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RESTORATION OF POOL FUNCTION TYPE BEHAVIOR TO SUCCINATE DEHYDROGENASE HAVING LATENT RECONSTITUTIVE ACTIVITY IN UBIQUINOL-CYTOCHROME c REDUCTASE COMPLEX

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Summary Mitochondrial ubiquinol-cytochrome c reductase complex contains small amounts of succinate dehydrogenase. Estimates from electrophoresis indicate there is one dehydrogenase per eight complexes. This dehydrogenase transfers electrons to the b-c_1 complex poorly, as judged by low succinate-ubiquinone and succinate-cytochrome c reductase activities. Electron transfer to the b-c_1 complex is restored by reconstitution of the complex with phospholipid. This phospholipid dependent restoration of electron transfer indicates that either reconstitutive activity of the dehydrogenase is preserved under conditions where electron transfer is absent, or that addition of phospholipid allows one dehydrogenase to transfer electrons to multiple b-c_1 complexes.

Introduction

The mechanism by which succinate dehydrogenase (SDH) transfers electrons to the b-c_1 segment of the mitochondrial electron transport chain is not understood. Recent experimentation has focused on the importance of the high potential iron-sulfur center, S-3, in reconstitution of SDH to the b-c_1 segment. Consequently, there is extensive evidence that reconstitution of soluble SDH to the membranous b-c_1 complex depends on the integrity of center S-3, which is labile in the soluble dehydrogenase and stabilized upon its rebinding to the membrane (1,2).

Coincident with binding to the membrane and stabilization of center S-3, SDH acquires succinate-ubiquinone reductase activity, which is notably absent in the soluble dehydrogenase (3). Thus it would appear that reconstitution of SDH to the b-c_1 complex is a process in which stabilization of center S-3 and its reconstitutive capability is synonomous with conferral of succinate-ubiquinone reductase activity. The purpose of this communication is to report

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that succinate dehydrogenase associated with resolved ubiquinol-cytochrome c reductase complex (complex III) retains reconstitutive capability under conditions where electron transfer activity to ubiquinone and the b-c_1 complex is absent.

Materials and Methods

Complex III was prepared according to Rieske (4) from phosphate washed bovine heart mitochondria (5). The preparations of complex III had ubiquinol-cytochrome c reductase activity of 25-30 units per mg and contained 1.8-2.0 nmol of cytochrome c_1 and 3.4-3.6 nmol of cytochrome b per mg of protein.

Succinate-cytochrome c reductase complex was prepared according to Yamashita and Racker (5), except the amount of cholate used in the initial extraction was lowered to 0.35 mg per mg of protein.

Succinate dehydrogenase was purified from succinate-cytochrome c reductase complex by a procedure recently developed in this laboratory (B. Trumpower and M. Fried, manuscript in preparation). The dehydrogenase, which was reconstitutively active, was 40-60 percent pure by nonheme iron, acid-labile sulfide, and flavin analysis, although electrophoresis profiles suggest a higher degree of purity (see Figure 1).

Phospholipids were purified from soybean phosphatides (6). Acrylamide gel electrophoresis was performed with gels containing 10 percent acrylamide (7); the samples were denatured in 5 percent dodecyl sulfate-5 percent mecroptoethanol by heating 30 min at 85°C.

Succinate-ubiquinone reductase activity was measured with 48 μM DBH, a decyl substituted analog of ubiquinone-2 (8), using 52 μM dichloroindophenol (DCPIP) as terminal acceptor (3). This same concentration of DCPIP was used to measure succinate dehydrogenase activity, using phenazine methosulfate (PMS) as a mediator (9,10). Succinate-cytochrome c reductase activity was measured as previously described (11). All activities were measured at 30°C and rates are expressed in units of one electron equivalent, a unit being defined as one microgram equivalent per minute.

Results and Discussion

In preparing complex III from bovine heart mitochondria we have consistently obtained preparations which appear to contain small amounts of succinate dehydrogenase when analyzed by acrylamide gel electrophoresis in dodecyl sulfate. The SDH associated with complex III can be tentatively identified by the appearance of the M_R = 70,000 flavoprotein subunit, which is quantitatively the most intensely stained polypeptide of purified SDH (Figure 1a) and which is detectable as a minor polypeptide in profiles of complex III (Figure 1b).

Other laboratories have also commonly observed SDH in preparations of bovine complex III (12-15) and, most recently, Siedow and coworkers (16) noted that SDH was the most difficult contaminant to remove in preparing

