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Electron-transfer complexes of *Ascaris suum* muscle mitochondria. II. Succinate-coenzyme Q reductase (complex II) associated with substrate-reducible cytochrome *b*-558

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A succinate-coenzyme Q reductase (complex II) was isolated in highly purified form from *Ascaris* muscle mitochondria by detergent solubilization, ammonium sulfate fractionation and gel filtration on a Sephadex G-200 column. The enzyme preparation catalyzes electron transfer from succinate to coenzyme Q_1 with a specific activity of 1.2 μmol coenzyme Q_1 reduced per min per mg protein at 25°C. The isolated complex II is essentially free of NADH-ferricyanide reductase, reduced CoQ_2-cytochrome *c* reductase and cytochrome *c* oxidase and consists of four major polypeptides with apparent molecular weights of 66000, 27000, 12000 and 11000 and two minor ones with M_r of 36000 and 16000. The complex II contained cytochrome *b*-558, a major constituent cytochrome of *Ascaris* mitochondria, at a concentration of 3.6 nmol per mg protein, but neither other cytochromes nor quinone. The cytochrome *b*-558 in the complex II was reduced with succinate. In the presence of *Ascaris* NADH-cytochrome *c* reductase (complex I–III) (Takamiya, S., Furushima, R. and Oya, H. (1984) Mol. Biochem. Parasitol. 13, 121–134), the cytochrome *b*-558 in complex II was also reduced with NADH and reoxidized with fumarate. These results suggest the cytochrome *b*-558 to function as an electron carrier between NADH dehydrogenase and succinate dehydrogenase in the *Ascaris* NADH-fumarate reductase system.

Introduction

The *Ascaris* NADH-fumarate reductase system is associated with the worm's mitochondrial membrane [1,2]. This system is physiologically significant in anaerobic carbohydrate metabolism of the Ascarid worm, which resides in the host's small intestine where oxygen is quite limited. The electron-transfer reaction, in which fumarate functions as a terminal acceptor of NADH-derived electrons to form succinate, is coupled to ATP synthesis [3–6].

Although the electron-transport system of *Ascaris* muscle mitochondria has been extensively studied using whole mitochondria or submitochondrial particles, the physiological function of the cytochrome components is still obscure [7–18]. Little is known about the protein structure and stoichiometry of the essential redox components of the *Ascaris* NADH-fumarate reductase system. Lack of isolated, active electron-transfer complex has made studies at the molecular level rather difficult. Resolution of the *Ascaris* respiratory chain into electron-transfer complexes has thus become an essential step in the study of the worm's reductase system.

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Abbreviation: CoQ, coenzyme Q.

Recently, we isolated two electron-transfer complexes, NADH-cytochrome *c* reductase (complex I–III) [19] and succinate-coenzyme Q reductase (complex II) from *Ascaris* muscle mitochondria. In this paper, we report the isolation and certain properties of *Ascaris* succinate-CoQ reductase associated with substrate-reducible cytochrome *b*-558 and present evidence as to the probable function of cytochrome *b* in the *Ascaris* NADH-fumarate reductase system.

Materials and Methods

Ascaris muscle mitochondria and NADH-cytochrome *c* reductase (complex I–III) were prepared as previously described [19] and stored at −80°C until use.

