CHARACTERISTICS OF A SUCCINATE-DICHLOROPHENOLINDOPHENOL REDUCTASE RECONSTITUTED WITH BOVINE HEART ELECTRON TRANSPORT COMPONENTS[1]

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The reconstituted succinate-dichlorophenolindophenol reductase complex described previously (1) has been further characterized. The complex is very sensitive to 4,4,4 trifluoro-1-(2-thienyl)-1,3 butanedione (TTB), being inhibited 50% by a concentration of 2.5 μM. Combinations of complex III (reduced ubiquinone-cytochrome c reductase) and the DCPIP reductase catalyze a TTB and antimycin A sensitive succinate-cytochrome c reductase. These observations suggest that the reconstructed succinate-DCPIP reductase is very similar in properties to isolated complex II (succinate-ubiquinone reductase).

Succinate dehydrogenase, when solubilized and purified by the method of King (2) can be recombined with other membrane components or with alkali treated submitochondrial particles to yield either succinate-ubiquinone reductase (3), or succinate oxidase (2, 4) activities. The capacity of the enzyme to combine with other membrane components and catalyze a mitochondrial related respiratory chain activity has been termed the reconstitution property of succinate dehydrogenase (2)

The soluble enzyme quickly loses the ability to recombine with mitochondrial electron transport components to catalyze the above activities if it is stored in an air atmosphere. The membrane associated enzyme maintains its stability indefinitely, however. In a recent study we established that a combination of phospholipid and a cytochrome b enriched fraction will stabilize the reconstitution property of solubilized succinate dehydrogenase (1). The resulting complex between the above membrane components catalyzes a succinate-DCPIP[2] reductase activity.

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[2]Abbreviations: DCPIP, dichlorophenolindophenol; UQ_{10}, ubiquinone with 10 isoprenoid units; TTB, 4,4,4 trifluoro-1-(2-thienyl)-1,3 butanedione; SDH, succinate dehydrogenase

This paper describes the characteristics of this reconstituted complex which establishes that it is similar in properties to intact complex II (5).

MATERIALS AND METHODS

Sodium dodecyl sulfate, cholic acid, and deoxycholic acid were obtained from Matheson, Coleman, and Bell and were recrystallized from 95, 70, and 70 percent ethanol, respectively. Sigma supplied cytochrome c (Type II), UQ_{10}, DCPIP, and antimycin A. Fisher Scientific furnished TTB. Asolectin was obtained from Associated Concentrates, Inc.

Protein, flavin, and cytochrome b analyses were carried out by methods referred to previously (1). Succinate dehydrogenase (2), the cytochrome b enriched fraction, and liposomes were prepared as described earlier (1). The flavin content of the succinate dehydrogenase was 5.9 nmol/mg protein. The cytochrome b enriched fraction used in this study contained 6.2 μmol heme/g protein corresponding to a purity of 17%, assuming the pure cytochrome b has a heme content of 36 μmol/g protein (6). Complex III was prepared as described by Rieske (7).

Preparation of the succinate-DCPIP reductase complex and its assay were described earlier (1). Preparation of the succinate-cytochrome c reductase complex from the succinate-DCPIP reductase complex and complex III was as follows: To 1 mg of the cytochrome b enriched fraction was added 0.03 ml of 0.25 M sucrose - 5 mM NaPi, pH 6.5, 3.5 mg asolectin, and 0.75 mg succinate dehydrogenase in the sequence listed. An immediate precipitate appeared upon addition of the succinate dehydrogenase. This precipitated material is the succinate-DCPIP reductase complex, as has been established previously. To this suspension was added 1.75 mg of complex III. The final volume was 0.25 ml and the protein concentration was 13.5 mg/ml. The mixture was allowed to incubate for 8 minutes at 30°. Some samples were then brought to 20 mM with respect to succinate and incubated 15 min at room temperature. Other samples were adjusted to a protein concentration of 5 mg/ml with 20 mM succinate - 1 mM EDTA - 0.1 M NaPi, pH 6.5 and incubated for 1 hour at 4°. Variations in these procedures are noted in Table 1. All samples were assayed for succinate-cytochrome c reductase activity at 22° as described by Yamashita and Racker (4), but with the assay mixture being brought to a NaCN concentration of 0.15 mM.

