Characterization of A Novel Respiratory Complex II in *T. cruzi* with a Heterodimeric Ip Subunit

ヘテロ二量体 Ip サブユニットをもつ新規 *T. cruzi* 呼吸鎖複合体 II の特性評価

指導教員: 北　潔　教授
東京大学大学院医学系研究科
平成19年4月進学
博士後期課程
国際保健学専攻
Jorge Jesus Morales Freites
ホルヘ　ヘスス　モラレス　フレイテス

Background and Objective

Trypanosoma cruzi is the etiological agent of the Chagas disease which affects 16-18 millions people in Latin American countries causing an estimated of 2.74 million disability-adjusted life year which is equivalent to $6.4 billions/year of economic loss for the countries affected. The energy metabolism this parasite is characterized the incomplete oxidation of D-glucose to partially reduced-end products, mainly succinate and acetate and to minor extent alanine, pyruvate, and ethanol, and the presence of a modified peroxisome called the glycosome where are compartmentalized the first seven enzymes of the glycolytic pathway. The respiratory chain of *T. cruzi* does not contain the canonical complex I (NADH-ubiquinone reductase) and relies mostly on the succinate oxidation by the complex II (succinate-ubiquinone oxidoreductase) for maintenance of

the mitochondrial membrane potential and the oxidative phosphorylation. The respiratory complex II plays an essential role on the energy metabolism of living cells and generally consists of a soluble domain composed of two subunits (SDH1 and SDH2) facing the mitochondrial matrix (eukaryotes) or cytosol (bacteria and archea), SDH1 contains a covalently linked FAD cofactor and is known as flavoprotein (Fp) subunit while SDH2 is a iron-sulphur containing protein known as Ip subunit that contains [2Fe-2S], [4Fe-4S] and [3Fe-4S] clusters. The membrane anchor domain contains one or two peptides (SDH3 or CybL and SDH4 or CybS) including five to six transmembrane regions that may or may not coordinate the ligation of a heme B molecule. Given the absence of the respiratory complex I, the respiratory complex II must play an essential role for the energy metabolism of parasite which in turns makes it a potential chemotherapeutic target against the Chagas disease. The present work describes the purification and characterization of the respiratory complex II from *T. cruzi*.

Results and Discussion

Essential Role of the Complex II in T. cruzi—*T. cruzi* epimastigotes grown in axenic cultures in the presence atpenin A5, a potent complex II inhibitor, showed and 4-fold increased of their doubling time indenpendently of the strain tested. The growth rate was not recovered in the presence of high concentration of glucose indicating that the parasite depends mostly on the mitochondrial energy metabolism. Furthermore, inhibition of the complex II by 25 μM atpenin A5

inhibited 57 and 67% of the NADH-dependent and succinate-dependent oxygen consumption of the intact parasites which clearly shows that this enzyme is the main entry point of electrons to its respiratory chain. These results together suggest a central role of the respiratory complex II for the energy metabolism of the parasite.

Purification and Subunit Composition of T. cruzi Complex II—The intact respiratory complex II was purified in a three-step procedure that included a anionic exchanger (Source 15Q) step followed by two consecutive gel filtrations through a superdex 200 pg. The intact complex II was recovered as a homogeneous peak of activity around 500 kDa at the final step of purification. Alternatively, analysis of the native complex was performed by phase separation by Triton X-114 of the mitochondrial fraction obtained from axenically cultured epimastigote forms of *T. cruzi*.

High-resolution clear native electrophoresis of both, the purified enzyme and the detergent rich fraction from the Triton X-114 phase separation of the mitochondrial fraction, showed a single band at 550 kDa that stained by both comassie blue stain and succinate dehydrogenase activity stain. Second dimensional Tricine-SDS electrophoresis analysis of the purified enzyme or the enzyme recovered in the detergent rich fraction of the Triton X-114 phase separation of the mitochondrial fraction consisted of twelve subunits. Addition of each subunit gives a calculated molecular weight of 286 kDa for the respiratory complex II of the parasite which indicated that the enzyme is a dimmer. N-terminal or internal peptide sequencing of each of the subunits allowed the identification of their respective encoding genes in the *T. cruzi* genome database.