Solubilization and purification of succinate-CoQ reductase

All operations were carried out 0–4°C. The frozen mitochondria (1200 mg) was thawed and centrifuged for 15 min at 15000 × *g* in a refrigerated centrifuge (TOMY Seiko, Ltd. Model RS-20GL). The pellet was resuspended in 50 mM potassium phosphate buffer (pH 7.2) and adjusted to a protein concentration of 20 mg/ml. To the mitochondrial suspension were added 10% (w/v) potassium deoxycholate solution (pH 8.0) and 20% (v/v) Triton X-100 to a final concentration each of 1%. The suspension was allowed to stand for 15 min and centrifuged for 30 min at 17000 × *g* and the resulting pellet was removed. To the supernatant obtained was added glycerol to a final concentration of 20% (v/v) to protect the membrane proteins. The preparation at this step was designated as the DOC-Triton X-100 fraction. This fraction was brought to 30% saturation with solid ammonium sulfate. The pH of the solution was adjusted to 7.5 by the addition of 1 M KOH. The turbid mixture was stirred for 30 min and spun for 30 min at 17000 × *g*. Following removal of the precipitate and floating pellet, the supernatant was brought to 45% saturation with solid ammonium sulfate. The mixture was stirred and centrifuged as in the preceding step. The brownish precipitate and floating pellet were combined and dissolved in 3.5 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 0.05% (w/v) potassium deoxycholate, 0.05% (v/v) Triton X-100 and 20% (v/v) glycerol to give a reddish brown solution. The solution was initially dialyzed against 1 l of the same buffer for 3 h and overnight after one change of the buffer. The dialyzed sample was applied at the bottom of a Sephadex G-200 column (2.6 × 59 cm) previously equilibrated with 50 mM potassium phosphate, 0.05% (w/v) potassium deoxycholate, 0.05% (v/v) Triton X-100 and 20% (v/v) glycerol, and eluted with the same buffer in an up-flow manner at a flow rate of 2.2 ml/h. On this chromatography, the fraction was separated into two colored peaks, I and II, in the order of elution (Fig. 1). Peak II showed succinate-CoQ_1 reductase activity whereas peak I, NADH-ferricyanide reductase activity (Fig. 1). The peak II fractions (fraction nos. 48–55) were pooled, concentrated to 3.3 ml through an ultrafiltration membrane (YM 10, Amicon Corporation, MA) and rechromatographed in the same manner as described above. The eluted succinate-CoQ_1 reductase activities were combined, concentrated to 1.5 ml through

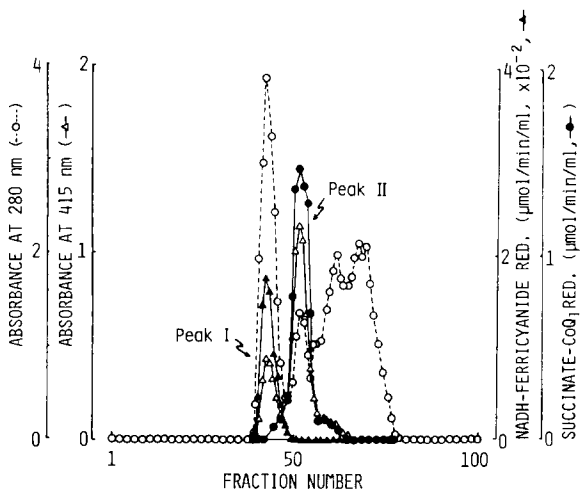

Fig. 1. The first chromatography of the ammonium sulfate fraction on the Sephadex G-200. The dialyzed ammonium sulfate fraction (30–45% saturation) was applied onto a column (2.6 × 59 cm), previously equilibrated with 50 mM potassiumphospahte buffer (pH 7.5) containing 0.05% (w/v) potassium deoxycholate, 0.05% (v/v) Triton X-100 and 20% (v/v) glycerol, and eluted with the same buffer in an up-flow manner at a flow rate of 2.2 ml/h. Absorbances at 280 nm (○------○) and 415 nm (△———△) of each eluted fraction were measured with the elution buffer as the control. ▲———▲, NADH-ferricyanide reductase; ●———●, succinate-CoQ_1 reductase.

the membrane and further characterized or stored at −80°C.

Enzyme assay

Succinate-CoQ reductase activity was determined at 25°C by monitoring the reduction of CoQ_1. The reaction mixture consisted of the enzyme preparation; 20 mM succinate/80 μM CoQ_1/100 mM phosphate buffer (pH 7.4)/0.3 mM EDTA/1 mM potassium cyanide in a total volume of 1 ml. The reaction was initiated by the addition of succinate and the decrease in absorbance at 275 nm against a reagent blank was recorded. An extinction coefficient at 275 nm of 14.0 $mM^{-1} \cdot cm^{-1}$ was used for calculating the enzyme activity [20]. NADH-ferricyanide reductase activity was measured at 25°C by following the reduction of ferricyanide. The reaction mixture contained the enzyme preparation; 1 mM NADH/1 mM ferricyanide/100 mM phosphate buffer (pH 7.4)/0.3 mM EDTA, in a final volume of 1 ml. The reaction was initiated by the addition of NADH and absorbance decrease due to the reduction of ferricyanide at 420 nm was monitored. Using the extinction coefficient of 1.0 $mM^{-1} \cdot cm^{-1}$, the NADH-ferricyanide reductase activity was determined by subtracting the rate of the nonenzymatic reduction of the dye from that of the enzymatic reaction. Cytochrome *c* oxidase [19] and reduced CoQ_2-cytochrome *c* reductase [21] were assayed according to the reported method.