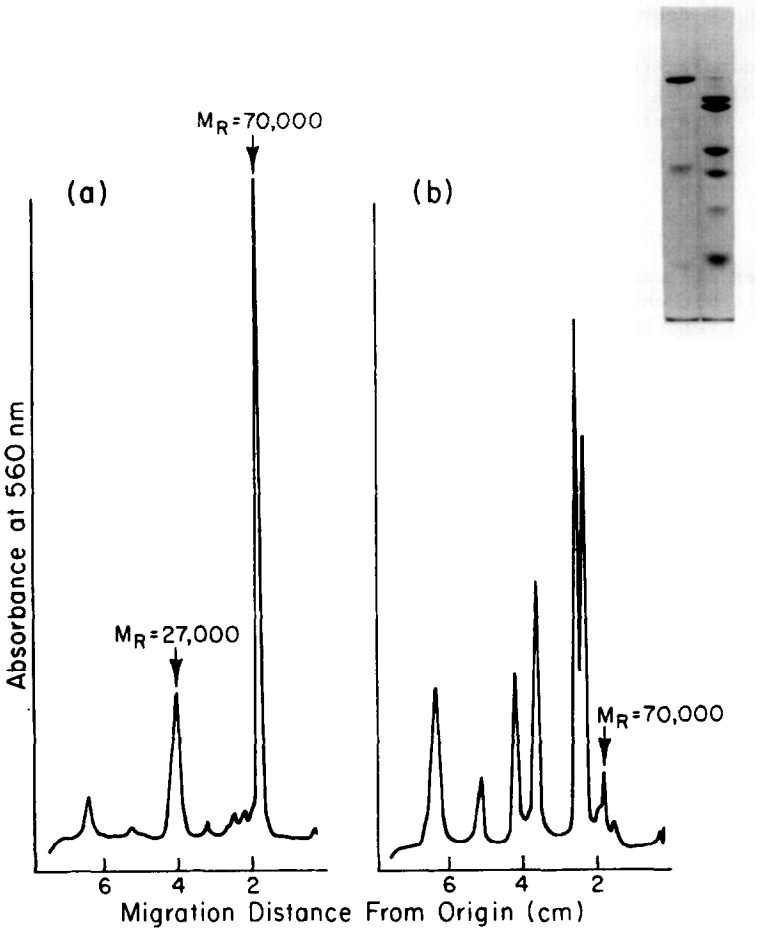

Figure 1. Polyacrylamide-SDS gel electrophoresis (7) profiles of succinate dehydrogenase (a) and complex III (b). As discussed in the text, the amount of SDH present in complex III can be estimated from the staining intensity of the M_R = 70,000 flavoprotein subunit of SDH which is detectable in the profile of complex III and which can be compared to the intensity of the M_R = 30,600 heme-containing cytochrome $\underline{c}_1$ polypeptide (15), which is located 3.6 cm from the origin in (b).

complex III from yeast. Whether this persistant association of SDH with complex III reflects a permanent structural association of the dehydrogenase with the $\underline{b}$-$\underline{c}_1$ complex $\underline{in\ situ}$, or is due to a coincidental association during isolation of complex III is not known.

To estimate the amount of SDH associated with complex III, soluble SDH of known nonheme iron, acid-labile sulfide, and flavin content (Figure 1a) was

used to establish a quantitative relationship between staining intensity and amount of flavoprotein subunit on electrophoresis. Applying this method to complex III, whose molarity was determined by measuring cytochrome $\underline{c}_1$ content spectrophotometrically, and assuming the M_R = 70,000 polypeptide in complex III is due solely to SDH, the complex III in Figure 1b was estimated to contain one SDH per 8.4 cytochrome $\underline{c}_1$. This would correspond to 0.45 nmol of SDH per mg of complex. Although this method of quantitating the content of dehydrogenase is perhaps less exact than measurement of histidyl flavin, it seems clear that one SDH is obtained coincidently with multiple $\underline{b}$-$\underline{c}_1$ complexes.

Although a small amount of SDH is commonly noted in electrophoresis profiles of complex III (12-16), little attention has been given to whether this dehydrogenase is active. Various laboratories have noted that soluble SDH, which is capable of reconstituting electron transfer to membranes depleted by alkaline treatment, is not capable of reconstitution with complex III (17,18). Thus it is of interest to determine whether SDH which is indigenous to preparations of complex III has retained reconstitutive activity, as measured by its ability to transfer electrons to the $\underline{b}$-$\underline{c}_1$ complex.

As shown in Table I, SDH associated with complex III has succinate-PMS-DCPIP reductase activity of approximately 3 units per mg. However, there is very low succinate-ubiquinone reductase or succinate-cytochrome $\underline{c}$ reductase activity. By comparison, in resolved succinate-cytochrome $\underline{c}$ reductase complex the succinate-PMS-DCPIP and succinate-ubiquinone-DCPIP reductase activities are usually equivalent and 50 percent to 2 fold greater than the cytochrome $\underline{c}$ reductase activity (Table I).