RESULTS AND DISCUSSION

In a previous paper (1) we reported that the reconstituted succinate-DCPIP reductase complex was not always dependent on the addition of external UQ_{10} for enzymatic activity. In fact, complexes reconstituted with preparations of the cytochrome b enriched fraction which had been stored frozen for several days demonstrated no requirement for added UQ_{10} in the assay system. The activities obtained, however, were comparable to those noted for complexes made with fresh preparations of the cytochrome b enriched fraction. This observation raised some question as to the physiological significance of the reconstructed complex. The following experiments demonstrate, however, that the reconstituted complex has

the properties of the succinate-ubiquinone segment of the chain. Figure 1 illustrates the sensitivity of the reconstructed complex to TTB. In this experiment the succinate-DCPIP reductase was assayed in the absence of added UQ_{10} and under these conditions 50% of the activity was inhibited in the presence of 2.5 μM TTB. Much larger amounts are required to inhibit 90% of the activity, as is shown in this figure. The sensitivity observed is higher than that demonstrated in mitochondria. Tappel (8) reported that 50% inhibition of succinate-ubiquinone reductase in intact mitochondria was observed with addition of 10 μM TTB, a concentration four times that required in the present study. The increased amounts needed for mitochondria may be related to the possibility that TTB can be bound to the membrane at sites other than the succinate-ubiquinone segment of the electron transport chain.

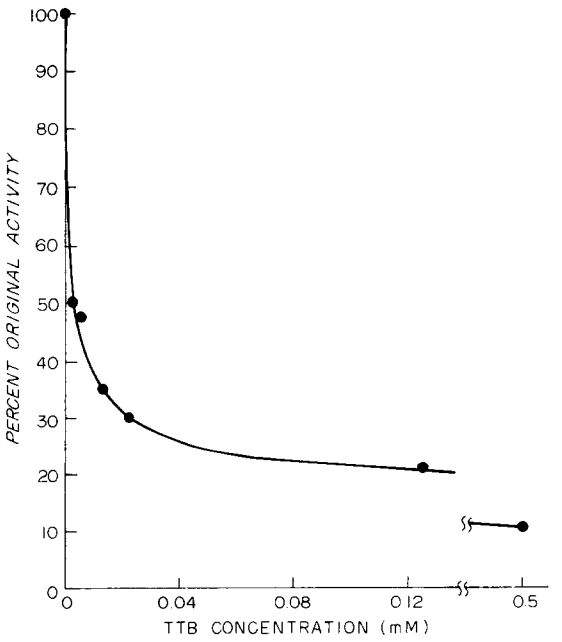

Fig. 1. Inhibition of succinate-DCPIP reductase activity with TTB. Sixty-four μg complex protein was added to each assay. The average specific activity for all the samples assayed was 161 nmol succinate oxidized/min/mg complex protein. Ubiquinone was not added in these assays.

The reconstituted succinate-DCPIP reductase can also combine with complex III to form a succinate-cytochrome c reductase complex, as is shown in Table I. The prior reconstruction of the succinate-DCPIP reductase appears to be a prerequisite for the formation of succinate-cytochrome c reductase. As shown in the table, if the components of the DCPIP reductase are added to complex III the resulting activity is relatively low. Moreover, addition of succinate to activate succinate dehydrogenase or dilution of the mixture has very little effect on the activity of this particular complex. If, however, the succinate-DCPIP reductase is formed before addition of complex III to the incubation mixture activities are significantly higher. Furthermore, diluting the resulting complex to 5 mg/ml results in a dramatic increase in cytochrome c reductase activity. These results suggest that the intact and organized succinate-ubiquinone segment is required for proper

Table I. Preparation of succinate-cytochrome c reductase from the succinate-DCPIP reductase complex and complex III[a]

First Addition	Second Addition	Final concentration of incubation mix (mg protein/ml)	μmol cyt c reduced /min/mg complex
complex III	SDR[b] components	13.5	0.33
complex III	SDR components +20mM succinate	11.3	0.37
complex III	SDR components	5	0.41
SDR components	complex III	13.5	0.76
SDR components	complex III +20mM succinate	11.3	0.88
SDR components	complex III	5	2.33

[a]The components of the succinate-cytochrome c reductase complex were introduced into the incubation mixture as indicated above, with the components of the succinate-DCPIP reductase complex being added in the order listed in the Experimental Procedure.

