Novel Mitochondrial Complex II Isolated from Trypanosoma cruzi Is Composed of 12 Peptides Including a Heterodimeric Ip Subunit*

Jorge Morales†, Tatsushi Mogi‡, Shigeru Mineki§, Eizo Takashima¶, Reiko Mineki†, Hiroko Hirawake‡, Kimitoshi Sakamoto, Satoshi Ōmura, and Kiyoshi Kita

From the †Department of Biomedical Chemistry, Graduate School of Medicine, the University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, the ‡Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, the §Division of Proteomics and BioMolecular Science, Juntendo University Graduate School of Medicine, Hongo, Bunkyo-ku, Tokyo 113-8421, and the ¶Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Minato-ku, Tokyo 108-8641, Japan

Mitochondrial respiratory enzymes play a central role in energy production in aerobic organisms. They differentiated from the α-proteobacteria-derived ancestors by adding non-catalytic subunits. An exception is Complex II (succinate:ubiquinone reductase), which is composed of four α-proteobacteria-derived catalytic subunits (SDH1–SDH4). Complex II often plays a pivotal role in adaptation of parasites in host organisms and would be a potential target for new drugs. We purified Complex II from the parasitic protist Trypanosoma cruzi and obtained the unexpected result that it consists of six hydrophilic (SDH1, SDH2N, SDH2C, and SDH5–SDH7) and six hydrophobic units (SDH3, SDH4, and SDH8–SDH11) nucleus-encoded subunits. Orthologous genes for each subunit were identified in Trypanosoma brucei and Leishmania major. Notably, the iron-sulfur subunit was heterodimeric; SDH2N and SDH2C contain the plant-type ferredoxin domain in the N-terminal half and the bacterial ferredoxin domain in the C-terminal half, respectively. Catalytic subunits (SDH1, SDH2N, plus SDH2C, SDH3, and SDH4) contain all key residues for binding of dicarboxylates and quinones, but the enzyme showed the lower affinity for both substrates and inhibitors than mammalian enzymes. In addition, the enzyme binds protoporphyrin IX, but SDH3 lacks a ligand histidine. These unusual features are unique in the Trypanosomatida and make their Complex II a target for new chemotherapeutic agents.

The parasitic protist Trypanosoma cruzi is the etiological agent of Chagas disease, a public health threat in Central and South America. These parasites are normally transmitted by reduvid bugs via the vector feces after a bug bite and also via transfusion of infected blood. About 16–18 million people are infected, and 100 million are at risk, but there are no definitive chemotherapeutic treatments available (1). Despite having potential pathways for oxidative phosphorylation (2), all trypanosomatids (Trypanosoma and Leishmania species) analyzed so far are characterized by incomplete oxidation of glucose with secretion of end products, such as succinate, alanine, ethanol, acetate, pyruvate, and glycerol (3, 4) (Fig. 1). Major routes for formation of succinate in Trypanosoma brucei are via NADH-dependent fumarate reductase in glycosomes and mitochondria (5, 6). In trypanosomatid mitochondria, the Krebs cycle is inefficient, and pyruvate is principally converted to acetate via acetate:succinate CoA transferase (7). A part of the Krebs cycle operates the utilization of histidine in the insect stage of T. cruzi (8).

Mitochondrial Complex II (succinate:quinone reductase (SQR) and succinate dehydrogenase (SDH)) serves as a membrane-bound Krebs cycle enzyme and often plays a pivotal role in adaptation of parasites to environments in their host (9, 10). In general, Complex II consists of four subunits (11). A flavoprotein subunit (SDH1, Fp) and an iron-sulfur subunit (SDH2, lp) form a soluble heterodimer, which then binds to a membrane anchor heterodimer, SDH3 (CybL) and SDH4 (CybS). SDH1 contains a covalently bound FAD and catalyzes the oxidation of succinate to ubiquinone via the [2Fe-2S] cluster in the N-terminal plant-type ferredoxin domain (lpFp) and the [4Fe-4S] and [3Fe-4S] clusters in the C-terminal bacterial ferredoxin domain (lpFpC). Ubiquinone is bound and reduced in a pocket provided by SDH2, SDH3, and SDH4 (12–14). SDH3 and SDH4 contain three transmembrane helices and coordinate protoporphyrin IX via histidine in the second helices of each subunit (11–14).

