RECONSTITUTION OF THE ELECTRON TRANSPORT SYSTEM II. Reconstitution of DPNH - Cytochrome c Reductase*, Succinic-Cytochrome c Reductase and DPNH, Succinic-Cytochrome c Reductase

Y. Hatefi, A. G. Haavik** and D. E. Griffiths**

Institute for Enzyme Research, University of Wisconsin
Madison, Wisconsin

Received March 27, 1961

In the preceding communication (Hatefi et al. 1961a), the preparation, the properties and the component entities of DPNH-Q*** reductase (I), succinic-Q reductase (II) and QH_2-cyt. c reductase (III) have been summarized. When I and III are combined, a highly active DPNH-cyt. c reductase is reconstituted, whose activity, as in mitochondria, is inhibited by Amytal and antimycin A (Table I). When II and III are combined, a highly active succinic-cyt. c reductase is formed (Table II). The activity of this system is in turn inhibited by thenoyltrifluoroacetone as well as by antimycin A. The only requirement for reconstitution, as outlined in the preceding communication, is that the daughter particles should be mixed together in relatively high concentration. The enzyme preparations are usually suspended in a buffered sucrose solution (0.66 M sucrose, 0.05 M Tris-Cl, pH 8.0) and are mixed together at 0°. After mixing, the solution is diluted with Tris-sucrose to 0.1 mg protein/ml and assayed at 0.5-1.0 μg enzyme protein/ml.

As seen in Table III, essentially no reconstitution occurs unless the daughter particles are mixed together in high concentration. However, once reconstitution is achieved in this manner, subsequent dilution of the solution does not cause the dissociation of the reconstituted particles. Thus the suspension of the

---

* DPNH-cyt. c reductase is defined as the Amytal and antimycin A sensitive system, which contains the functional components (DPNH-flavoprotein, non-heme iron, coenzyme Q, cyt. b and cyt. c_1) found in intact mitochondria.

** Postdoctoral trainees of the University of Wisconsin Institute for Enzyme Research.

*** For abbreviations cf. Hatefi et al. 1961a.

TABLE I

Reconstitution of the DPNH-Cytochrome c Reductase System

Enzyme Preparation	Specific Activity
I	8.9
III	0.0
I + III	57.4
I + III + Amytal	0.0
I + III + Antimycin A	0.0

After combining I and III, the mixture was diluted to 0.1 mg protein/ml and assayed at 1 μg protein/ml. Assay conditions were the same as described previously by Hatefi et al. (1961b). Specific Activity is expressed as μmoles cyt. c reduced/min/mg protein of I at 38°.

TABLE II

Reconstitution of the Succinic-Cytochrome c Reductase System

Enzyme Preparation	Specific Activity
II	0.0
III	0.0
II + III	48.4
II + III + antimycin A	< 2.0
II + III + Thenoyltrifluoroacetone	0.0

Assay conditions were the same as in Table I.

reformed particle can be diluted and assayed at 1 μg protein per ml without decline of activity with time.

Although reconstitution does not take place in dilute solutions of the reacting particles, strictly speaking it necessitates the presence in high concentration of only one of the daughter particles. That is, so long as one of the daughter particles is present in high concentration, the other can be

TABLE III

Effect of Enzyme Concentration on Reconstitution

Enzymes I and III (μg/ml of each)	DPNH-cyt. c Reductase Activity	Enzymes II and III (μg/ml of each)	Succinic-cyt. c Reductase Activity
1	4.5	1	0.9
10	6.4	10	2.6
5,000	43.2	100	13.2
		6,000	39.0

Specific Activity is expressed as μmoles cyt. c reduced/min/mg protein of I or II. Other details of the assay are given in Table I.

