Reconstitution of Succinate-Coenzyme Q Reductase (Complex II) and Succinate Oxidase Activities by a Highly Purified, Reactivated Succinate Dehydrogenase*

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SUMMARY

Succinate dehydrogenase has been isolated in a highly purified form from succinate-coenzyme Q reductase preparations. The enzyme contains 1 mole of covalently bound flavin, 8 g atoms of iron, and 8 moles of acid-labile sulfide per 150,000 g of protein. It catalyzes succinate oxidation in the presence of phenazine methosulfate as electron acceptor at a rate of 26 to 32 amoles per min × mg of protein (V_{\text{max}} \approx 37). The succinate dehydrogenase thus prepared does not couple to the electron transport system, but it can be made to do so after treatment with Na₂S, ferrous ions, and mercaptoethanol. Thus, both the succinate-coenzyme Q reductase and the succinoxidase systems have been reconstituted by combining the reactivated succinate dehydrogenase with appropriate segments of the respiratory chain. These results permit a better understanding of the composition and the electron transfer pathway of the mitochondrial respiratory chain from succinate to coenzyme Q.

Since its recognition in 1909 (1) and extraction in soluble form in 1954 (2), succinate dehydrogenase has been the subject of intensive study and numerous reports and review articles (3-5). Nevertheless, some of the most fundamental characteristics of this enzyme, such as molecular weight, composition, enzymatic activity, and mode of interaction with the components of the respiratory chain, have remained unsettled. In order of complexity and ability to interact with natural electron acceptors, the preparations of succinate dehydrogenase reported to date may be divided into three categories.

Classical preparations of succinate dehydrogenase, which contain 1 mole of flavin, 4 g atoms of iron, and 4 moles of acid-labile sulfide per 200,000 g of protein, catalyze electron transfer from succinate to phenazine methosulfate, and less efficiently to ferricyanide, but they do not react with the components of the electron transport system (3, 4).

The succinate dehydrogenase preparations of King (5-7) extracted from mitochondrial particles at alkaline pH in the presence of succinate contain, on the average, 1 mole of flavin, 8 g atoms of iron, and 8 moles of labile sulfide per 320,000 g of protein (8), and, in addition to dyes, they interact with electron transport particles the succinoxidase activity of which has been impaired by pretreatment with alkali. The reconstitution activity1 of such preparations is extremely labile and depends on the presence of succinate during extraction from mitochondria. In the absence of succinate, an enzyme is obtained which is incapable of reconstitution, but according to the work of King, has the same spectral properties and dye reductase activity as the succinate-treated extract (6). The reason that one extract is active for reconstitution and another is inactive has not been explained.

The succinate-coenzyme Q reductase of Ziegler and Doeg (9, 10) contains 1 mole of flavin, 1 mole of cytochrome b₈, 8 g atoms of iron, and 8 moles of labile sulfide2 per 240,000 g of protein. It reacts with PMS3 and coenzyme Q as electron acceptors and recombines with the appropriate complexes of the electron transport system to reconstitute highly active succinate-cytochrome c reductase and succinoxidase particles (11-13). The succinate-coenzyme Q reductase reaction is specifically inhibited by 2-thienyltrifluoroacetone.

This article provides new information regarding the molecular weight, composition, and spectral properties of succinate dehydrogenase, the composition and electron transfer pathway of succinate-coenzyme Q reductase, and the site of action of thenoyltrifluoroacetone. More significantly, it demonstrates the interaction of the soluble dehydrogenase with the electron transport system and shows that reconstitution is the only reliable test for activity of the isolated components of the respiratory chain.

1 In this communication, reconstitution activity is defined as the ability of soluble succinate dehydrogenase to transfer electrons to the respiratory chain.
2 Determined in this laboratory.
3 The abbreviations used are: PMS, phenazine methosulfate; Q, coenzyme Q (ubiquinone); reactivated succinate dehydrogenase, succinate dehydrogenase reactivated for reconstitution; ETP, electron transport particle prepared from mitochondria by sonic disruption; DCI, 2,6-dichloroindophenol; TTA, 2-thienyltrifluoroacetone.
MATERIALS AND METHODS