[b]Succinate-DCPIP reductase.

orientation with complex III. Presumably when the components of the succinate-DCPIP reductase complex are added to complex III before being added together they do not orient properly for formation of the succinate-ubiquinone segment of the electron transport chain. The characteristics of this reconstitution procedure are reminiscent of those observed when succinate-cytochrome c reductase is reconstituted from isolated complex II and complex III. In an earlier study by Tzagoloff, et al (9) combinations of these latter complexes demonstrated high activity if they were added together at high concentration and then diluted out. Likewise, dilution of mixtures of the DCPIP reductase and complex III increases the activity of the resulting succinate-cytochrome c reductase (Table I) and indicates that the reconstructed succinate-DCPIP reductase complex behaves similarly to isolated complex II in reconstitution procedures.

The succinate-cytochrome c reductase complex reconstituted under conditions for maximal activity is sensitive to both TTB and antimycin A, as is demonstrated in Table II. Furthermore, the succinate-DCPIP reductase complex has no TTB or antimycin A sensitive succinate-cytochrome c reductase activity. The complex III preparation utilized in these experiments does have a TTB and antimycin A sensitive cytochrome c reductase activity, but it is only 17% of the activity reconstituted with succinate-DCPIP reductase and complex III.

The specific activities reported for succinate oxidation by the reconstituted succinate-DCPIP reductase and succinate-cytochrome c reductase complexes appear to be low when calculated on a mg complex protein basis. Flavin and cytochrome b analyses of representative reconstituted DCPIP reductase complexes reveal, however, that only part of the succinate dehydrogenase added is being incorporated. The DCPIP reductase contains an average of 0.55 nmol flavin/mg protein (1). Utilizing this number for the flavin content of the complex, values of 3.8 and 6.1 μmol succinate oxidized/min/mg SDH have been calculated as the average and maximal specific activities, respectively, at 22°. In arriving at these values only those activities that were inhibited over 85 percent with TTB were utilized in the calculations. The maximal activity is about half that noted by Davis and Hatefi

Table II. Characteristics of the reconstituted succinate-cytochrome c reductase system[a]

Sample	μmol cyt c reduced/min/mg complex protein		
	no inhibitor	0.25mM TTB	1μg Antimycin A
Succinate-cyt c reductase	3.09	0.09	
Succinate-cyt c reductase	3.47		0.03
Succinate-DCPIP reductase	0.01	0.08	
Succinate-DCPIP reductase	0.01		0.01
Complex III	0.55	0.08	
Complex III	0.56		0.11

[a]The succinate-cyt c reductase and succinate-DCPIP reductase complexes were prepared as described in the Experimental Procedure.

(10) who reported a specific activity of 13.4 when using the King preparation of SDH in their experiments on reconstitution of alkali treated complex II.

Analysis of the purified succinate dehydrogenase revealed a flavin content of 5.9 μmol/g of protein. Using this value and the data in Table II an activity of 13.7 μmol succinate oxidized/min/mg SDH was obtained for the flavoprotein in the succinate-cytochrome c reductase complex. This value was obtained assuming that all the flavoprotein added was functional in the reconstructed complex. Davis and Hatefi (10), in a similar experiment, obtained a value of 13.2 μmol succinate oxidized/min/mg SDH in a succinate-cytochrome c reductase complex reconstituted by recombining the King preparation of SDH, alkali treated complex II, and complex III. The specific activities we have calculated for succinate oxidation and those reported by Davis and Hatefi (10) may not be directly comparable since different methods were used to estimate succinate dehydrogenase concentrations of the reconstituted complexes; the similar activities do suggest, however, that the complexes prepared with succinate dehydrogenase, phospholipid, and the cytochrome b enriched fraction are comparable in activity to those

prepared by a combination of the King preparation of SDH and alkali treated complex II.

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