Primary structure analysis of the amino acid sequence for each subunit of the respiratory complex II from *T. cruzi* suggested that the soluble and the membrane anchor domains of this enzyme are composed of six subunits each. The soluble domain contains a Flavoprotein subunit (Fp), a unique heterodimeric Ip subunit that consists of two hemifunctional monomers containing the "plant ferredoxin" type domain, in the case of $SDH2_N$, and the "bacterial ferredoxin" type domain, in the case of $SDH2_C$, and three trypanosome specific subunits. On the other hand, the homologues for CybL and CybS subunits and the presence of four trypanosome-specific subunits were identified in the membrane anchor domain. All the orthologue genes for the twelve subunits in the *T. cruzi* complex II were found in its close relatives *T. brucei* and Leishmania species genome databases which suggests that this structure is conserved among the trypanosomatid family of organisms.

Properties of T. cruzi Complex II—The spectra of the air-oxidized and the dithionite reduced purified enzyme showed a red shift of the soret band from 413 to 426 nm and the appearance of a β and α peaks at 529 and 561 nm respectively which is consistent with the bi-axial ligation of protoheme-IX. Stoichiometry of the heme:protein content indicates the ligation of one protohem-IX molecule per monomer of the enzyme.

T. cruzi respiratory complex II has all the amino acids responsible for the ligation of dicarboxilates at the Fp subunit and for the ubiquinone coordination, and reduction on the $SDH2_C$, CybL and CybS subunits. Kinetic analysis of the succinate ubiquinone reductase (SQR) activity

shows a difference in the values of *K*m for ubiquinone-1 (33.9±3.6 μM) and ubiquinone-2 (18.8±6.4 μM), indicating that the 6-polyprenyl group of ubiquinone contributes to the binding affinity. In contrast the *K*m for succinate (1.26 mM) is comparable to that one reported for the helminth parasite *Ascaris summ* (610 μM) where this enzyme functions as a quinol-fumarate reductase (the reverse reaction of the SQR) under hypoxic habitats inside the host organisms. The *K*m values for succinate and ubiquinone-2 of the *T. cruzi* complex II, 1.26 mM and 18.8 μM, respectively, were higher than those reported for the bovine counter part, 130 and 71 μM.

Finally, the effect of several ubiquinone binding site inhibitors was analyzed and found that only atpenin A5 was an effective inhibitor of the SQR activity of the complex II of *T. cruzi*. The IC_{50} of aptenin for the parasite enzyme was 6 μM which is over 1000 times higher than the IC_{50} for the rat enzyme (4 nM) suggesting the presence of important differences at the ubiquinone binding site between the parasite and the mammalian respiratory complex II.

Conclusions

The present work describes for the first time the full characterization of a respiratory complex II from a protozoan organism. It has been shown that the respiratory complex II from *T. cruzi* is a novel enzyme that is composed of twelve subunits among which stands out the presence of a unique heterodimeric Ip subunit and the presence of seven trypanosome-specific subunits of unknown function. The structural features of *T. cruzi* complex II are shared across the trypanosomatid family

of organisms which are exclusively composed of parasites including human pathogens such as *T. brucei* and Leishmania ssp.

The novel subunit organization of the *T. cruzi* complex II suggests that the addition of extra-subunits could have been a process driven by the splitting of the original Ip subunit gene into two hemifunctional monomers. Each Ip monomer conserved a non-functional domain or “ghost domain” (C-terminal in the case of $SDH2_N$ and N-terminal in the case of $SDH2_C$) as a remnant of the original Ip gene which could have retained its capacity to interaction with other proteins. Conversely, the presence of a heterodimeric Ip subunit at the soluble domain of the enzyme included new structural constrains that led to the recruitment of the trypanosome-specific subunits SDH5-7 and SDH8-11 to stabilize the newly formed trimer SDH1-$SDH2_N$-$SDH2_C$.

The unique structural features and biochemical properties of the respiratory complex II from *T. cruzi* and the central role it plays for the energy metabolism of the parasite makes this enzyme a potential chemotherapeutic target against the Chagas disease.