added in smaller amounts and reconstitution achieved to the extent that the concentration of the second particle is the limiting factor. Thus, when small amounts of QH_2-cyt. c reductase are added to a concentrated solution of DPNH-Q reductase, the emergent DPNH-cyt. c reductase activity increases linearly as the level of QH_2-cyt. c reductase is increased (Fig. 1). When optimal amounts of the latter are added, the concentration of the reconstituted particles (the DPNH-cyt. c reductase) becomes maximal and further additions of QH_2-cyt. c reductase to the mixture are ineffective. Per mg protein, QH_2-cyt. c reductase is far more active (i.e. in terms of its capacity for catalyzing electron flux from donor to acceptor) than DPNH-Q reductase (Hatefi et al. 1961a). However, as seen in Fig. 1, reconstituted activity is a function of protein concentration of the daughter particles (i.e. the number of particles of each) rather than a function of equal activity units of each. Similar results have been obtained when succinic-Q reductase is titrated with QH_2-cyt. c reductase. These results indicate that reconstitution as described above is the result of interaction between the two particulate segments of the electron transport chain to reform the structured unit from which they were derived.

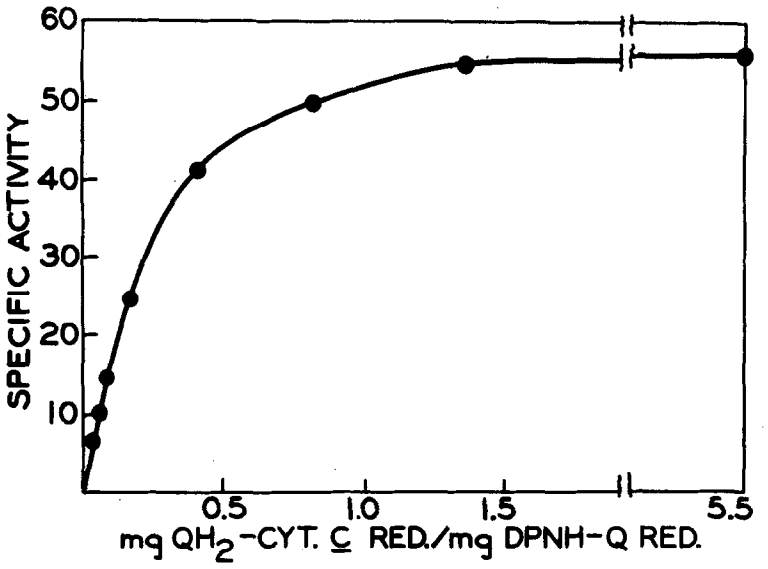

FIGURE 1

Reconstitution of DPNH-cyt. c reductase as a function of QH_2-cyt. c reductase concentration - specific activity and other details of the assay are the same as in Table I. The residual cyt. c reductase activity of DPNH-Q reductase alone was subtracted from the experimental values obtained.

Reconstitution of DPNH, Succinic-Cytochrome c Reductase.

The reconstitution of the particle with both DPNH and succinic-cyt. c reductase activity is achieved by mixing together nearly equal amounts of the enzyme complexes I, II and III. Thus, as seen in Table IV a cyt. c reductase particle is obtained which is highly active with respect to both succinate and DPNH as substrates. When succinate is the substrate, electron flow to cyt. c is inhibited by thenoyltrifluoroacetone and antimycin A but not by Amytal. When DPNH is the substrate, it is the latter two compounds that are fully effective as inhibitors.

The possibility has been considered that by mixing together enzyme preparations I, II and III, two separate particles are reconstituted, one capable of catalyzing DPNH-cyt. c reduction and the other succinic-cyt. c reduction. However, this does not appear to be the case. When DPNH-cyt. c reductase and succinic-cyt. c reductase particles are separately reconstituted and then

TABLE IV

Reconstitution of the DPNH, Succinic-Cytochrome c Reductase System

Enzyme Preparation	Inhibitor	Specific Activity Succinic-Cyt. c	Specific Activity DPNH-Cyt. c
DPNH-Q reductase (I)	---	0.0	8.9
Succinic-Q reductase (II)	---	0.0	0.0
QH_2-Cyt. c reductase (III)	---	0.0	0.0
I + III	---	0.0	57.4
II+ III	---	48.4	0.0
I + II + III	---	56.6	42.9
I + II + III	Antimycin A	< 4.0	0.0
I + II + III	Amytal	52.0	0.0
I + II + III	Thenoyltrifluoroacetone	0.0	---