Preparation of Succinate-Coenzyme Q Reductase—Succinate-coenzyme Q reductase (Complex II) was isolated from bovine heart mitochondria essentially according to the procedure of Ziegler and Doeg (9). The following modifications resulted in better reproducibility. Mitochondria were isolated in the presence of 0.5 mM sodium succinate. The mitochondrial paste was resuspended in three-fourths of its weight of the original supernatant and adjusted to 70 mg of protein per ml. The 5-hour dialysis was performed against a solution 0.25 M in sucrose and 10 mM in potassium phosphate, pH 7.4, and for the final deoxycholate-ammonium sulfate treatment the protein concentration was adjusted to 22 to 25 mg per ml instead of 30 mg per ml. Finally, the removal of detergent with ammonium acetate in the presence of urea was replaced with filtering the preparation through a coarse Sephadex G-25 column equilibrated and eluted with 20 mM potassium phosphate, pH 7.4. The trailing end of the Complex II band containing bile salts was eliminated; Complex II was collected by centrifugation for 1 to 2 hours at 105,000 × g, resuspended in phosphate buffer at the desired concentration, and kept frozen under purified nitrogen. For the preparation of succinate dehydrogenase, the Complex II pellet was resuspended in 50 mM Tris-HCl, pH 8.0, and kept frozen as before.

Preparation of Succinate Dehydrogenase—Complex II preparations were suspended in Tris-HCl (buffer pH was adjusted to 8.0 at room temperature), as described above, at a protein concentration of 15 to 20 mg per ml. The suspension was gassed with purified nitrogen, stored for 24 to 48 hours at −20°C, and then thawed and centrifuged for 2 hours at 105,000 × g. The supernatant was collected and reconstituted under nitrogen, and the pellet was extracted once again with Tris-HCl. The extracts were pooled and fractionated with neutral, saturated ammonium sulfate. Fractions precipitating at 41.2 and 44.6% salt saturation were removed, and the fraction precipitating at 54.6% saturation was collected and dissolved in 20 mM potassium phosphate, pH 7.4, at a concentration of at least 5 mg of protein per ml. The succinate dehydrogenase thus prepared is stable for weeks at 20°C under nitrogen. When kept in the presence of oxygen, the activity of succinate dehydrogenase diminishes rather rapidly, and the decrease in activity parallels the loss of labile sulfide. This behavior is similar to the rapid decay of activity and loss of labile sulfide of the mitochondrial DPNH dehydrogenase at pH 4.8 to 5.0 (14). Under conditions described elsewhere (15), starch gel electrophoresis of fresh succinate dehydrogenase gave a single band, and parallel protein-activity profiles (each as a single band) were obtained when succinate dehydrogenase was chromatographed on Sephadex G-100.

The cytochrome-rich pellet of Complex II and the first and second ammonium sulfate fractions still contain a considerable amount of succinate dehydrogenase, which can be further purified as above. Presence of succinate in Complex II suspensions inhibits the extraction of succinate dehydrogenase by Tris-HCl. The resolution of Complex II with respect to succinate dehydrogenase is also possible by chaotropic agents (16). Thus, at 37°C, the presence of 2.5 mM urea or 0.3 to 0.5 M guanidine hydrochloride results in extraction of succinate dehydrogenase from Complex II preparations, but the extraction procedure described above is definitely milder.

Preparation of Succinate Dehydrogenase Active for Reconstitu-
was treated at pH 9.4 according to the procedure of King (6). Alkali
treatment of the cytochrome fraction of Complex II was
either by application of the procedure of King (6) or by double
extraction of the particles at room temperature with about 200
volumes each time of 50 mM Tris-HCl, pH 9.4. Such cytochrome
preparations still retained 25 to 50% of the Complex II flavin
but were completely inactive in the succinate-coenzyme Q re-
ductase assays. After alkali treatment, all the particles were
sedimented by centrifugation. The cytochrome fractions of
Complex II were resuspended in 50 mM phosphate, pH 7.2, and
ETP was re suspended in a solution of the
same buffer containing
in addition, 0.25 M sucrose. Because the alkali-treated
cytochrome fraction of Complex II is especially rich in cyto-
chrome b (>5 nmoles per mg of protein), it will be referred to
hereafter as the cytochrome b fraction.

