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Isolation and Properties of Complex II (succinate-ubiquinone reductase) in the Mitochondria of *Paragonimus westermani*

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Abstract

Complex II (succinate-ubiquinone reductase) was purified from the mitochondria of *Paragonimus westermani* adult and its properties were compared with those of *Ascaris* muscle and beef heart. All three complex II had similar specific activities of succinate-ubiquinone reductase, but significant difference in the sensitivities to thenoyltrifluoroacetone were observed. The degree of sensitivity decreased in beef heart, *P. westermani* and *Ascaris* muscle in that order. Complex II of *P. westermani* contained succinate reducible b-type cytochrome with a peak at 558 nm in the difference spectrum at room temperature. The peak splitted into two peaks at 552 nm and 556 nm at low temperature. The purified preparation was composed of four polypeptides with molecular weights of 69, 27, 14.5 and 12 K daltons. A comparative study showed that two smaller subunits differed in their molecular weights in the three species, while two larger subunits had similar values.

Key words: Paragonimus westermani, mitochondria, complex II, succinate-ubiquinone reductase, fumarate reductase system

Introduction

The energy metabolism of parasites varies widely, because the establishment of life in a host organism depends on their ability to sustain life processes in this environment.

In mammals, the mitochondria is the organelle which contains the enzymes of the tricarboxylic acid cycle and the carriers of the electron transport system, and plays a central role in aerobic energy metabolism. In parasites, profound modifications of the mitochondrial functions have evolved in response to their environment and their electron transport systems are different from those of mammalian cytochrome systems by being branched and possessing multiple terminal oxidases (Cheah, 1973; Kronick *et al.*, 1974; Cheah and Prichard, 1975; McManus, 1986).

Adult lung flukes of genus *Paragonimus* reside in worm cysts of the lungs of mammals. Morphological and spectral properties were studied on the mitochondria of *P. miyazakii* (Hamajima *et al.*, 1982), *P. westermani* (Hamajima *et al.*, 1982) and *P. ohirai* (Yamagami *et al.*, 1984). These results demonstrated the presence of cytochrome b, c_1, c and aa_3, which account for the aerobic component of respiration, and an additional b-type cytochrome, which was reducible by succinate. Similar b-type cytochromes were found in the mitochondria of *Ascaris* (Cheah, 1973, 1976; Hayashi and Oya, 1978), *Moniezia expansa* (Cheah and Bryant, 1966), *Fasciola hepatica* (Cheah and Prichard, 1975), *Dirofilaria immitis*, *Clonorchis sinensis* and *Hymenolepis diminuta* (Oya and Kita, 1987), and were thought to be one of the components of branched respiratory chain in the mitochondria of parasites.

Recently, this additional b cytochrome in *Ascaris* muscle mitochondria, cytochrome b_{558},

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was found to be associated with complex II (succinate-ubiquinone reductase) and to participate in the NADH-fumarate reductase system, which is a terminal step of the phosphoenolpyruvate carboxykinase-succinate pathway (Takamiya *et al.*, 1986). Thus, complex II acts as fumarate reductase in the anaerobic respiration of *Ascaris* muscle, including the reverse reaction of succinate dehydrogenase, and plays a very special and important role in anaerobic energy metabolism. In actual fact, fumarate reductase activity and cyanide insensitive respiration have been demonstrated (Hamajima, 1982; Takamiya *et al.*, 1985) suggesting that adult worms are adaptable to anaerobic conditions, as well as aerobic conditions, as mentioned previously.

The present study reports the isolation of complex II from *P. westermani* and compares its properties with those of the mitochondria of beef heart, which functions as succinate-ubiquinone reductase, and of *Ascaris* muscle, which functions as fumarate reductase, in order to determine the relations between the molecular composition and the functions of complex II in aerobic and anaerobic respiration.

Materials and Methods

Isolation of mitochondria

Mitochondria were isolated from adult worms of *P. westermani*, which were obtained from dogs infected with metacercariae sixteen months before they were sacrificed.

Mitochondria were prepared in a homogenizing medium containing 210 mM mannitol, 70 mM sucrose, 0.5 mM EDTA-(K^+), 0.4 mM procaine, 1% (W/V) bovine serum albumin, and 5 mM Tris-HCl at pH 7.4. The washing medium was of similar content, except that bovine serum albumin was omitted.