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† The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

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§ To whom correspondence may be addressed. Tel.: 81-3-5841-3526; Fax: 81-3-5841-3444; E-mail: tmogi@m.u-tokyo.ac.jp.

¶ Present address: Dept. of Microbiology, School of Life Dentistry at Tokyo, Nippon Dental University, Tokyo 102-8159, Japan.

† To whom correspondence may be addressed. Tel.: 81-3-5841-3526; Fax: 81-3-5841-3444; E-mail: kitak@m.u-tokyo.ac.jp.

‡2 Shigeru Mineki

‡4 Reiko Mineki

§2 Satoshi Ōmura

¶2 Kiyoshi Kita

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§ Present address: Dept. of Microbiology, School of Life Dentistry at Tokyo, Nippon Dental University, Tokyo 102-8159, Japan.

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The abbreviations used are: SQR, succinate:quinone reductase; hrCNE, high resolution clear native electrophoresis; IC50, the 50% inhibitory concentration; lpFp, the N-terminal plant-type ferredoxin domain; lpFpC, the C-terminal bacterial ferredoxin domain; DCIP, 2,4-dichlorophenolindophenol; SML, sucrose monolaurate; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; MOPS, 3-(N-morpholino)propanesulfonic acid; Qn, ubiquinone-n.
12-Subunit Complex II from T. cruzi

Parasitic nematodes adapted to hypoxic host environments often have modified respiratory chains. Many adult parasites perform fumarate respiration by expressing a stage-specific isoform of Complex II (9, 10). Hemonchus contortus uses an isoform for SDH2 (9), whereas Ascaris suum uses isoforms for SDH1 and SDH4 (10). To explore the adaptive strategy in a parasitic protist, we isolated mitochondria from axenic culture of T. cruzi epimastigotes and characterized the purified Complex II. Our results demonstrated for the first time that T. cruzi Complex II is an unusual supramolecular complex with a heterodimeric iron-sulfur subunit and seven novel noncatalytic subunits. Purified enzyme showed reduced binding affinities for both substrates and inhibitors. Because this novel structural organization is conserved in all trypanosomatids (2, 15, 16), parasite Complex II would be a potential target for the development of new chemotherapeutic agents for trypanosomiasis and leishmaniasis.

EXPERIMENTAL PROCEDURES

Preparation of Mitochondria—T. cruzi strain Tulahuhen was grown statically for 6–7 days at 26 °C in 300-cm² cell culture flasks (Falcon, BD Biosciences) containing 250 ml of the modified LIT medium (17), supplemented with 0.1% (w/v) glucose, 0.001% (w/v) hemin (Sigma), and 5% (v/v) fetal bovine serum (MP Biomedicals). Mitochondria were isolated from epimastigotes by the differential centrifugation method (18) with slight modifications. Parasites grown to 6–8 × 10⁷ cells/ml were washed with buffer A (20 mM Tris-HCl, pH 7.2, 10 mM NaH₂PO₄, 1 mM sodium EDTA, 1 mM dithiothreitol, 0.225 M sucrose, 20 mM KCl, and 5 mM MgCl₂). Cells were disrupted by grinding with silicon carbide (Carborundum 440 mesh; Nacalai Tesque, Kyoto, Japan) in the presence of a minimum volume of buffer B (25 mM Tris-HCl, pH 7.6, 1 mM dithiothreitol, 1 mM sodium EDTA, 0.25 M sucrose, and EDTA-free Complete protease inhibitor mixture (Roche Applied Science)). The mixture was stirred for 30 min and centrifuged at 200,000 × g for 1 h. The supernatant was loaded at 1 ml/min onto a Source 15 Q column (1.6 inner diameter × 10 cm; GE Healthcare), equilibrated with buffer C containing 0.1% SML. After washing with 5 volumes of the same buffer, proteins were eluted with a 200-ml linear gradient of NaCl from 0 to 150 mM at 0.25 ml/min in 20 mM MOPS-NaOH, pH 7.2, containing 1 mM sodium EDTA, 1 mM sodium malonate, 150 mM NaCl, and 0.1% SML. Peak fractions were rechromatographed as above, and purified enzyme was concentrated and stored at −80 °C until use.

