RECONSTITUTION OF THE ELECTRON TRANSPORT SYSTEM

III. RECONSTITUTION OF DPNH OXIDASE, SUCCINIC OXIDASE, AND DPNH, SUCCINIC OXIDASE

by L. R. Fowler* and Y. Hatefi

Institute for Enzyme Research
University of Wisconsin, Madison, Wisconsin

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It was shown, in the two preceding communications in this series (Hatefi et al. 1961 a,b), that the mitochondrial electron transport system could be divided into four functional complexes: DPNH-coenzyme Q reductase (I), succinic-coenzyme Q reductase (II), reduced coenzyme Q (QH_2)-cytochrome c reductase (III), and cytochrome oxidase (IV). Complexes I and III were derived from a highly active preparation of DPNH-cytochrome c reductase (V) (Hatefi et al. 1961 a,c). Complex II was prepared in this laboratory by Ziegler and Doeg (1959).

It has been demonstrated (Hatefi et al. 1961 b) that (a) by combining I and III, a highly active DPNH-cytochrome c reductase unit is reconstituted, (b) when II and III are combined, a comparably active succinic-cytochrome c reductase is obtained, and (c) combination of I, II and III results in a unit with both DPNH and succinic-cytochrome c reductase activities. The conditions for the attainment of reconstitution and other characteristics of these reconstructed enzyme systems have already been discussed. The present communication deals with the recombination of cytochrome oxidase** (Complex IV) with Complexes I, II and

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* Post-doctoral trainee of the University of Wisconsin Institute for Enzyme Research.

**The cytochrome oxidase preparation used in these experiments was purified from the "green residue" obtained in the initial step during the isolation of DPNH-cytochrome c reductase from mitochondria (Hatefi et al. 1961 c). Fresh preparations of the enzyme catalyzed the oxidation of ferrocytochrome c at a rate of 75 μmoles cytochrome c oxidized by molecular oxygen/min/mg protein. The heme a content of the cytochrome oxidase, as calculated from direct spectrophotometric measurements was 5.9 mμmoles/mg protein. (For procedures, see Griffiths and Wharton, 1961).

III, or with DPNH-cytochrome c reductase (V), to yield reconstituted units with the respective DPNH oxidase, succinic oxidase, and DPNH, succinic oxidase activities.

Reconstitution of DPNH Oxidase

When IV, V and cytochrome c (0.45 mg/mg protein of IV plus V) are combined, a highly active DPNH oxidase system is reconstituted. IV or V in individual combination with cytochrome c showed little or no activity (Table I). It was necessary to premix the above components at high concentration for appreciable reconstitution to occur (Table II). (See Hatefi et al. 1961 a, for a discussion of this point.) As in mitochondria, the DPNH oxidase activity of the reconstituted particle was strongly inhibited by azide, cyanide, antimycin A and amytal.

TABLE I

Reconstitution of the DPNH Oxidase System from Three Components

Enzyme Preparation	Specific Activity
(V + cyt. c)	0.0
(IV + cyt. c)	1.8
(IV + V + cyt. c)	45.8
" + azide	2.6
" + cyanide	0.0
" + antimycin A	0.0
" + amytal	0.0

DPNH-oxidase activities are expressed as μmoles DPNH (1 electron equivalents) oxidized/min/mg cytochrome oxidase (IV) protein, at 38° C. The reaction was followed spectrophotometrically, by the decrease in absorbancy at 340 mμ. on addition of reconstituted enzyme (containing 2.5 μg of IV) to a total volume of 1.0 ml of an aqueous medium containing 20 μmoles KPO_4, pH 7.0; 52 μg asolectin lipid; 0.01 μmole EDTA; and 0.2 μmole DPNH.