After minced into small pieces and washed several times with the homogenizing medium, the worms were suspended in three volumes (W/V) of the same medium and homogenized in a glass pestled glass homogenizer run at 800–1,000 rpm with three strokes. After another six volumes of medium were added, the suspension was homogenized again in a teflon pestled glass homogenizer under the same speed with six strokes.

The homogenate was centrifuged at 650 x g for 7 min by a RS-20 GL TOMY SEIKO centrifuge, and the resulting supernatant was recentrifuged at the same condition. The supernatant was centrifuged once more under 9,000 x g for 7 min and the resultant pellet consisted of three layers: an upper, fluffy layer of light brown colour, a lower pellet layer of dark grey, and a middle dark brown layer. The middle layer, which contained the majority of mitochondria, was collected. After several washings, the mitochondria were suspended in 0.5 mM EDTA containing 210 mM mannitol and 70 mM sucrose for further purification of complex II. The mitochondria from *Ascaris* muscle and beef heart were prepared as described previously (Takamiya *et al.*, 1984; Crane *et al.*, 1956) with the exception that Nagase treatment was eliminated.

Isolation of complex II (succinate-ubiquinone reductase)

Complex II of *P. westermani* was purified by the same method used for *Ascaris* muscle (Takamiya *et al.*, 1986), except that Sephacryl was used instead of Sephadex in order to get better separation, and a smaller column (1.5 cm x 45 cm) was used because of the limitations of sample availability. The mitochondria were adjusted to 20 mg/ml by 50 mM potassium phosphate buffer. Deoxycholate (neutralized to pH 7.5 by potassium hydroxide) and Triton X-100 were added to final concentrations of 1%, respectively, and then glycerol was added to 20% (V/V). After incubation in an ice bath for 30 min with gentle stirring, the mixture was centrifuged to remove the undissolved debris. To the supernatant solid ammonium sulfate was added to 30% saturation and was then centrifuged at 14,000 rpm for 30 min. The resultant of centrifugation was composed of the clear supernatant and a floating layer with dark brown colour. The aqueous portion was collected and the ammonium sulfate was brought to 45% saturation using the same method, and centrifuged again. The supernatant of yellow colour, was aspirated

and to the dark reddish-brown floating material was added a small volume of buffer, which contained 50 mM potassium phosphate, 0.05% deoxycholate, 0.05% Triton X-100, and 20% glycerol (pH 7.5). After removing the ammonium sulfate by dialysis against the same buffer overnight, the sample was applied to a column of Sephacryl S-200 and eluted by the same buffer at a flow rate of 1.5 ml/hr. The eluted fractions were assayed for succinate-ubiquinone reductase and NADH-ferricyanide oxidase activities (Takamiya *et al.*, 1986). The first peak with NADH-ferricyanide oxidase activity was removed, and the second peak, which contained succinate-ubiquinone reductase activity, was collected and concentrated by ultrafiltration for rechromatography. The fractions from the second column which had succinate-ubiquinone reductase activity were collected and concentrated for further experiments.

Complex II of beef heart was purified in almost the same manner as for *Ascaris* muscle (Takamiya *et al.*, 1986) using a Sephadex column and three repetitions of rechromatography were essential for removal of complex I.

Enzyme activity and spectrophotometry

Enzyme activities and spectra were measured with a dual wavelength spectrophotometer (model DW-2, American Instrument Co., Silver Spring, MD) as described previously (Takamiya *et al.*, 1984, 1986). The contents of cytochrome b and flavin were calculated from room temperature reduced minus oxidized difference spectra with molar extinction coefficients of 17.9 mM^{-1} cm^{-1} (Takamiya *et al.*, 1984) and 11.0 mM^{-1} cm^{-1} (Chance, 1957), respectively. Low temperature difference spectra at 77 K with liquid nitrogen were obtained according to the procedure of Estabrook (1956) with a low temperature cuvette having a 2 mm-light path.