Isolation of Complex II—All steps were carried out at 4 °C. Mitochondrial fraction (~300 mg of protein from 10 liters culture) was brought to 70 ml with buffer C (10 mM KPO₄. pH 7.5, 1 mM sodium EDTA, 1 mM sodium malonate, EDTA-free Complete protease inhibitor mixture (Roche Applied Science) (2 tablets/50 ml), 1% (w/v) sucrose monolaurate SM-1200 (SML) (Mitsubishi-Kagaku Foods Co., Tokyo, Japan)). The mixture was stirred for 30 min and centrifuged at 200,000 × g for 1 h. The supernatant was loaded at 1 ml/min onto a Source 15 Q column (1.6 inner diameter × 10 cm; GE Healthcare), equilibrated with buffer C containing 0.1% SML. After washing with 5 volumes of the same buffer, proteins were eluted with a 200-ml linear gradient of NaCl from 0 to 150 mM at 0.25 ml/min. Active fractions were concentrated to ~250 μl by ultrafiltration with Amicon Ultra-4 (molecular weight cutoff 100,000, Millipore) and subjected to gel filtration FPLC with a Superdex 200-250-mg column (1 cm inner diameter × 30 cm; GE Healthcare) at 0.25 ml/min in 20 mM MOPS-NaOH, pH 7.2, containing 1 mM sodium EDTA, 1 mM sodium malonate, 150 mM NaCl, and 0.1% SML. Peak fractions were rechromatographed as above, and purified enzyme was concentrated and stored at −80 °C until use.

Identification of Complex II Subunits—The purified enzyme was subjected to 12.5% SDS-PAGE, and subunits were transferred to an Immobilon-P membrane (Millipore), followed by

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**TABLE 1**

| Step       | Protein | Succinate:DCIP reductase | Yield | Purification |
|------------|---------|--------------------------|-------|-------------|
| Mitochondria | 314 | 27 | 0.085 | 100 | 1.0 |
| SML extract  | 141 | 22 | 0.16 | 83 | 1.9 |
| Source 15Q  | 7.8 | 7.6 | 0.97 | 28 | 12 |
| Superdex 200 (1st) | 1.3 | 1.4 | 1.09 | 5.3 | 13 |
| Superdex 200 (2nd) | 0.15 | 0.43 | 2.87 | 1.6 | 34 |

**FIGURE 1.** Metabolic pathways in T. cruzi. Incomplete oxidation of glucose takes place in glycosomes and mitochondria, and end products such as succinate, l-alanine, and acetate are excreted from parasites (3, 4). Cytoplasmic dihydroorotate (DHO):fumarate reductase (DHOD) contributes succinate production (6).

**FIGURE 2.** Elution profile of T. cruzi Complex II on Superdex 200 chromatography. Complex II fractions from the first gel filtration chromatography with a Superdex 200-250-mg column were concentrated and rechromatographed at the flow rate of 0.25 ml/min. Aliquots were collected every 0.5 ml. Elution profiles for proteins and cytochromes were monitored at 280 (A) and 412 nm (C), respectively, and the enzyme activity (●) was measured as decylquinone-mediated succinate:DCIP reductase.
staining with Coomassie Brilliant Blue R-250 (19, 20). Five or ten N-terminal amino acid residues were determined with a Procise 494 HT (Applied Biosystems) or an Hp G1005A (Hewlett-Packard Co.) Protein Sequencing System at the BioMedical Research Center of Juntendo University or APRO Life Science Institute, Inc. (Tokushima, Japan). When the N terminus was blocked, protein bands were digested with trypsin, and internal peptide sequences were determined (20). Genes coded for Complex II subunits were identified with BLASTP in the T. cruzi genome data base (15).