TABLE II

Effect of Concentration on DPNH Oxidase Reconstitution

Enzyme Preparation	Specific Activity
IV + V + cyt. c	5.8
(IV + V) + cyt. c	23.2
(IV + V + cyt. c)	32.8

Components enclosed in parentheses in this and subsequent tables were mixed at high concentration (ca. 20 mg/ml of I-V) before addition to the assay medium. Components not in parentheses were added separately to the assay system. The final concentration of each component in the assay medium was the same in all experiments.

Reconstitution of the DPNH oxidase system is achieved also by admixture of I, III and IV, in the presence of cytochrome c (0.45 mg/mg protein of I + III + IV) (Table III). In this case also, the premixing of the above components at high concentration was mandatory for maximal reconstitution.

TABLE III

Reconstitution of DPNH Oxidase from Four Components

Enzyme Preparation	Specific Activity
I + III + IV + cyt. c	2.6
(I + III) + IV + cyt. c	8.9
(I + III + IV + cyt. c)	29.3

Assay conditions were the same as in Table I.

Reconstitution of Succinic Oxidase

Reconstitution of the succinic oxidase system was achieved by combining

II, III and IV in the presence of cytochrome c (0.11 mg/mg protein of II + III + IV) (Table IV). Attention is drawn to the fact that the reconstituted activity of this system is also blocked by the typical inhibitors of the succinoxidase system of mitochondria.

Keilin and King (1958) have recently reported on the reconstitution of a succinic oxidase system from a soluble succinic dehydrogenase and an alkali-treated heart muscle preparation. Since sufficient data have not been given for the composition and the various activities of these materials, no comparison with our studies can be made at this time.

TABLE IV

Reconstitution of the Succinic Oxidase System

Enzyme Preparation	Specific Activity
(II + cyt. c)	0.0
(III + cyt. c)	0.0
(II + III) + IV + cyt. c	2.6
(II + III + IV + cyt. c)	18.7
" + azide	< 1.0
" + cyanide	0.0
" + antimycin A	0.0
" + thenoyltrifluoroacetone	< 1.0

Succinic oxidase activity is expressed as μmoles succinate (1 electron equivalents) oxidized/min/mg protein of IV, at 38° C. The reaction was followed by measuring the disappearance of oxygen in the assay medium, by means of an oxygen electrode. The enzyme, containing 73 μg cytochrome oxidase (IV) protein, was added to a total of 1.6 ml of an aqueous medium, containing 30 μmoles KPO_4, pH 7.4; 52 μg asolectin lipid; 0.01 μmole EDTA; and 20 μmoles Na succinate.

Reconstitution of DPNH, Succinic Oxidase

When I, II, III and IV are combined in the presence of cytochrome c

(0.18 mg/mg protein of I + II + III + IV), a unit with both DPNH oxidase and succinic oxidase activity is reconstituted (Table V). Both activities are inhibited by cyanide and antimycin A. DPNH oxidase, but not succinic oxidase, is inhibited by amytal, while the latter activity is specifically inhibited by thenoyltrifluoroacetone.

TABLE V

Reconstitution of DPNH, Succinic Oxidase

Enzyme Preparations	Specific Activity	
	Succinic Oxidase	DPNH Oxidase
IV + cyt. c	0	0
(I + II + III) + cyt. c	3.1	2.9
(I + II + III + IV + cyt. c)	28.7	14.5
" + cyanide	0	0
" + antimycin A	0	0
" + amytal	25.7	0
" + thenoyltrifluoro-acetone	0.7	---

The assay conditions were the same as in Table IV. Substrates were 20 μmoles Na succinate, and 0.75 μmole DPNH. Specific activities are expressed as μmoles substrate (1 electron equivalents) oxidized/min/mg protein of IV at 38° C.

Conclusions

The electron transport system of mitochondria can be divided into four enzyme complexes. These complexes are capable of recombining with one another, and, depending on the units involved, the following enzyme systems can be reconstituted:

1. DPNH-cytochrome c reductase
2. Succinic-cytochrome c reductase
3. DPNH, succinic-cytochrome c reductase
4. DPNH oxidase
5. Succinic oxidase
6. DPNH, succinic oxidase

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