The effect of thenoyltrifluoroacetone (TTFA) on complex II was compared using a ubiquinone mediated succinate 2,6-dichlorophenolindophenol (DCIP) assay system which used DCIP as an electron acceptor, because TTFA interfered with the measurements of the absorbance of ubiquinone as a result of its high absorption in the ultra violet region. A decrease of absorption at 600 nm was recorded and an extinction coefficient at 600 nm of 21 mM^{-1} cm^{-1} was used for the calculation of activities (King, 1967).

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970) using 15% acrylamide. The molecular markers used were phosphorylase (94,000), bovine serum albumin (68,000), ovalbumin (43,000), chymotrypsinogen (25,000) and cytochrome c (12,000).

Protein determination

Protein concentrations were determined according to the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Chemicals

Ubiquinone-1 was kindly provided by Eisai Co., Ltd., Tokyo. Deoxycholate was purchased from Sigma Chemical Company (St. Louis, MO) and recrystallized from 70% ethanol. Triton X-100 was obtained from Packard Instrument Company, Inc. (Downers Grove, IL). Sephacryl S-200 was purchased from Pharmacia, Sweden.

All other chemicals used were of an analytical grade.

Results

Purification of Complex II

From 298 worms (wet weight 23.7 g) was obtained 171 mg of mitochondrial protein. Complex II was isolated from the mitochondria as succinate-ubiquinone reductase. Complex II was solubilized with deoxycholate and Triton X-100, and applied on the column of Sephacryl S-200 after ammonium sulfate fractionation. Sample with more than 70% of the solubilized succinate-ubiquinone reductase activities was applied on the column, and Figure 1 shows the elution diagram of complex II. Complex II was eluted as a single peak with a symmetrical pattern, indicating the homogeneity of the preparation. The activity of NADH-ferricyanide reductase, co-purified with succinate-ubiquinone reductase up to the step of ammonium sulfate fractionation, was eluted faster than complex II from this column, and was removed

by repeated gel filtrations. Table 1 summarizes the purification. Adhering to the standard procedure described under "Materials and Methods" is critical in order to obtain reproducible results. More than 18-fold purification was attained, with a recovery of 10%. Cytochrome oxidase activity was removed by ammonium sulfate fractionation.

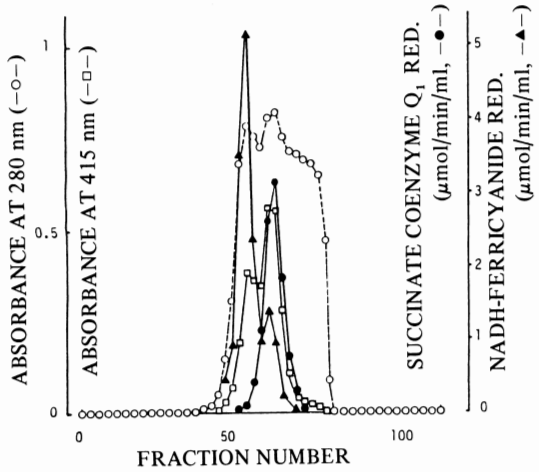

Fig. 1 The first chromatography of the ammonium sulfate fraction prepared from mitochondria of *Paragonimus* on the Sephacryl S-200. The dialyzed fraction (30–45% saturation) was applied on a column (1.5 cm x 45 cm), previously equilibrated with 50 mM potassium phosphate buffer (pH 7.5) containing 0.05% (W/V) deoxycholate, 0.05% (W/V) Triton X-100 and 20% (V/V) glycerol, and eluted with the same buffer at a flow rate of 1.5 ml/hr. Absorbances at 280 nm (—○—) and 415 nm (—□—) of each eluted fraction were measured. NADH-ferricyanide reductase, —▲—; succinate-ubiquinone reductase —●—.

Succinate-ubiquinone reductase activities of Complex II isolated from P. westermani, beef heart and Ascaris muscle

The procedure for isolation of complex II is applicable to other sources of mitochondria, thus giving us the unique chance to characterize and compare the complexes at the same time and under the same conditions.

The kinetic data of succinate-ubiquinone reductase activity in complex II obtained from the three species did not vary greatly with respect to specific activities and Km values (Table 2). When *P. westermani, Ascaris* muscle and beef heart were compared, the specific activity of succinate-ubiquinone reductase in complex II of *P. westermani* was slightly higher than that of *Ascaris,* but lower than that of beef heart. The enzyme of *P. westermani* had the highest affinity for succinate.