**Assay Conditions**—Succinate dehydrogenase activity was
measured spectrophotometrically at 38° by the phenazine metho-
sulfate-mediated reduction of DCI at 600 mU. The reaction
mixture contained 50 mM potassium phosphate (pH 7.4), 20 mM
sodium succinate, 0.1 mM EDTA, and 0.1% bovine serum
albumin. Immediately before addition of enzyme, 70 pM DCI
and 1.65 mM PMS were added to the reaction mixture. Succe-
inate dehydrogenase at about 4 to 8 mg of protein per ml of 20
mM phosphate, pH 7.4, was initially incubated with 20 mM
succinate for 5 min at 37°, then diluted and assayed at about 4
µg per ml of the reaction mixture. The PMS-DCI reductase
activity of Complex II preparations was assayed in the same
manner, except that the reaction mixture also contained 1 mM
TTA in a final volume of 1% ethanol.

Succinate-coenzyme Q reductase activity was measured by the
coenzyme Q-dependent reduction of DCI or ferri cyanide (9).
The DCI reaction mixture contained 50 mM potassium phos-
phate (pH 7.4), 20 mM succinate, 0.1 mM EDTA, and, immedi-
ately before addition of enzyme, 70 pM DCI and 50 pM coenzyme
Q2 in a final volume of 2% ethanol were also added. The ferri-
cy anide reaction mixture contained the same concentra-
tions of buffer, EDTA and coenzyme Q2, but the pH of the
medium was 7.6, the experimental cell contained 1.6 M potas-
sium ferri cyanide, and the reference cell half as much.

Succinooxidase assays were conducted at 30° (6) on a Gilson
Medical Electronics oxygraph fitted with a Clark electrode.
Flavin, iron, and labile sulfide were estimated as described pre-
viously (15). Cytochrome c1 was estimated according to the
procedure of Basford *et al.* (20) and coenzyme Q according to the
method of Hat efi (21). Succinate dehydrogenase and coenzyme
Q reductase rates were calculated from changes in the absorbance
of DCI at 600 mU (ε600 21) and of ferri cyanide at 420 mU (ε420
1.0). Except where indicated in Table III, all activities are
expressed as micromoles of succinate oxidized per min × mg of
total protein.

All chemicals were reagent grade. PMS was obtained from
Sigma. Coenzyme Q2 was generously supplied by Dr. O.
I sler, of Hoffman-La Roche, and Dr. K. Folkers, of Stanford
Research Institute.

**RESULTS**

**Succinate-Coenzyme Q Reductase**—The Complex II prepara-
tions obtained in this laboratory have essentially the same
composition as those of Ziegler and Doeg (9, 10) with respect to
flavin, cytochrome b, iron, and labile sulfide. They also reduce
coenzyme Q at a ratio of 50 to 55 µmoles per min × mg of protein
at 38°. In contrast to the reports of Ziegler and Doeg, we find
that Complex II preparations contain various amounts of cyto-
chrome c1 (maximum, 1.5 µmoles per mg of protein).4 Furthermore,
our studies have shown that the cytochrome b, but not the
cytochrome c1, of Complex II preparations is slowly reducible
by succinate (Fig. 1). Both the rate and the extent of cyto-
chrome b reduction are increased in the presence of added co-
enzyme Q2, and the reaction in the presence or absence of added
coenzyme Q is inhibited by TTA. In addition, a most surpris-
ing finding is that the reduction of cytochrome b, even in the
presence of added coenzyme Q, is severely inhibited by 1 to 2 µM
antimycin A. As has been reported already (22), these concen-
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Acid-unextractable flavin ........................................ 6–7
Acid-labile sulfide .................................................. 48–51
Iron ................................................................. 52–54

**Fig. 2.** The spectral characteristics of succinate dehydrogenase. The enzyme at a protein concentration of 1.8 mg per ml was dissolved in 15 mM Tris-HCl, pH 8.15, containing 1.68 mM urea. The presence of urea at slightly alkaline pH prevents turbidity development during various treatments. Trace 1, oxidized succinate dehydrogenase; Trace 2, difference spectrum of Trace 1 succinate dehydrogenase minus Trace 3 succinate dehydrogenase showing contribution of the iron-labile sulfide system to the spectrum of oxidized succinate dehydrogenase; Trace 4, oxidized succinate dehydrogenase treated with dithionite; and Trace 5, the spectrum of succinate dehydrogenase after destruction of the iron-labile sulfide system with mersalyl and reduction of the flavin with dithionite.