**Phase Partitioning of Mitochondrial Fraction with Triton X-114**—Phase partitioning by Triton X-114 was performed as described previously (21) with a slight modification. A total of 2–3 mg of mitochondrial fraction was resuspended in 1 ml of Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2 mM sodium dodecylmaltoside (SML, pH 7, and kept at 30 °C overnight. After thawing, the insoluble material was removed by centrifugation at 4 °C, and the supernatant was incubated for 10 min at 37 °C and centrifuged at 2000 × g for 10 min to separate the aqueous and detergent-rich phases. The aqueous phase was brought to 2% (v/v) Triton X-114, whereas the detergent-rich fraction was brought to 1 ml with the above buffer. After incubation on ice for 10 min, samples were incubated at 37 °C for 10 min and phases separated as before. This wash step was repeated three times. Finally, the samples were dialyzed and concentrated by Amicon Ultra-4 (Millipore) in the presence of 50 mM imidazole, 50 mM NaCl, 6 mM aminocaproic acid, 0.05% (w/v) deoxycholate, and 0.1% (w/v) SML, pH 7, and kept at −80 °C until use.

**Enzyme Assay**—Decylubiquinone-mediated succinate-2,4-dichlorophenolindophenol (DCIP) reductase activity was measured at 25 °C in 100 mM potassium phosphate, pH 7.4, containing 1 mM MgCl₂, 2 mM KCN, 0.1 mM antimycin A (Sigma), and 0.1% SML with 63 μM decylubiquinone (Sigma) plus 60 μM DCIP. After 2 min of incubation, reduction of DCIP (ε₆₀₀ = 21 mm⁻¹ cm⁻¹) was measured in the presence of 10 mM succinate. SQR activity was determined with 40 μM ubiquinone-2 (Q₂) (Sigma, ε₂₇₈ = 12.3 mm⁻¹ cm⁻¹). Kinetic analysis was done with KaleidaGraph version 4.0 (Synergy Software).

**Miscellaneous**—High resolution clear native electrophoresis (hrCNE) (22) was performed with 4–16% Novex gels (Invitrogen) using 0.02% dodecylmaltoside and 0.05% sodium deoxycholate for the cathode buffer additives, and the Complex II band was visualized by the activity staining (23) or Coomassie Brilliant Blue. Tricine-PAGE analysis was done with Novex 10–20% Tricine gels (Invitrogen), and proteins bands were sequentially stained by Sypro ruby (Invitrogen) and silver. During purification the succinate-decylubiquinone-DCIP reduc-

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**FIGURE 3. Electrophoresis analysis of T. cruzi Complex II**. A, purified Complex II (2 μg; lanes 1 and 3) and the detergent-rich fraction from the phase partitioning by Triton X-114 of the mitochondrial fraction (60 μg; lanes 2 and 4) were subjected to hrCNE. Proteins were stained by Coomassie Brilliant Blue (left panel), and Complex II was visualized by SDH activity staining (right panel). B, proteins of Complex II showing SDH activity in A were analyzed by 10–20% Tricine SDS-PAGE and visualized by silver stain (lane 5, pure complex; lane 6, detergent-rich fraction). C shows the subunit composition of the pure Complex II from T. cruzi stained by SYPRO ruby (lane 7) or silver stain (lane 8). Molecular weight standard used was NativeMark (Invitrogen, lane 1) and Mark 12 unstained standards (Invitrogen, lanes 5 and 7).

**TABLE 2**

| Subunit*  | Sequence confirmed | Accession number or RefSeq ID at NCBI (haplotype, Mₜ) | Identity% | TM* |
|-----------|-------------------|---------------------------------------------|----------|-----|
| SDH1      | [Ser¹⁰]–[Met¹⁹]   | AB031774 (NE, 66,974), XP_809281 (E, 18,231) | 59%      | 0   |
| SDH5      | [Ala³⁴]–[Leu³⁷]   | XP_818124 (NE, 53,831), XP_810172 (E, 20,788) | 16%      | 0   |
| SDH₂₂     | [Ser¹⁰]–[Arg²⁶], [Lys²⁷]–[Leu³¹] | XP_814994 (merged, 32,322) | 24%      | 37% |
| SDH₂₁     | [Pro]–[Leu]     | XP_803796 (NE, 21,352), XP_806126 (E, 21,379) | 25%      | 43% |
| SDH₆₆     | [Val²]–[Val²⁶]  | XP_809065 (NA, 36,077), XP_812789 (NA, 36,035) | 15%      | 0   |
| SDH₆₅     | [Val²]–[Val²⁶]  | XP_813603 (NA, 36,133), XP_813645 (NA, 36,039) | 14%      | 0   |
| SDH₇⁷     | [Ile³⁹]–[Leu]   | XP_813318 (NE, 28,218), XP_820239 (E, 28,202) | 22%      | 0   |
| SDH₉     | [Val]–[Phe]    | XP_809410 (NE, 12,176), XP_810064 (E, 12,204) | 29%      | 1   |
| SDH₄     | [Phe]–[Thr]   | XP_808211 (E, 13,957), XP_816430 (NE, 13,975) | 27%      | 2   |
| SDH₈     | [Gly]–[Met]   | XP_809192 (NE, 16,199), XP_817545 (E, 16,143) | ND*       | 2   |
| SDH₉     | [Ile]–[Pro]   | XP_807105 (merged, 15,736) | ND       | 1   |
| SDH₁₀    | [Phe]–[Val]   | XP_808894 (NE, 15,565), XP_808903 (E, 15,554) | ND       | 1   |