If phospholipid is added to complex III the succinate-ubiquinone and succinate-cytochrome $\underline{c}$ reductase activities are increased 6-7 fold (Table I), while there is only a 33 percent increase in succinate-PMS-DCPIP reductase activity. As a consequence of adding phospholipid the rates of these three reactions relative to each other are like those observed with resolved succinate-cytochrome $\underline{c}$ reductase complex, although the absolute rates of the

Table I

Demonstration of Latent Electron Transfer Activities in Complex III

Electron Transfer Reaction	Rates of Electron Transfer (units/mg)		
	No addition	Plus phospholipid	SCR
Succinate-PMS-DCPIP	2.92	4.00	9-11
Succinate-Q-DCPIP	0.59	4.11	9-11
Succinate-Cytochrome c	0.39	2.85	5-6

Complex III (300 µg) was incubated 30 min at 25°C in 200 µl of 40 mM Na phosphate-20 mM Na succinate-0.5 mM Na EDTA (pH 7.4). Where indicated, 50 mol phospholipid per mol of cytochrome c_1 was included in the incubation. The incubated samples were diluted to 2 ml by addition of the same buffer, previously chilled to 4°C, and 1.5 µg of complex III was withdrawn for assays. The activities shown for succinate-cytochrome c reductase complex (SCR) are the range of activities obtained with 5 preparations.

phospholipid reconstituted complex III are lower. Addition of ubiquinone-10 along with phospholipid had no effect beyond that observed with phospholipid alone (results not shown).

The latent, phospholipid dependent activities show the expected inhibition by antimycin and thenoyltrifluoroacetone as shown in Table II. The succinate-ubiquinone reductase activity is inhibited by thenoyltrifluoroacetone, but not by antimycin, while the succinate-cytochrome c reductase activity is inhibited by both compounds.

Figure 2 shows the relationship between restoration of latent electron transfer activities and amount of phospholipid added. Maximum activity is obtained with approximately 60 molecules of phospholipid per b-c_1 complex. Whether the stoichiometry of this phospholipid requirement is determined by the molarity of b-c_1 complex or dehydrogenase remains to be determined. These

Table II

Effect of Inhibitors on Reconstituted Latent Electron Transfer Activities

Electron Transfer Reaction	Rates of Electron Transfer (units/mg)		
	Control	+ Antimycin	+ TTFA
Succinate-Q-DCPIP	3.74	3.40	0.76
Succinate-Cytochrome c	3.50	0.28	0.13

Complex III was reconstituted with phospholipid as described in the legend to Table I. Where indicated, 4 μg of antimycin per mg of complex III was added after the 30 min incubation. Thenoyltrifluoroacetone (TTFA) was added directly to the assay mixture at a concentration of 500 μM.

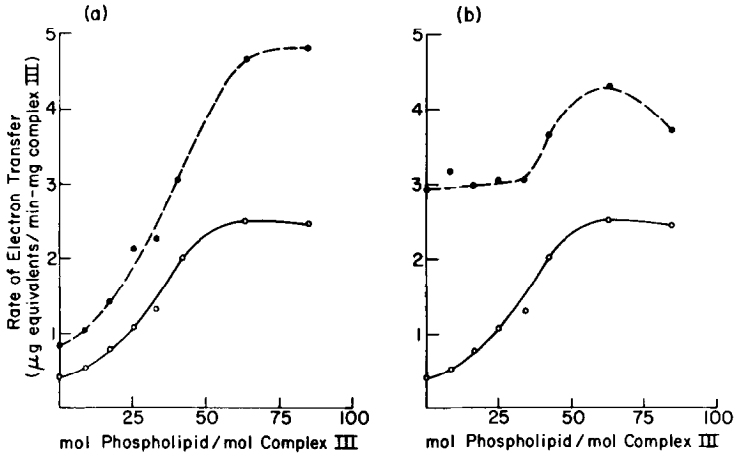

Figure 2. Titration curves showing the amount of phospholipid required to restore latent electron transfer activities to complex III. The curves in (a) show the coincident reconstitution of succinate-ubiquinone reductase (-●-●-) and succinate-cytochrome c reductase (-o-o-) activities. The curves in (b) show the effect of phospholipid on succinate-PMS-DCPIP reductase activity (-●--●-), compared to succinate-cytochrome c reductase activity (-o-o-).

results also show that the restoration of succinate-cytochrome c reductase activity is coincident with restoration of succinate-ubiquinone reductase activity. By contrast, there is no change in the succinate-PMS-DCPIP reductase activity at amounts of phospholipid up to 30 mol per mol of complex III, which causes a 3-4 fold increase in succinate-cytochrome c reductase activity.

One possible explanation for the results reported here is that the ability of SDH to reconstitute with the b-c_1 complex is stabilized under conditions where reconstitutive activity *per se* is not manifested. If such is the case it will be of interest to establish whether iron-sulfur center S-3 shows the kinetic (1) and spectroscopic (2) properties previously correlated with reconstituted dehydrogenase, or whether these properties of center S-3 are likewise latent.

A second possibility is that the SDH found in complex III is fully reconstituted to the b-c_1 complex before addition of phospholipid, but in a manner such that one SDH can transfer electrons to only one b-c_1 complex. Thus the activities which are restored by addition of phospholipid might result from nonstoichiometric interaction of one SDH with multiple b-c_1 complexes. The 6-7 fold increase in activity resulting from phospholipid addition, when compared to the estimated ratio of SDH to b-c_1 complex, is consistent with this possibility. This pool function type of activity, if applicable, might be due to a mobile pool of ubiquinone (19,20) which depends on addition of phospholipid, or due to phospholipid dependent mobility of the dehydrogenase, as recently proposed to explain the pool function type interaction of NADH dehydrogenase with complex III which is dependent on added phospholipid (21).

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