Reactivation of Succinate Dehydrogenase—Succinate dehydrogenase, isolated from Complex II preparations, has the composition shown in Table I. Per milligram of protein, the enzyme has a much higher concentration of acid-unextractable flavin, iron, and labile sulfide than the preparations of others (4, 8). It has no detectable cytochromes, and its ratio of iron to labile sulfide to flavin is approximately 8:8:1. In the presence of PMS as electron acceptor, the enzyme catalyzes the oxidation of succinate at a rate of 26 to 32 pmoles per min x mg of protein at 38° (V _max_ \( \approx 37 \)). The comparable rate in the presence of ferricyanide as acceptor is 2 to 2.5. Similar to purified preparations of the mitochondrial DPNH dehydrogenase (15, 23), the activity of succinate dehydrogenase is slowly diminished upon incubation at room temperature with 5 mM o-phenanthroline (50% inhibition after 1 hour; 85% inhibition after 3 hours), but is little affected upon treatment with bathophenanthroline or Tiron.

The spectral characteristics of succinate dehydrogenase are shown in Fig. 2. Trace 1 is the spectrum of oxidized succinate dehydrogenase and Trace 1 the spectrum of dithionite-reduced succinate dehydrogenase. Trace 2 is the contribution of the iron-labile sulfide system to the oxidized spectrum, and Trace 3 is the contribution of flavin. Trace 3 shows the spectrum of succinate dehydrogenase after destruction of the iron-labile sulfide system with sodium mersalyl and reduction of the flavin with dithionite. It is seen that (a) the 550 nm shoulder and the 490 nm peak in Trace 1 are attributable to the iron-labile sulfide system of succinate dehydrogenase and the 450 nm shoulder due to both iron-labile sulfide and flavin, (b) the contribution of the iron-labile sulfide system to the absorbance of the oxidized enzyme at 450 nm amounts to a molar extinction coefficient of approximately 2300 per atom of iron, and (c) succinate dehydrogenase has essentially no characteristic absorption between 400 and 700 nm other than those contributed by flavin and iron-labile sulfide. These results are qualitatively very similar to those obtained with the mitochondrial DPNH dehydrogenase (15). The electron spin resonance spectrum of succinate-treated succinate dehydrogenase at 90° K is shown in Fig. 3. The flavin free radical at g = 2 is marked and the iron-labile sulfide signal at g = 1.94 is clearly seen at higher field strength.

Preparations of succinate dehydrogenase are completely inactive for reconstitution. However, as will be seen below, this property is easily restored to the enzyme.

**Table I**

<table>
<thead>
<tr>
<th>Component</th>
<th>Nanomoles (or nanoatoms)/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-unextractable flavin</td>
<td>6–7</td>
</tr>
<tr>
<td>Acid-labile sulfide</td>
<td>48–51</td>
</tr>
<tr>
<td>Iron</td>
<td>52–54</td>
</tr>
</tbody>
</table>

Fig. 2. The spectral characteristics of succinate dehydrogenase. The enzyme at a protein concentration of 1.8 mg per ml was dissolved in 15 mM Tris-HCl, pH 8.15, containing 1.68 mM urea. The presence of urea at slightly alkaline pH prevents turbidity development during various treatments. Trace 1, oxidized succinate dehydrogenase; Trace 2, difference spectrum of Trace 1 succinate dehydrogenase minus Trace 3 succinate dehydrogenase showing contribution of the iron-labile sulfide system to the spectrum of oxidized succinate dehydrogenase; Trace 4, oxidized succinate dehydrogenase treated with dithionite; and Trace 5, the spectrum of succinate dehydrogenase after destruction of the iron-labile sulfide system with mersalyl and reduction of the flavin with dithionite.

Preparations of succinate dehydrogenase are completely inactive for reconstitution. However, as will be seen below, this property is easily restored to the enzyme.

**Reactivation of Succinate Dehydrogenase for Reconstitution**—Treatment of succinate dehydrogenase with ferrous ammonium sulfate, Na2S, and mercaptoethanol as described under “Methods and Materials” restores to succinate dehydrogenase the ability to interact with alkali-inactivated ETP or with the cytochrome b-rich fraction of Complex II, respectively, to reconstitute succinoxidase or succinate-coenzyme Q reductase activity. As seen in Table II, incubation of ETP at 38° and pH 9.4 for 60 min completely destroys the succinoxidase activity of the particles. Addition of succinate dehydrogenase to alkali-treated ETP elicits no oxidation of succinate, but the addition of reactivated succinate dehydrogenase completely restores the succinoxidase activity of the particles. Similar results for reconstitution of succinate-coenzyme Q reductase activity are shown in Table III. It is seen that only the combination of reactivated succinate dehydrogenase and the cytochrome b-rich fraction of Complex II leads to reconstitution. Reactivated succinate dehydrogenase and the cytochrome b fraction can be added separately to the reaction mixture, but reactivation of succinate dehydrogenase is required in each case.
Fig. 3. The electron spin resonance spectrum of succinate-treated succinate dehydrogenase. The enzyme at 15.45 mg of protein per ml was initially incubated for 3 min at 35° with 0.1 mM succinate before it was frozen in liquid nitrogen.