* Alleles were named as SDH3-1 (XP_809410) and SDH3-2 (XP_801064) in the order of the accession numbers, except for SDH5.
* These are N-terminal sequences except for SDH₂₂ and SDH₈, where the N-terminal residues were blocked.
* Homologous alleles located in a merged assembly of Esmeraldo (E) and non-Esmeraldo (NE) homologous sequences whose different copies were merged during the genome assembly are indicated by "merged." Haplotypes for gene with more than two copies in the genome that does not belong to a merged region are not assigned (NA).
* Identity% to counterparts in human were as follows: SDH1 ([D30648], SDH2 ([P21912], SDH3 ([Q99643]), or SDH4 ([O14521]). In parentheses, the identity% of SDH2N and SDH2C that correspond to either Met¹–Pro¹⁴ (IpN domain) or Tyr¹⁵⁶–Val²⁸⁰ (IpC domain), respectively, of human SDH2 is shown. Identity% for truncated forms of SDH1 and SDH5 (SDH₁₁ and SDH₂) in the Esmeraldo haplotype was 66 and 20%, respectively.
* Transmembrane segments (TM) were estimated with TMHMM (52) and SOSUI (53).
* SDH₂₂N from other trypanosomatids lack Met¹ to Arg²⁷ of TcSDH₂N.
* ND indicates not determined because these hydrophobic sequences are a highly divergent form of mammalian sequences.
tase activity was monitored in a microplate spectrophotometer (Benchmark Plus, Bio-Rad). Kinetics and UV-visible absorption spectra were determined at room temperature with a V-660 UV-visible spectrophotometer (Jasco, Tokyo, Japan). Protoheme IX and protein concentrations were determined by pyridine hemochromogen method (24) and the micro BCA method (Pierce), respectively. Sequence alignment was done with ClustalX 2.0 (25).

RESULTS AND DISCUSSION

Isolation of T. cruzi Complex II—To determine the molecular organization of T. cruzi Complex II, we purified this enzyme from epimastigote mitochondria by ion-exchange and gel filtration chromatography using the nonionic detergent sucrose monolaurate (Table 1). Decylubiquinone-mediated succinate:DCIP reductase activity was eluted as a single peak at each step.
and co-eluted with proteins and β-type cytochrome(s) at the second Superdex 200 chromatography (Fig. 2). Specific activity was increased 34-fold to 2.9 units/mg proteins, and the yield was/H11011 2%. A hrCNE of the pure protein identified T. cruzi Complex II as an/H11011 550-kDa complex (Fig. 3, lanes 1 and 3), which is 4-fold larger than bovine and yeast Complex II (130 kDa) and potato Complex II (150 kDa) (26, 27). Upon phase partitioning of the mitochondrial fraction with Triton X-114, the Complex II of T. cruzi was found only in the detergent-rich fraction (data not shown). Analysis of the detergent-rich fraction by hrCNE showed the Complex II as a single band at the same position as the pure enzyme (H11011 550 kDa) (Fig. 3, lanes 2 and 4). These results indicated that the purified Complex II was obtained in its intact form. Interestingly, second dimensional analysis of both the purified Complex II and the detergent-rich fraction from phase partitioning with Triton X-114 with SDH activity showed that T. cruzi Complex II is composed of 12 subunits (Fig. 3, lanes 5 and 6). The same subunit composition was obtained by immunoaffinity purification of the partially purified enzyme (data not shown). The apparent molecular weight of the subunits ranges from 7.3 to 63 kDa (Fig. 3, lanes 7 and 8). Assuming the presence of equimolar amounts of subunits, a total molecular mass of Complex II would be 286.5 kDa, indicating that T. cruzi Complex II is a homodimer.