Spectrophotometry

All absorption spectra were recorded on a dual-wavelength spectrophotometer (model DW-2, American Instrument Co., Silver Spring, MD).

Determination of heme and quinone contents

The contents of heme B and heme C were determined essentially according to the method of Basford et al. [22]. The purified complex II was dialyzed against 10 mM potassium phosphate buffer (pH 7.0) to remove glycerol. To the dialyzed complex II (0.8 ml, 1.3 mg protein) was added 4 ml of acidic acetone (a mixture of 5 ml acetone and 0.05 ml of 2.4 M HCl) to extract heme B. The mixture was centrifuged at 9000 × *g* for 15 min and the resulting supernatant reserved. To the precipitate was added 4 ml of the acidic acetone solution and the mixture was centrifuged at 9000 × *g* for 15 min following homogenization in a glass homogenizer with a teflon pestle. The supernatant was reserved and the procedure described above was repeated again (total 3 times extraction). The collected supernatant was concentrated to 0.5 ml by a rotary evaporator and mixed with 1.5 ml of pyridine. To the resulting mixture was added 2 ml of 0.2 M KOH to make a solution of pyridine hemochromogen. The content of heme B was determined from the absorption spectra of the pyridine ferrohemochrome using the molecular extinction coefficient of 34.7 $mM^{-1} \cdot cm^{-1}$. The final precipitate was dissolved in 2 ml of a mixture of pyridine and 0.2 M KOH (1 : 1) to determine heme C.

Quinone was determined according to the method of Kröger [23]. $2\frac{1}{2}$ ml 60% (v/v) methanol in light petroleum were added to the complex II (0.5 ml, 0.9 mg) previously dialyzed against 10 mM potassium phosphate buffer (pH 7.0). The mixture was then agitated for 10 min and centrifuged at 1500 × *g* for 2 min. The upper petroleum layer was reserved. To the lower layer and precipitate was added 1 ml of light petroleum. The mixture was agitated for 10 min and centrifuged at 1500 × *g* for 2 min. The combined petroleum layer was evaporated at 35°C using a rotary evaporator and the resultant residue was dissolved in 2 ml ethanol. The quinone content was estimated from the sodium borohydride-reduced minus non-treated difference spectra of the ethanol solution.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was performed according to the method of Swank and Munkres, using 10% acrylamide gels [24]. The gels were stained with Coomassie brilliant blue and scanned at 570 nm using a Shimadzu dual-wavelength TLC scanner (type CS-910, Shimadzu Seisakusho Ltd., Kyoto). The standard proteins used for the molecular weight determination of complex II components were bovine serum albumin (68 000), ovalbumin (43 000), chymotrypsinogen (25 700), horse heart cytochrome *c* (12 300) and bovine insulin (5700).

Protein determination

Protein was determined by the method of Lowry

et al. [25] with bovine serum albumin as the standard.

Chemicals

CoQ_1 and CoQ_2 were kindly provided by Eisai Co., Ltd. Tokyo. Deoxycholic acid was purchased from Sigma Chemical Company (St. Louis, MO) and recrystallized from 70% (v/v) ethanol. Triton X-100 was obtained from the Packard Instrument Company, Inc. (Downers Grove, IL). Sephadex G-200 was purchased from Pharmacia. All other chemicals were of analytical grade.

Results

Table I summarizes the purification of complex II from *Ascaris* muscle mitochondria. The specific activity of succinate-CoQ_1 reductase was 31.5-times higher in the purified complex than in the mitochondria. The NADH-ferricyanide reductase activity co-purified with succinate-CoQ_1 reductase up to the step of ammonium sulfate treatment was completely removed by repeating the chromatography (Fig. 1, Table I). The purified complex II was essentially free from cytochrome *c* oxidase and reduced CoQ_2-cytochrome *c* reductase activity.