### Table II

**Reconstitution of succinate oxidase activity**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP</td>
<td>0.50</td>
</tr>
<tr>
<td>Alkali-treated ETP</td>
<td>0.00</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>0.00</td>
</tr>
<tr>
<td>Reactivated succinate dehydrogenase</td>
<td>0.00</td>
</tr>
<tr>
<td>Alkali-treated ETP + succinate dehydrogenase</td>
<td>0.00</td>
</tr>
<tr>
<td>Reactivated ETP + succinate dehydrogenase</td>
<td>0.48</td>
</tr>
<tr>
<td>Reactivated ETP + reactivated succinate dehydrogenase + TTA</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### Table III

**Reconstitution of succinate-coenzyme Q reductase activity**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP</td>
<td>0.02</td>
</tr>
<tr>
<td>Alkali-treated ETP</td>
<td>0.02</td>
</tr>
<tr>
<td>Reactivated succinate dehydrogenase</td>
<td>0.02</td>
</tr>
<tr>
<td>Cytochrome b</td>
<td>0.02</td>
</tr>
<tr>
<td>Succinate dehydrogenase + cytochrome b</td>
<td>0.03</td>
</tr>
</tbody>
</table>

the reaction mixture (Table III, Experiment II), or premixed and sedimented by centrifugation and then added to the reaction mixture as a particulate complex (Table III, Experiment III).

Figs. 4 and 5 show reconstitution of succinoxidase and Complex II activities as a fixed amount of each reacting partner (i.e. alkali-treated ETP and reactivated succinate dehydrogenase or the cytochrome b fraction of Complex II and reactivated succinate dehydrogenase) is titrated with increasing amounts of the other. It is seen that at low reactivated succinate dehydrogenase concentration, maximum activity is easily reached as the particle concentration (ETP or cytochrome b fraction) is increased, but the saturation of particles (especially of ETP) with reactivated succinate dehydrogenase requires a higher ratio of the dehydrogenase to the particles. The reconstituted succinoxidase activity is completely inhibited by 0.1 mM TTA, and the reconstituted succinate-coenzyme Q reductase activity is inhibited by 2 mM TTA to the same extent (80%) as is the activity of unfractionated Complex II preparations.

Omission of Na2S from the "reactivation" mixture (as in Preparation A) resulted in only 20% reconstitution activity in the succinate-coenzyme Q reductase assay as compared to the complete system, and omission of ferrous ions in only 13% ac-
Reconstitution of Succinate-Coenzyme Q Reductase

FIG. 4. Reconstitution of succinate oxidase activity as a function of relative concentrations of reactivated succinate dehydrogenase and alkali-treated ETP.

FIG. 5. Reconstitution of succinate-coenzyme Q reductase activity as a function of relative concentrations of reactivated succinate dehydrogenase and alkali-treated ETP.

is performed under mild conditions, a considerable loss of iron and labile sulfide (11, 15, 28). Thus, preparations of DPNH dehydrogenase with as little as 25% labile sulfide and 50% iron have been reported by others. The situation with succinate dehydrogenase seems very similar. The preparation of succinate dehydrogenase reported here has an iron to labile sulfide to flavin ratio of 8:8:1, but the earlier preparations of others have had only 2 to 4 μg of iron per mole of covalently bound flavin (24). Nevertheless, as has been shown above, even our preparation of succinate dehydrogenase appears to have sustained some damage during isolation. This damage is undetectable by the PMS reductase activity of the enzyme, but it is clearly evident from the inability of succinate dehydrogenase to interact with the components of the respiratory chain. The damage appears to be on the iron-sulfide system, as it is easily repaired by treatment of succinate dehydrogenase with NaS, ferrous ions, and mercaptoethanol. Furthermore, the damage appears to be slight even though it completely renders the enzyme impotent for reconstitution. This is because as far as the accuracy of the analytical methods permits, the ratio of iron-labile sulfide-flavin is the same in both Complex II and succinate dehydrogenase.