Identification of Genes Coded for Subunits—We determined N-terminal sequences (or internal peptide sequences in case of SDH2N and SDH18) of all subunits and identified genes coded for SDH1-1, SDH2N, SDH2C, SDH5–SDH7 (hydrophilic sub-
12-Subunit Complex II from T. cruzi

units), SDH3, SDH4, and SDH8–SDH11 (hydrophobic subunits) (Table 2). All subunits, except SDH1-1, are trypanosomatid-specific and structurally unrelated to plant-specific soluble subunits (AtSDH5–AtSDH8, 5–18 kDa) (27–29). All genes (except SDH6 with four copies) are present as two copies, which are assigned to either Esmeraldo or non-Esmeraldo haplotype (haploid genotype) in T. cruzi subgroup Ile. In contrast, only one copy each of the orthologues is present in T. brucei, Leishmania major, Leishmania infantum, and Leishmania braziliensis (supplemental Table S1). N-terminal sequence analysis of SDH3 and SDH7 showed that yields of two isoforms are similar (i.e. SDH3-1:SDH3-2 = 63:37, and SDH7-1:SDH7-2 = 54:46), indicating that isoforms are expressed from each haplotype. Because truncated isoforms for SDH1 and SDH5 in the Esmeraldo haplotype (see below) are not assembled into the 12-subunit complex and SDH2<sub>N</sub> and SDH9 isoforms have the identical sequence, 512 (= 1<sup>4</sup> × 4<sup>1</sup> × 2<sup>12–15</sup>) kinds of heterogeneity may exist in the T. cruzi Complex II monomer (Table 2).

Flavoprotein Subunit—SDH1-1 (63-kDa band in Tricine-PAGE) cross-reacted with the antiserum against bovine SDH1 (data not shown) and is highly homologous to counterparts in PAGE) cross-reacted with the antiserum against bovine SDH1 (Table 2).

Iron-Sulfur Subunit—Sequence analysis of the 25- and 21-kDa band proteins revealed that they contain the plant ferredoxin domain (Ip<sub>N</sub>) and bacterial ferredoxin domain (Ip<sub>C</sub>) of canonical SDH2 (Ip) in the N- and C-terminal half, respectively (Fig. 4). Sequence identities of Ip<sub>N</sub> and Ip<sub>C</sub> are 37 and 43%, respectively, to those of human SDH2 (Table 2), and the Ip<sub>N</sub> and Ip<sub>C</sub> domains contain all amino acid residues responsible for binding of iron-sulfur clusters and ubiquinone (12, 13, 30) (Fig. 4). Such a heterodimeric Ip subunit can be found in T. brucei (31), T. cruzi, L. major, L. infantum, and L. braziliensis (Tables 2), which belong to the order Trypanosomatida. Thus, we named these subunits as SDH2<sub>N</sub> and SDH2<sub>C</sub>, respectively.

Splitting of mitochondrial membrane proteins has been reported for cytochrome c oxidase CoxII in Apicomplexa and Chlorophyceae (32, 33), and ATP synthase α subunit in Leishmania tarentolae and T. brucei (34, 35). The former occurs at the gene level and the latter by post-translational cleavage. Sequence analysis indicates that heterodimeric SDH2 and CoxII have emerged from gene duplication followed by degeneration of the N- or C-terminal half of the duplication products. Conserved domains in degenerated duplilcons, which have arisen from mitochondrion-to-nucleus transfer of the dupli-
brucei SDH4 needs to be tested in future studies. It is also possible that trypanosomatid-specific subunits could be assembled as a jigsaw puzzle-like membrane anchor.

Spectroscopic Properties of T. cruzi Complex II—Pyridine ferrrohemochrome analysis showed that T. cruzi Complex II binds a stoichiometric amount of protoheme IX (0.85 heme/monomer of enzyme) indicating that monomer enzyme complex contains one heme. At room temperature, the air-oxidized and fully reduced forms of the purified enzyme showed peaks at 413 and 426, 527, and 561 nm, respectively (Fig. 6). Peak positions are similar to those reported for Complex II from E. coli (40), adult A. suum (41), and bovine (42, 43), where heme is ligated via histidine in the second helices of SDH3 and SDH4. Although heme has an important role in the assembly of Complex II, it is not essential for the reduction of ubiquinones (43, 44).