A difference in the sensitivity to TTFA, which is known as a potent inhibitor for complex II in mammalian mitochondria was observed (Table 3). The succinate-ubiquinone reductase activity of *Ascaris* complex II was not inhibited with 100 μM TTFA. On the other

Table 1 Summary of the purification of *P. westermani* succinate ubiquinone reductase (complex II)

Fraction	Total protein (mg)	Succinate ubiquinone reductase	NADH-ferricyanide reductase	Cytochrome oxidase
		(μmol acceptor red. min^{-1} mg^{-1} prot.)		(sec^{-1} mg^{-1} ml^{-1})
Mitochondria	171	0.13	0.51	0.05
DOC-Triton X-100	78	0.30	0.85	0.13
$(NH_4)_2SO_4$ (30-45%)	11	1.44	2.44	N. D.
1st Sephacryl S-200	2.68	2.81	0.61	N. D.
2nd Sephacryl S-200	0.92	2.41	N. D.	N. D.

N.D.: not detectable

Table 2 Succinate ubiquinone reductase of complex II purified from *Ascaris* muscle, *P. westermani* and beef heart mitochondria

	Ascaris muscle	*P. westermani*	beef heart
Specific activity*	2.04	2.41	3.90
Km for succinate†	6.70	2.10	4.40

*μmol ubiquinone-1/min/mg protein.
†μM, activity was measured by DCIP in the presence of 80 μM ubiquinone-1.
All the complex II were purified and analysed under the same condition in a series of experiments.

Table 3 Effect of TTFA on succinate ubiquinone reductase activity of complex II purified from *Ascaris* muscle, *P. westermani* and beef heart mitochondria

TTFA (μM)	Residual activity (%)		
	Ascaris muscle	*P. westermani*	beef heart
10	104	73	73
30	103	68	55
100	104	48	30

Activity was measured by DCIP in the presence of 80 μM of ubiquinone-1.

Table 4 Contents of prosthetic group in complex II purified from *Ascaris* muscle, *P. westermani* and beef heart mitochondria

	Ascaris muscle	*P. westermani*	beef heart
Cytochrome b	5.30	2.02	2.76
Flavin	9.34	3.07	3.00

hand, the enzyme activity of *P. westermani* decreased to 48% with the same condition of inhibitor, but it was less sensitive than that of beef heart.

Spectral properties

Complex II isolated from *P. westermani* contained flavin and b-type cytochrome, as well as that of beef heart and *Ascaris* muscle. The absolute spectra of complex II at room temperature are shown in Figure 2. In oxidized form, complex II exhibits a Soret peak at 415 nm and a broad absorption of the oxidized form of flavin around 460 nm. By the addition of suc-

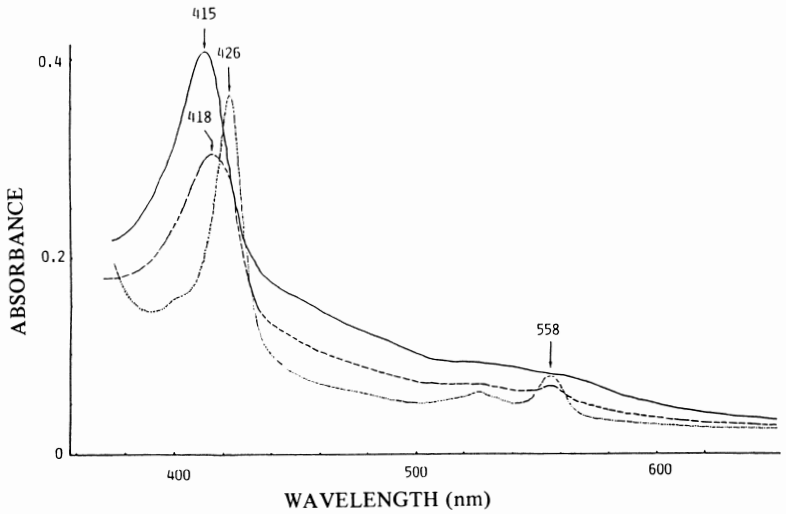

Fig. 2 Room temperature absolute spectra of *P. westermani* complex II. Complex II (0.9 mg/ml) was oxidized by air before assay and elution buffer was used as the reference (solid line). After recording, 20 μl of 1M succinate was added to the sample cuvette, then five minutes later, the dashed line was recorded. The dotted line represents succinate plus dithionite reduced spectrum.