Spectral properties

Fig. 2 shows the reduced-minus-oxidized difference spectra at 77 K of *Ascaris* complex II. On reducing this complex with succinate, the spectrum exhibited two α-bands at 552 and 558 nm and a γ-band at 424 nm (Fig. 2A), indicating that double-peaked cytochrome *b*-558, a major constituent cytochrome of *Ascaris* muscle mitochondria [19], was fractionated in the complex II and that the cytochrome was succinate-reducible as in the mitochondria [18]. With the further addition of dithionite to the sample mixture, these peaks were intensified without the appearance of other peaks or shoulders (Fig. 2B). The absolute spectra of *Ascaris* complex II at room temperature are shown in Fig. 3, where the oxidized form has a γ-band at 413 nm and broad absorption around 460 nm. The dithionite-reduced form exhibited an α-band at 560 nm with a shoulder at 553 nm and a γ-band at

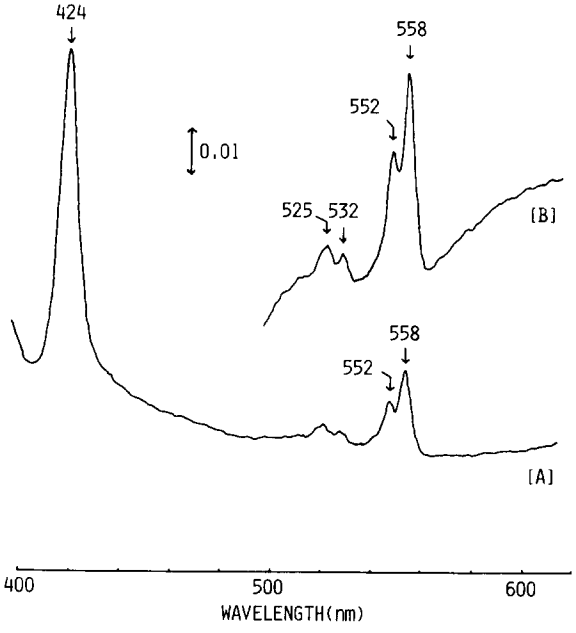

Fig. 2. Low-temperature difference spectra of the *Ascaris* complex II. Trace A: spectrum of the succinate-reduced minus oxidized complex II. Oxidized complex II was prepared by treatment with a small amount of ferricyanide followed by dialysis against a suspending buffer. Complex II in the sample cuvette was reduced with 10 mM succinate for 3 min at room temperature before freezing in liquid nitrogen. Both the sample and reference cuvettes contained complex II (0.13 mg protein per ml)/16.7 mM potassium phosphate buffer (pH 7.4)/0.016% (w/v) deoxycholate/0.016% (v/v) Triton X-100/50% (v/v) glycerol. Trace B: after recording spectrum A, both the sample and reference materials were thawed and the sample materials was treated with a few grains of dithionite. Both materials were then refrozen and the spectrum was recorded again. The light path of the cuvette was 2 mm.

TABLE I

SUMMARY OF THE PURIFICATION OF *ASCARIS* SUCCINATE COENZYME Q REDUCTASE (COMPLEX II)

Fraction	Total protein (mg)	Specific activities of reductases (μmol acceptor red. per min per mg protein)	
		Succinate-CoQ_1	NADH-ferricyanide
Mitochondria	1200	0.038	2.6
DOC-Triton X-100	800	0.088	4.6
$(NH_4)_2SO_4$ (30–45%)	130	0.43	16
1st Sephadex G-200	19	1.3	2.0
2nd Sephadex G-200	4.7	1.2	0.0