Specific Activity is expressed as μmoles cyt. c reduced/min/mg protein of I or II at 38°. Other details of the assay are given in Table I.

together added to the assay medium, their respective cyt. c reductase activities are additive, which means that the two enzyme systems function separately. However, when enzymes I, II and III are combined and the DPNH, succinic-cyt. c reductase unit is reconstituted, the DPNH-cyt. c and the succinic-cyt. c reductase activities of the system are not additive. This observation argues against the possibility that two reconstituted particles (a DPNH-cyt. c reductase and a succinic-cyt. c reductase) are separately formed. On the contrary, it suggests that a rate limiting step (possibly the reduction of coenzyme Q) is shared by both dehydrogenases. We also have evidence, inter alia, that the bound coenzyme Q of I is shared by both the DPNH-cyt. c and the succinic-cyt. c reductase pathways when enzymes I, II and III are combined.

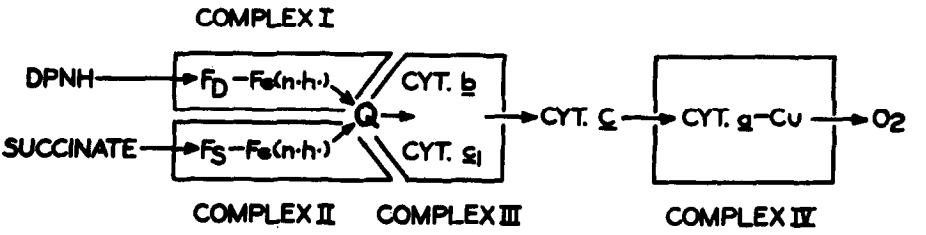

FIGURE 2

Schematic Representation of the Functional Complexes and their Arrangement in the Electron Transport System.

CONCLUSIONS

The studies reported in this and the preceding communication indicate that the terminal electron transport system of mitochondria can be divided into four discrete units. These units are the enzyme systems I, II and III described above, and the cytochrome oxidase unit which contains cyt. a and copper (Hatefi, 1959; Griffiths and Wharton, 1961). Each unit may be considered as a complex of enzymes, coenzymes and a paramagnetic metal ion capable of undergoing oxidation-reduction. Electron transport activity in each unit is a function of the special structural arrangement of its components. There is some evidence that suggests further resolution of the above complexes results in irreversible loss of the properties that are characteristic of the corresponding activities in the intact electron transport system.

Since the reaction catalyzed by cytochrome oxidase has been thoroughly investigated and defined (Griffiths and Wharton, 1961), the overall structure of the electron transport system, composed of the above four complexes, may be summarized as shown in Figure 2. Functionally the reconstituted cytochrome c reductase systems can be linked to oxygen by adding cytochrome oxidase and cyt. c to the reaction mixture. While this coupling of activity is not reconstitution as defined earlier (Hatefi et al. 1961a), it is hoped that this final step of the terminal electron transport system can be also reconstituted; however, as yet no definitive experiments in this direction have been performed.

REFERENCES

Griffiths, D. E. and Wharton, D. C., J. Biol. Chem. (1961), in press.

Hatefi, Y., Biochim. Biophys. Acta, 30, 648 (1959).

Hatefi, Y., Haavik, A. G. and Griffiths, D. E., Biochim. Biophys. Res. Comm. 441 (1961a).

Hatefi, Y., Haavik, A. G. and Jurtshuk, P. (1961b), submitted to Biochim. Biophys. Acta.

Hatefi, Y., Jurtshuk, P. and Haavik, A. G. (1961c), submitted to Biochim. Biophys. Acta.