cinate, b cytochrome was partially reduced and showed an α-absorption peak at 558 nm and a Soret peak at 418 nm. In the spectrum of the reduced form, obtained with sodium dithionite, the peaks increased in their absorption intensities and the Soret peak was shifted to 426 nm. The broad absorption of the oxidized form of flavin disappeared after reduction. The difference spectra at room temperature reduced with succinate or dithionite, also had an α-absorption peak at 558 nm (data not shown).

In the low temperature difference spectra obtained with succinate or dithionite, two α-absorption peaks at 552 and 556 nm, and a Soret peak at 424 nm were observed (Figure 3A, B). This indicated that complex II of *P. westermani* has a double-peaked b-type cytochrome, cytochrome b_{556}, which is reducible by succinate, and this property is the same as that with *Ascaris* muscle. No cytochrome c or aa_3 were found either in the room temperature or in low temperature spectra.

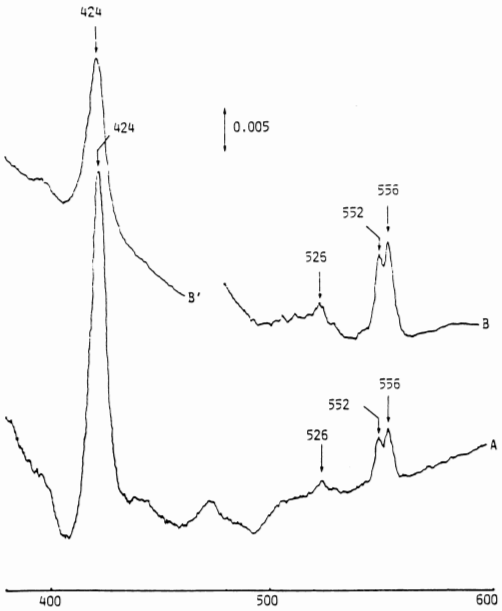

Fig. 3 Low temperature difference spectra of *P. westermani* complex II. All the low temperature spectra were performed with 2 mm light path cuvettes. Both the assay and reference cuvettes contained complex II (0.15 mg/ml), 0.025% (W/V) deoxycholate, 0.025% (W/V) Triton X-100, 25 mM potassium phosphate, pH 7.4, and 50% glycerol. Trace A was obtained by adding 10 μl of 1M succinate and 5 μl of 1M KCN to the sample cuvette, and 15 μl of D.W. was added to the reference cuvette before they were frozen in liquid nitrogen. After recording and thawing, a few grains of dithionite were added to the sample cuvette. After being well-mixed and frozen, traces B and B' were recorded.

From these spectra, the contents of cytochrome b and flavin in complex II were calculated and compared with those of beef heart and *Ascaris* muscle prepared by our method. Complex II of beef heart contained b-type cytochromes with an equimolar amount of flavin, and this result was consistent with the result of a previous report (Hatefi *et al.*, 1978). In the case of *P. westermani* and *Ascaris* muscle, the content of flavin was rather higher, when compared with that of cytochrome b. The specific content of cytochrome b and flavin in *Ascaris* complex II was the highest among the three species. This may be due to the fact that complex II is one of the major components of mitochondria in *Ascaris* muscle (Takamiya *et al.*, 1984, 1986); the amount of complex II in *Ascaris* muscle mitochondria was calculated to be about 3.5-fold that in mammalian mitochondria (Oya and Kita, 1987).

Subunit structure of complex II and homology among the species

The isolated complex II of *P. westermani* was composed of four major polypeptides (Figures 4 and 5), and this molecular organization of subunits was identical to the complex II purified from the mitochondria of beef heart (Hatefi *et al.*, 1980), *Ascaris* muscle (Takamiya *et al.*, 1986), bacterial succinate dehydrogenase complex and fumarate reductase complex (Crowe *et al.*, 1983; Cole *et al.*, 1985).