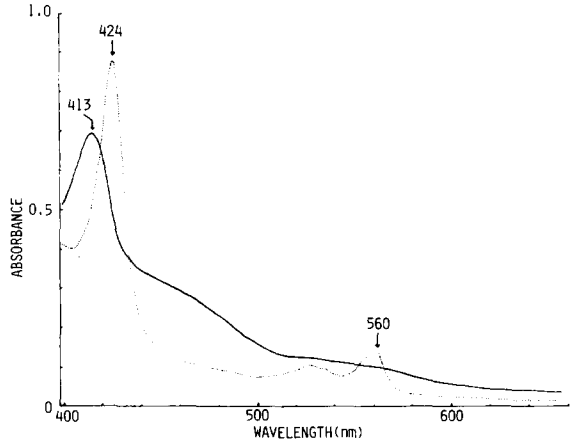

Fig. 3. Absolute spectra of *Ascaris* complex II at room temperature. The purified complex II was diluted to 0.96 mg/ml in 50 mM potassium phosphate buffer (pH 7.5) containing 0.05% (w/v) potassium deoxycholate, 0.05% (v/v) Triton X-100 and 20% (v/v) glycerol. The spectra were measured at 25°C using cuvettes with a light path of 1 cm. The solid (———) and dotted (· · · · · ·) lines represent the oxidized and dithionite-reduced forms, respectively.

424 nm. These spectra were similar to those of bovine heart complex II fractionated from succinate-cytochrome *c* reductase by Yu [26]. The room temperature difference spectrum of *Ascaris* complex II reduced with dithionite also had an α-band at 560 nm with a shoulder centred at 553 nm and γ-band at 424 nm (data not shown).

Oxidation-reduction kinetics of cytochromes b in the mixed system

Oxidation-reduction behaviour of cytochrome *b*-558 in *Ascaris* complex II was further studied in the presence of the worm complex I–III. As shown in Fig. 4a, the addition of NADH to the reaction mixture containing complexes I–III and II resulted in the biphasic reduction of cytochromes *b* in the system; an immediate reduction following addition of the substrate continued for about 3 min. There was also a slow reduction which attained state [A] within a reaction time of 28 min. The difference spectrum of this state (Fig. 4bA) exhibited two α-bands at 554 and 561 nm and a γ-band at 426 nm and showed the cytochrome *b*-558 in the complex II to be reduced with NADH though the peak positions of the α- and γ-bands in the mixed