Composition of Mitochondrial Succinate-Coenzyme Q Reductase System—The low iron and labile sulfide content of earlier preparations of succinate dehydrogenase, the inactivity of such preparations for reconstitution, and the reconstitution activity of the low flavin (3.2 nmoles per mg of protein)-high iron-sulfide (26 nanoatoms and 24 nmoles per mg of protein, respectively) preparation of King (8) had allowed the possible presence of a separate iron-sulfur protein in the Complex II system. However, the fact that the iron-sulfide-flavin ratio of Complex II (8:8:1) is recovered in a highly purified succinate dehydrogenase (6.5 to 7 nmoles of flavin per mg of protein) makes the presence of an additional iron-sulfur protein in Complex II rather unlikely.

Electron Transfer Pathway from Succinate to Coenzyme Q—Among the known components of Complex II preparations, only flavin, the iron-labile sulfide system of succinate dehydrogenase and cytochrome b are reduced by succinate. The rate of reduction of cytochrome b is too slow, however, to support coenzyme Q reduction by Complex II. On the other hand, the reconstitution studies reported above suggest that the iron-sulfide system of succinate dehydrogenase plays a significant role in the reduction of coenzyme Q by succinate. Thus, it appears that, as far as the known carriers of Complex II are concerned, electron transfer from succinate to coenzyme Q involves only the components of succinate dehydrogenase. However, if this reasoning is correct, then why is reactivated succinate dehydrogenase unable to reduce coenzyme Q by itself? One possible explanation might be in the altered catalytic properties of the soluble succinate dehydrogenase as compared to the particle-bound enzyme (25). As mentioned above, such changes have been extensively documented in the case of the mitochondrial DPNH dehydrogenase ([15, 23]; see also Reference 14 for other examples). The low dye reductase activity of soluble succinate dehydrogenase as compared to the particle-bound enzyme (4) might also be an indication of such allotopic (dislocation) changes.

Site of Action of Thenoyltrifluoroacetone—It has been shown

This mixture was not tested in the succinate-coenzyme Q reductase assay system because of the nonenzymatic interaction of free mercaptoethanol with DCI.

* As pointed out earlier (17), this argument assumes that succinate dehydrogenase itself is a single protein and not a complex of a flavoprotein and an iron-sulfur protein.
that in Complex II the reduction of both coenzyme Q and cytochrome b is inhibited by TTA. As far as can be determined, succinate dehydrogenase is the only component preceding cytochrome b and coenzyme Q in the succinate-coenzyme Q reductase system (see also Reference 17). These results suggest, therefore, that the site of action of TTA is on succinate dehydrogenase itself. The fact that TTA does not inhibit PMS reduction further suggests that TTA acts on the iron-sulfide system of succinate dehydrogenase rather than on its flavin.

Activity of Reconstituted Systems—It has been shown in Table III that succinate dehydrogenase plus cytochrome b exhibits a TTA-sensitive Q reductase activity of about 0.4, whereas reactivated succinate dehydrogenase plus cytochrome b has an activity of 11.5. These results indicate that, in reconstitution experiments, it is extremely important to keep in mind the turnover capacity of the system and attempt to recover activities commensurate with the enrichment of components in the reconstituted system as compared to ETP preparations. That reconstruction of highly active systems is possible has been reported in earlier work from this laboratory on the reconstitution of the entire electron transport system from purified complexes of the respiratory chain (11-13). The above example indicates that, had we been satisfied with the activity of 0.4 pmole of succinate oxidized per min, we should never have really reconstituted the succinate-coenzyme Q reductase system.

Acknowledgments—We thank Dr. A. Bearden for making the electron spin resonance spectrophotometer available to us and Dr. W. G. Hanstein for recording the electron spin resonance spectrum of succinate dehydrogenase. We also thank P. Tejada and C. Munoz for expert technical assistance.

*Note in Fig. 5 that, with a constant amount of reactivated succinate dehydrogenase and increasing cytochrome b, the TTA-uninhibited rate is constant, but with constant cytochrome b and increasing reactivated succinate dehydrogenase, the TTA-uninhibited rate also increases.

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