For further investigation on the homology and difference of the subunit structure, molecular weights of individual polypeptides in complex II purified from *P. westermani*, *Ascaris* and beef heart were determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (Figure 5). The molecular weight of the largest subunits was almost the

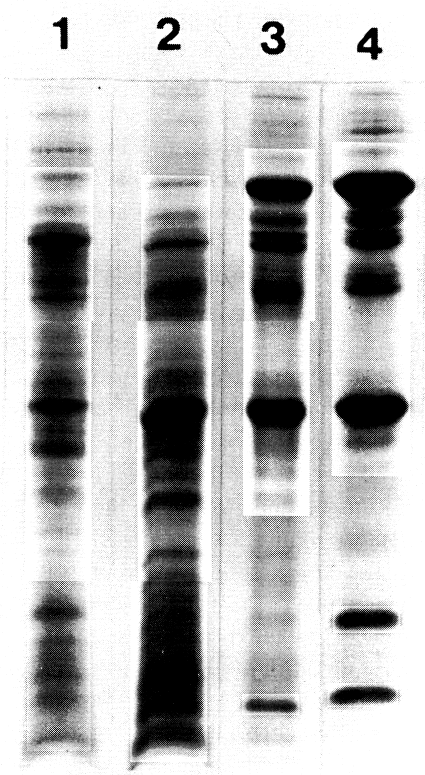

Fig. 4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the fractions during the purification of complex II from mitochondria of *P. westermani*. Lane 1; deoxycholate-Triton X-100 extract (53 μg), lane 2; floating precipitate of 45% ammonium sulfate (40 μg), lane 3; peak fraction from first Sephacryl S-200 (60 μg), lane 4; peak fraction from second Sephacryl S-200 column (12 μg).

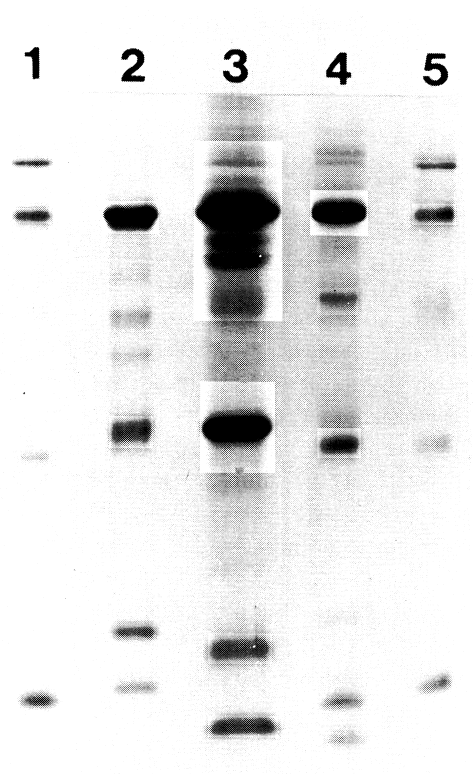

Fig. 5 Comparison of molecular weights of individual subunits in complex II purified from *Ascaris* muscle, *P. westermani* and beef heart by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Lanes 1 and 5 were molecular markers, as mentioned in "Materials and Methods". Lane 2, *Ascaris* complex II, 8.8 μg, lane 3, *P. westermani* complex II, 17.8 μg, and lane 4, beef heart muscle complex II, 6 μg. 15% of acrylamide was used and Commassie Brilliant Blue was used for protein staining.

same in all three preparations, with values of 68, 69 and 68K for *Ascaris* muscle, *P. westermani* and beef heart complex II, respectively. With regard to the second subunit, molecular weight of them was also similar, although that of beef heart was somewhat smaller (24K) than that of the other two (26K in *Ascaris* and 27K in *P. westermani*). On the other hand, in two smaller subunits, the differences were more remarkable. The molecular weight of the third and smallest subunit decreased in the order of *Ascaris* to *P. westermani,* and beef heart. The molecular weights of the third subunit were determined to be 15, 14.5 and 12.5K and those of the smallest subunits were determined to be 13.5, 12 and 11.5K, respectively. The minor bands between the largest and the second subunits were degradated products from the largest subunits, because by the freezing and thawing of the preparation, these bands increased their intensities with decreases in intensity of the largest subunit, and the anti-*Ascaris* largest subunit monoclonal antibody crossreacted with these bands as well as with the largest subunit (manuscript in preparation). The properties of

the largest subunit of complex II, which is susceptible to proteolysis, was also reported in the case of bacteria (Condon *et al.*, 1985).