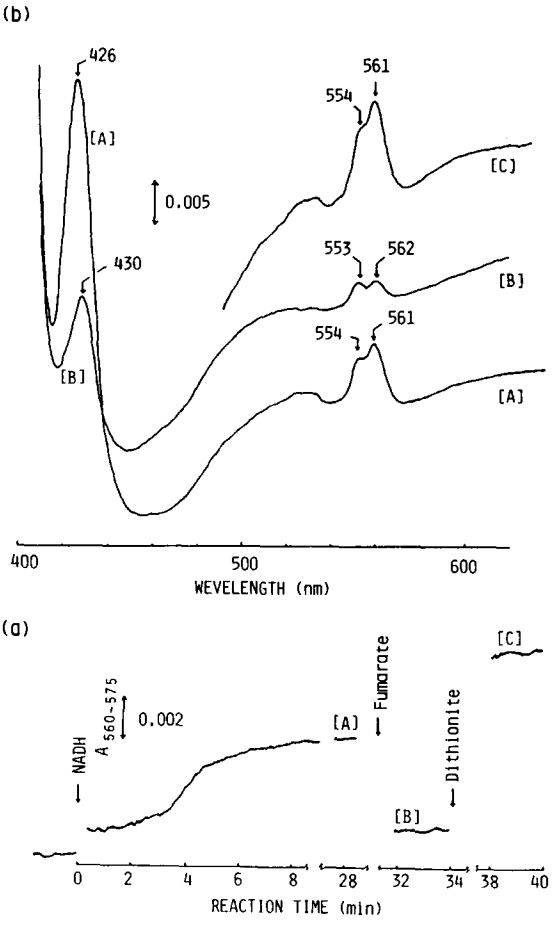

Fig. 4. Oxidation-reduction kinetics of the cytochromes in the mixed system. The reaction was carried out in a Thunberg type cuvette (volume, 0.5 ml; light path, 1 cm) with a silicon-rubber injection port to avoid any possible effects of oxygen. The main chamber of the cuvette contained 25 mM Tris-HCl (pH 8.0)/0.33 M sucrose/2% (v/v) glycerol/0.5 mM histidine/5 mM potassium phoasphate/*Ascaris* complex II (42.5 μg)/complex I–III (125 μg) in a final volume of 0.495 ml and the side chamber 0.005 ml of 200 mM NADH. The cuvette was evacuated for 5 min under reduced pressure and then flushed with argon gas. The evacuation and flushing procedure was repeated three times. Reduction of cytochromes *b* in the system, initiated by the addition of NADH, was monitored at 25°C in a dual-wavelength mode with wavelength pairs of 560 and 575 nm (Fig. 4a). When the reduction attained level A (Fig. 4aA), the difference spectrum of the mixed system was recorded using the reaction mixture without NADH as a reference cuvette (Fig. 4bA). Where indicated, 0.005 ml of 1 M fumarate was added through the silicon-rubber injection port with a microsyringe and the difference spectrum was recorded as described above (Fig. 4bB). After recording the spectrum, the reduction of cytochromes *b* was measured as described above (Fig. 4aB). To determine the full level of reduction of cytochromes *b*, a few grains of dithionite were added and the level and differnece spectrum were recorded (Fig. 4aC and 4bC).

system shifted to the longer wavelength by 1 or 2 nm from those of complex II (α-band at 560 nm and γ-band at 424 nm) owing to the contribution of cytochromes in complex I–III. No reduction of the cytochromes was observed with NADH in the absence of complex I–III. The addition of fumarate to the system at state [A] resulted in the rapid oxidation of *b* cytochrome(s) to state [B] whose reduction level was similar to that of the initial reduction (Fig. 4aB). The difference spectrum of state [B] exhibited two α-peaks at 553 and 562 nm and a γ-peak at 430 nm (Fig. 4bB), indicating the fumarate-oxidizable component(s) not to be cytochromes in complex I–III (cytochromes *b*-559.5 and c_1-550.5) but cytochrome *b*-558 in complex II. In fact, the room temperature difference spectrum of complex I–III reduced with NADH had two α-bands (553 and 562 nm) and a γ-band (430 nm) (unpublished observation). As shown in [C] of Fig. 4a and b, the addition of dithionite resulted in the full reduction of the cytochromes in the system, indicating about 50% of cytochrome *b*-558 to be reduced with NADH.

Heme and quinone contents

The pyridine ferrohemochrome of the acidic acetone extract from *Ascaris* complex II had an α-band at 557 nm, indicating the complex II to contain a type of protoheme. The heme content estimated was 3.6 nmol per mg protein. However, no heme was detected in the pyridine solution of the protein precipitate though its absolute spectrum was characteristic of a flavin, which was covalently bound to the succinate dehydrogenase of the complex II. These results clearly showed that *Ascaris* complex II contained no cytochromes other than cytochrome *b*-558. In fact, cytochromes *b*-559.5, c_1-550.5 and *b*-563, previously suggested to be localized in the worm complex III (reduced CoQ_2-cytochrome *c* reductase) [19], were removed from the succinate-CoQ_1 reductase activity as the peak I fraction on the gel chromatography (data not shown).

Ascaris mitochondria has been reported to contain rhodoquinone-9 instead of ubiquinone-10 [27]. In the present study, the occurrence of the rhodoquinone was confirmed in *Ascaris* mitochondria. However, no quinone was detected in the purified complex II.

Polypeptide composition

Fig. 5 shows the polypeptide composition of the purified *Ascaris* complex II. Four major peptides, with relative mobilities (Rm) to a marker dye of 0.156, 0.381, 0.587 and 0.604 and two minor ones with Rm of 0.31 and 0.514 were observed in the SDS-polyacrylamide gel electrophoresis. These values correspond to apparent molecular weights of 66000, 27000, 12000 and 11000, and 36000 and 16000, respectively. Among four major proteins, two larger (66000 and 27000) and two smaller (12000 and 11000) ones seem to be comparable to the two subunits of succinate dehydro-

