μM)	0.0
Q_1	Amytal (3 mM)	< 2.0
Q_1	Demerol (0.5 mM)	< 2.0
Ferricyanide	--	208.0

The assay for DPNH-Q reductase activity was the same as described previously (Hatefi et al. 1960). Conditions for DPNH-ferricyanide reductase assay - 40 μmoles Tris-Cl, pH 7.5, 0.15 μmole DPNH, 1.6 μmoles $K_3Fe(CN)_6$ in the experimental cuvette and 0.9 μmole in the blank cuvette and water to 1.0 ml. The experiments were carried out at 38° and the specific activity is expressed as μmoles substrate (one electron) reduced or oxidized/min/mg enzyme protein.

reductase activity is inhibited by Amytal and p-CMS but not by antimycin A. Only the isoprenologues of coenzyme Q are rapidly reduced by the enzyme; compounds such as 2,3-dimethoxy-5,6-dimethylbenzoquinone (MeQ_0) and 2,3-dimethoxy-5-methylbenzoquinone (Q_0) are poor substitutes (Hatefi et al. 1960).

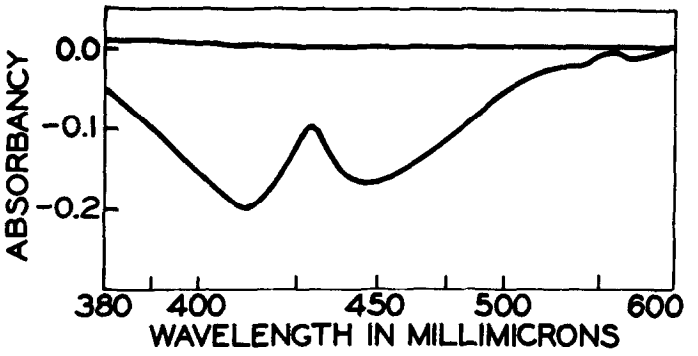

Figure 1 - Difference spectrum of DPNH - Q reductase - 5 mg enzyme protein, 20 μmoles K-phosphate, pH 8.0, and 0.66 M sucrose to 1.0 ml. The preparation was reduced with 0.15 μmole DPNH.

Reduced Q (QH_2) - Cytochrome c Reductase

This enzyme complex constitutes the second half of the DPNH-cyt. c reductase and remains in the supernatant after precipitation of DPNH-Q reductase by $(NH_4)_2SO_4$. QH_2-cyt. c reductase is precipitated by further addition of $(NH_4)_2SO_4$ to the medium. This enzyme complex contains cyt. b, cyt. c_1 and non-heme iron, and catalyzes only the reduction of cyt. c by QH_2 (Table II). This reaction is inhibited by antimycin A but not by Amytal. The spectrum of the preparation is given in Fig. 2.

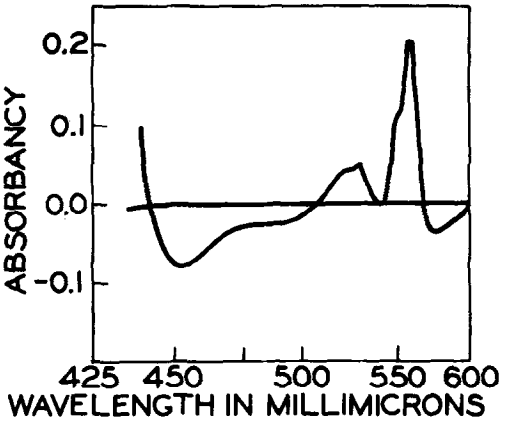

Figure 2 - Difference spectrum of QH_2 - cyt. c reductase - 1.8 mg enzyme protein, 20 μmoles K-phosphate, pH 8.0, and 0.66 M sucrose to 1.0 ml. The preparation was reduced with $Na_2S_2O_4$.

TABLE II

Enzymic Properties of QH_2 - Cytochrome c Reductase

Substrate	Inhibitor	Specific Activity
QH_2	---	420.0
QH_2	Antimycin A (2 µg/ml)	< 20.0
Succinate	---	0.0
DPNH	---	0.0

Conditions for QH_2-cyt. c reductase assay* - 40 µmoles K phosphate, pH 7.4, 0.1 µmole EDTA, 20 µmoles Na Azide, 50 µg Q_2H_2 (in ethanol), 1.5 mg cyt. c. Specific Activity is expressed as µmoles cyt. c reduced per min. per mg. protein at 38°. Succinic-cyt. c reductase and DPNH-cyt. c reductase assays have already been described (Hatefi et al. 1961b).

* We are indebted to Dr. J. Rieske for performing these assays.

Succinic-Q reductase has been prepared by Ziegler and Doeg (1959) in this laboratory. The preparation contains succinic flavoprotein, non-heme iron and little or no Q. It also contains cyt. b whose functional link to the dehydrogenase has been altered during isolation, rendering it non-reducible by succinate. Succinic-Q reductase activity is inhibited by thenoyltrifluoroacetone but not by Amytal or antimycin A. Other characteristics of this preparation have been described previously by Ziegler and Doeg (1959). The interactions of the above enzyme complexes have been of great value in the study of sequence of components and their functional interrelationships in the electron transport system of mitochondria.

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