Discussion

Complex II (succinate-ubiquinone reductase), was isolated from mitochondria of *P. westermani* and its properties were compared with those of complex II of *Ascaris* muscle and beef heart mitochondria, which have anaerobic and aerobic respiratory chains, respectively.

Yamagami and coworkers (Yamagami *et al.*, 1984) reported morphological and spectral properties of the adult worms of genus *Paragonimus,* and two types of mitochondria, lightweight mitochondria and heavyweight mitochondria, were obtained by centrifugation from the homogenate of adult *P. ohirai.* Whereas succinate-reduced cytochromes of the former mitochondria were reoxidized by air, those of the latter did so only sparingly, suggesting that aerobic energy metabolism is conserved in the lightweight mitochondria, while anaerobic energy is transduced in heavyweight ones. A similar phenomenon was observed in *P. miyazakii* (Hamajima *et al.*, 1982) but no information was available about *P. westermani.* In this study, the mitochondria for the preparation of complex II from *P. westermani* were obtained according to the method used in *Ascaris* (Takamiya *et al.*, 1986). As described in "Materials and Methods", three layers were observed after a second centrifugation at 9,000 xg, but only the bottom layer of dark grey color and the middle layer of brown color contained mitochondria. This result was the same as described previously (Hamajima *et al.*, 1982). Present authors also tried the procedure of Yamagami for the preparation of mitochondria from *P. westermani* (Yamagami *et al.*, 1984) and obtained same result of three layers. The electron-microscopic studies showed that the middle layer mainly contained mitochondria and the bottom layer actually contained lipid granules, pigments, debris of tissues and only little amounts of mitochondria, and the mitochondria in the two different layers were difficult to distinguish morphologically from each other. The succinate-ubiquinone reductase, succinate oxidase and NADH-ferricyanide reductase activities in the lower layer were only one-fifth of those in the middle layer and the relative activity among three enzymes were the same in both layers (manuscript in preparation). All of these data suggested that the homogenate of *P. westermani* seems to contain only one kind of mitochondria.

The specific activity of succinate-ubiquinone reductase of the isolated beef heart complex II was similar to that of Yu's (Yu *et al.*, 1977). One of the reasons for this similarity may be due to the lack of phospholipids, because with the addition of phospholipids, the activity increased more than 1.5 times (data not shown). Among the three species we compared, the highest activity of succinate-ubiquinone reductase was obtained in bovine heart (3.90 μmol/min/mg), followed by *P. westermani* and *Ascaris* with values of 2.40 and 2.04 μmol/min/mg, respectively. This result is reasonable because complex II physiologically functions in beef heart as succinate dehydrogenase (Hatefi, 1978, 1985), but as fumarate reductase in *Ascaris* muscle (Köhler *et al.*, 1976, Takamiya *et al.*, 1986). The ratios of flavin to cytochrome b in complex II from beef heart, *P. westermani* and *Ascaris* muscle were 1.08, 1.52 and 1.76, respectively. By comparing the results obtained from present study with those on the beef heart (Hatefi, 1978) and bacteria (Cole *et al.*, 1985), a conclusion that cytochrome b in complex II is present at a molar concentration close to that of flavin was introduced. Higher amounts of flavin than cytochrome b in *P. westermani* and *Ascaris* complex II in the present data may be due to the partial removal of heme b during purification or to the contamination of flavin to the preparations. The latter is more likely, because large amounts of flavin were observed in the mitochondria of these parasites (Takamiya *et al.*, 1984).