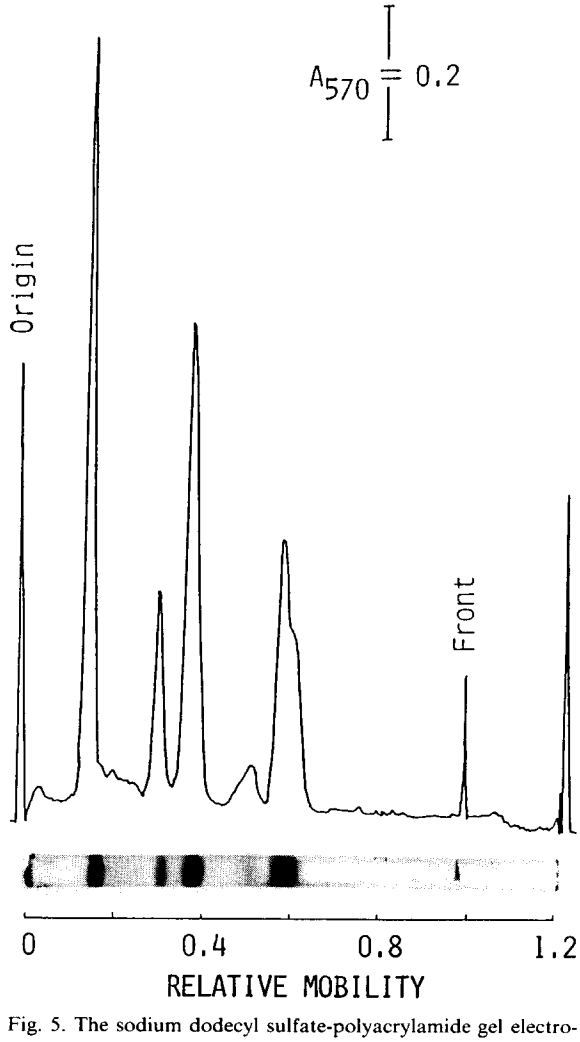

Fig. 5. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the *Ascaris* complex II (23 μg of protein) using 10% gel.

genase and the two peptides of cytochrome *b*-560 of bovine heart complex II, respectively [28]. It is likely, however, that the two minor components (36 000 and 16 000) were impurities.

Discussion

The present data clearly show that cytochrome *b*-558 is exclusively localized in complex II, i.e., associated with succinate dehydrogenase. Cytochrome *b*-558 in complex II had a main α-peak at 558 nm and a distinct sub-α-peak at 552 nm in the reduced minus oxidized difference spectra at 77 K (Fig. 2A and B). The previous spectroscopic observation, suggesting the presence of double-peaked cytochrome *b* in *Ascaris* muscle mitochondria [14], was directly confirmed by fractionation from other cytochromes in the worm mitochondria. Cheah purified a soluble cytochrome designated as *Ascaris* cytochrome *b*-560 from *Ascaris* muscle, whose reduced α-peak at 77 K was markedly split into two peaks [13,17]. The present cytochrome *b*-558, however, notably differs from Cheah's *Ascaris* cytochrome *b*-560 in its water-solubility and reduced α-peak characteristics. Cytochrome *b*-558 is tightly bound to the mitochondrial membrane; all attempts to extract it without detergents were unsuccessful. Cytochrome *b*-558, in the low temperature difference spectra exhibited double α-peaks (558 and 552 nm) whose positions were shorter by 1.5 and 0.5 nm than the respective positions of the split α-peak of *Ascaris* cytochrome *b*-560 (559.5 and 552.5 nm) [13]. Furthermore, the α-peak at the longer wavelength (558 nm) has an extinction higher than that at the shorter wavelength (552 nm) in the case of cytochrome *b*-558 (Fig. 2A and B) whereas, with *Ascaris* cytochrome *b*-560, hardly any difference in extinction was observed between the split peaks [13]. Cheah also reported the occurrence of a cytochrome *b* with a reduced α-peak at 558 nm in *Ascaris* muscle mitochondria and its reducibility by succinate [15]. As yet, it is unknown whether Cheah's cytochrome *b*-558 in the mitochondria is identical with the present cytochrome *b*-558. Cytochrome *b*-558 in complex II was not sensitive toward carbon monoxide; it is unlikely that the cytochrome is an *o*-type cytochrome or CO-reactive hemoprotein reported present in *Ascaris* mitochondria [11,15,16].