Enzymatic Properties of T. cruzi Complex II—We examined SQR activity of the purified enzyme and found the difference in apparent $K_m$ values between $Q_1$ (33.9 ± 3.6 μM) and $Q_2$ (18.8 ± 6.4 μM) (Fig. 7), indicating that the 6-polyprenyl group of ubiquinone contributes to the binding affinity. The apparent $V_{max}$ value of the T. cruzi Complex II was rather constant, 11.9 ± 2.2 for $Q_1$ and 11.5 ± 0.4 $Q_2$ units/mg proteins, respectively, and one-fourth of those reported for bovine and E. coli enzymes (45, 46). This is not surprising because T. cruzi complex II has about 2–3 times more proteins than the other enzymes. $K_m$ values for ubiquinone and succinate (18.8 ± 6.4 μM ($Q_2$) and 1.48 ± 0.17 mm, respectively) were higher than 0.3 and 130 μM, respectively, of bovine enzyme (45), and 2 and 277 μM, respectively, of the E. coli enzyme (46, 47). Notably, the $K_m$ value for succinate was comparable with 610 μM in adult A. suum (10), which expresses the stage-specific Complex II as quinol:fumarate reductase under hypoxic habitats in host organisms.

Then we examined effects of inhibitors for binding sites of quinones and dicarboxylates on SQR activity. Atpenin A5, a potent inhibitor for Complex II, inhibited the T. cruzi enzyme with the IC$_{50}$ value of 6.4 ± 2.4 μM, which is 3 orders of magnitude higher than that of bovine Complex II (4 nm) (48). Furthermore, carboxin, 2-thienyltrifluoracetone, plumbagin, and 2-heptyl-4-hydroxyquinoline N-oxide were ineffective (100 μM < IC$_{50}$). Structural divergence in trypanosomatid SDH3 and SDH4 could be the cause for lower binding affinities for both quinones and inhibitors. In addition, we found for the dicarboxylate-binding site that the IC$_{50}$ value for malonate (40 μM) was much higher than the $K_i$ value for bovine Complex II (1.3 μM) (45).

Structure of Trypanosomatid Complex II—To the best of our knowledge, this is the first report on the isolation of protist Complex II. T. cruzi Complex II has unusual subunit organization with six each of hydrophilic and hydrophobic subunits. Such a supramolecular structure and heterodimeric SDH2 (SDH2N and SDH2C) are conserved in the Trypanosomatida. Furthermore, SDH1, SDH2N, SDH2C, SDH3, SDH4, and SDH8–SDH10 can be identified in the ongoing genome projects on the evolutionary relatives, the photosynthetic free-living Euglena gracilis, and the nonphotosynthetic euglenoid Astasia longa in the Euglenida. Thus a part of these features are common in the Euglenozoa, a divergent lineage of eukaryotes (Fig. 8).

Accumulation of noncatalytic subunits through expanding the protein interaction network could be a driving force for protein evolution. Structural and catalytic features are unique, and thus this enzyme could be a potential target for novel chemotherapeutic agents for trypanosomiasis and leishmaniasis.

Conclusion—The parasitic protist T. brucei is a gold mine where unprecedented biological phenomena like RNA editing and transsplicing in mitochondria were originally discovered. It was found recently in Diplonema papillatum, a free-living evolutionary cousin,
that mature mRNA for cytochrome c oxidase Cox1 was assembled from nine gene fragments by a jigsaw puzzle mechanism (49). From a characterization of Complex II from \textit{T. cruzi}, we revealed a novel supramolecular organization, which is conserved in the \textit{Trypanosomatida}. Parasites have exploited unique energy metabolic pathways as adaptations to their natural habitats within their hosts (50, 51). In fact, the respiratory systems of parasites typically show greater diversity in electron transport pathways than those of host animals. As shown in this study, such is also the case with Complex II, which is a well known marker enzyme of mitochondria. Studies on the role of supramolecular Complex II in adaptation of \textit{trypanosomatids} is now underway in our laboratory.

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