The b cytochrome in purified complex II of *P. westermani,* cytochrome b_{556}, is very similar to those in *Ascaris* and beef heart, with respect to its peak position and its splitting into

two peaks at low temperature (Takamiya *et al.*, 1986; Hatefi *et al.*, 1980). However, cytochrome b_{556} of *P. westermani,* as well as the cytochrome b_{558} of *Ascaris,* is easily reducible by succinate (Takamiya *et al.*, 1986), while in beef heart is not (Hatefi and Galante, 1980). This difference seems to be due to the difference of midpoint potentials and correlate to the function of their complex II as succinate dehydrogenase and/or fumarate reductase. Succinate reducible b cytochrome was observed in the mitochondria of *P. westermani* (Hamajima *et al.*, 1982), and this b cytochrome may correspond to cytochrome b_{556} in complex II because its major absorption peak was 556 nm at low temperature. The fact that there was an absorption peak at 556 nm with a shoulder at 552 nm in the low temperature difference spectrum between the mitochondria reduced with NADH and those reoxidized with fumarate led us to the interpretation described above (data not shown). Complex II appears to be a highly conserved group of the enzyme, from bacteria to mitochondria, irrespective of its function and the direction of electron transfer (Cole *et al.*, 1985; Hatefi, 1985). The two large polypeptides are the subunits of SDH (succinate dehydrogenase) and the largest subunit carries the covalently bound flavin. Their molecular weights are very close to each other as shown in this work, and the anti-*Ascaris* largest subunit antibody crossreacted with others' largest subunits (manuscript in preparation). These results are consistent with the fact that an identical sequence of nine amino acids in the flavin attachment site of the largest subunit in beef heart complex II is conserved in those of succinate dehydrogenase and fumarate reductase in *E. coli* (Cole *et al.*, 1985). Moreover, the largest subunits of both enzymes in *E. coli* show marked homology at the total amino acids sequence level, too (Cole *et al.*, 1985).

At least one of the iron-sulfur clusters (S-3) is contained in the second subunit, and this is the site of inhibition by TTFA (Hatefi, 1985). Since succinate-ubiquinone reductase activities in three kinds of complex II showed different sensitivities to TTFA, the properties of the second subunit are not identical, though their molecular weights are quite similar.

Concerning the two smaller subunits, their function in complex II are still unclear. Several experiments support the hypothesis that the two smaller subunits play an important role in providing a binding site for the two larger subunits to the mitochondrial and bacterial membranes, and in stabilizing the subunit structure of complex II. Besides this kind of anchoring function, a function as an electron carrier has also been suggested by Hatefi (Hatefi and Galante, 1980). Cytochrome b_{560} purified from beef heart contains two small polypeptides, and these polypeptides are supposed to be essential for the electron transfer from succinate to ubiquinone, though the reduction rate of cytochrome b_{560} by succinate is very low. The rapid reduction of cytochrome b and re-oxidation by fumarate were observed in complex II of *Ascaris* and *P. westermani.* This is direct evidence for the participation of cytochrome b in electron transfer in complex II. In the case of mitochondria, it is possible to conclude that the cytochrome b in complex II which functions as fumarate reductase is reducible by succinate.

Yu and coworkers reported that the smallest subunit in complex II of beef heart is a ubiquinone binding protein, which is required for the reduction of ubiquinone by complex II (Yu and Yu, 1980). The requirement of the smallest subunit is also shown in the fumarate reductase system of *E. coli* by the use of mutant strains (Cecchini *et al.*, 1986). No homology was found in two small subunits between succinate dehydrogenase and fumarate reductase complexes of *E. coli* (Cole *et al.*, 1985), and differences in molecular weight were shown in small subunits among three species in the present study. Based on these observations present authors suggest that the two smaller subunits also have key functions in the regulation of enzyme activities between succinate dehydrogenase and fumarate reductase, and that they determine the type of respiration and direction of electron transfer in complex II.

Physiologically, complex II of *Ascaris* is found to act as fumarate reductase. On the

other hand, complex II of beef heart mitochondria acts as succinate-ubiquinone reductase. As shown in this work, complex II of *P. westermani* has intermediate properties between those of *Ascaris* muscle and beef heart in sensitivity to TTFA, reducibility of cytochrome b with succinate, and molecular weights of the two smaller subunits. A comparison of the properties of each subunit in complex II and elucidation of their functions will clarify which component and what kind of structure are important in determining the type of respiration and the direction of electron transfer in complex II.

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Abbreviations

TTFA. thenoyltrifluoroacetone; DCIP. 2,6-dichlorophenolindophenol; SDS. sodium dodecyl sulfate