The oxidation-reduction kinetics of cytochromes *b* in the mixed system containing complexes II and I–III (Fig. 4) indicate that electrons derived from NADH are transferred to cytochrome *b*-558 in complex II and then through this cytochrome to fumarate probably by reverse reaction of succinate dehydrogenase. However, the specific activity of electron transport from NADH to fumarate was much lower in the mixed system than in *Ascaris* submitochondrial particles (data not shown). Since complexes II and I–III used in this study possessed respective enzymatic activities enough to reconstitute the NADH-fumarate reductase, the failure in reconstitution seems to be ascribable to the deficiency in quinone, probably rhodoquinone [27], which may efficiently mediate electron transfer between NADH dehydrogenase and cytochrome *b*-558 in complex II. Indeed, in *Ascaris* submitochondrial particles and its S_1 fraction (complex I–II–III) [19], both of which contained rhodoquinone, cytochrome *b*-558 was more rapidly reduced with NADH than in the present mixed system (data not shown). In addition, the redox potential of rhodoquinone-rhodoquinol pair is much negative whereas that of ubiquinone-ubiquinol pair positive [29]. These findings imply that, unlike mammalian mitochondria, *Ascaris* mitochondria functions preferentially in the direction of fumarate reduction rather than succinate oxidation and that *Ascaris* cytochrome *b*-558 is also one of the electron carrieres between NADH dehydrogenase and succinate dehydrogenase.

Interestingly, bacteria possessing fumarate reductase systems contain *b* cytochromes [30,31], and succinate dehydrogenase-cytochrome *b* complexes have been isolated from *Bacillus subtilis* [32] and *Neurospora crassa* [33]. Furthermore, evidence suggesting the involvement of type *b* cytochrome in mitochondrial NADH-fumarate reductase system of other parasites [34], molluscan [35], oligochate [36] and rat tissues [37] has been reported though the molecular properties and localization of cytochrome *b* in electron transfer complexes are still obscure. In the case of bovine heart muscle, complex II is known to contain a type *b* cytochrome designated as cytochrome *b*-560 whose spectral properties are similar to those of

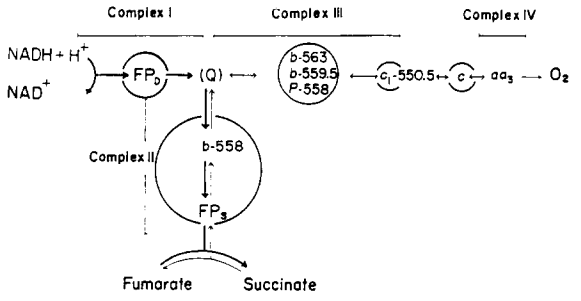

Fig. 6. A postulated scheme of the electron transfer pathway of the *Ascaris* NADH-fumarate reductase system. FP_D, NADH dehydrogenase; FP_S, succinate dehydrogenase; Q, quinone.

cytochrome *b*-558 in *Ascaris* complex II [26,28]. However, in contrast to *Ascaris* cytochrome *b*-558, bovine heart cytochrome *b*-560 has been reported to be hardly reduced, if at all, by succinate [26,28] though the dithionite-reduced cytochrome *b*-560 is reoxidized with fumarate [28]. This observation was confirmed using bovine heart complex II prepared by the present isolation procedure (data not shown). The role of bovine heart cytochrome *b*-560 in the mammalian respiratory chain is still unclear. A comparative study of complex II in *Ascaris* and bovine heart muscle is now in progress.

Based on the results in the present study, it is evident that, unlike mammalian mitochondria [38,39], the amount of complex II is greater than that of complex III in *Ascaris* muscle mitochondria since, in worm mitochondria, the content of cytochrome *b*-558 is about six times that of cytochrome *b*-559.5, a type *b* cytochrome localized in complex III [19]. This is also supported by comparative data indicating the activity ratio of succinate dehydrogenase to reduced CoQ_2-cytochrome *c* reductase to be about 20 in *Ascaris* submitochondrial particles, compared to 0.18 in rat liver particles [4]. From these and previous results [19], cytochrome localization in electron transfer complexes is established and a probable electron transfer pathway of the NADH-fumarate reductase system in *Ascaris* muscle mitochondria is proposed (Fig. 6).

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