論文の内容の要旨

論文課題

Crystal structure and catalytic mechanism of *Trypanosoma cruzi* dihydroorotate dehydrogenase

（アメリカ型トリパノソーマのジヒドロオロト酸脱水素酵素の結晶構造解析と反応メカニズムの解析）

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Dihydroorotate dehydrogenase (DHOD) catalyzes the oxidation of dihydroorotate to orotate, the fourth step and the only redox reaction in the de novo biosynthesis of pyrimidine. In addition of DHOD activity (first–half reaction), the *Trypanosoma cruzi* DHOD (TcDHOD) has fumarate reductase (FRD) activity (second–half reaction) reducing fumarate produced in the mitochondrion and producing succinate that is excreted from the parasite (Fig 1). It has been suggests that TcDHOD is involved not only in the de novo biosynthesis of pyrimidine but also in redox homeostasis of the parasite (Takashima, *et al.*, 2002).

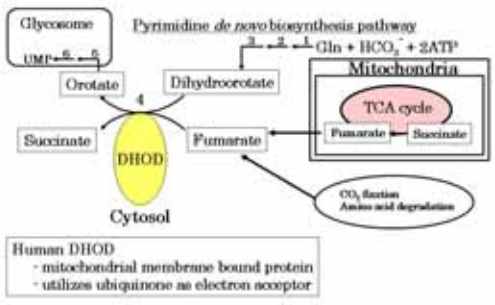

Figure 1. Schematic illustration of dihydroorotate dehydrogenase of *T. cruzi*

Biochemical analysis indicates that TcDHOD functions as the main soluble fumarate reductase in the parasite (Takashima, *et al.*, 2002) and TcDHOD knock down parasites could not survive even in the presence of exogenous pyrimidine bases and

nucleotides (Annoura, *et al.*, 2005). It suggests that the *Trypanosoma* parasite is more dependent on fumarate reductase activity to maintain the redox homeostasis than the dihydroorotate dehydrogenase activity.

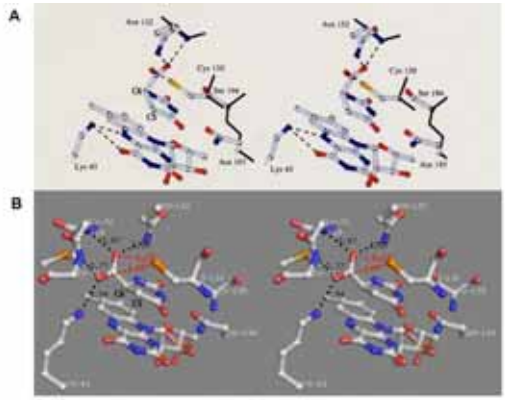

Figure 2. (A) A simple docking model of the binding of L-dihydroorotate in the active site (Rowland, et al., 1998). (B) The L-dihydroorotate complexed structure obtained in this work. The distance between Cys130 –SH group and the carboxyl group oxygens are shown in red dotted lines.

In this work, crystals of the TcDHOD in complex with orotate (product of its first–half reaction) and oxonate (a competitive inhibitor versus L-dihydroorotate) were obtained, and *X*-ray diffraction data were refined to final resolution of 1.80 and 1.12 Å resolution, respectively. To clarify the enzyme reaction mechanism the crystals of TcDHOD in complex with dihydroorotate, fumarate, succinate and the native conformation were also obtained by soaking method and refined to 1.20, 1.50, 1.38 and 1.5 Å resolution respectively. These high resolution crystal structures of the native TcDHOD and also in complex with its all physiological substrates and products, makes it possible to propose a molecular mechanism of first and second-half reaction for Family1A enzymes.

A mechanism of dihydroorotate oxidation had been proposed based on a computational docking model of the biding of L-dihydroorotate in the active site of *Lactococcus lactis* DHODA (Fig 2A). In this model the carboxylate group of L-dihydroorotate is positioned close to the –SH group of Cys130 promoting its deprotonation, and the formed $–S^-$ abstract the proton from the C5 of dihydroorotate with cooperative hydride transfer to flavin isoalloxazine ring N5 (Rowland, *et al.*, 1998). However the detailed structural analysis of the obtained TcDHOD structure in complex with L-dihydroorotate (Fig 2B) indicates that

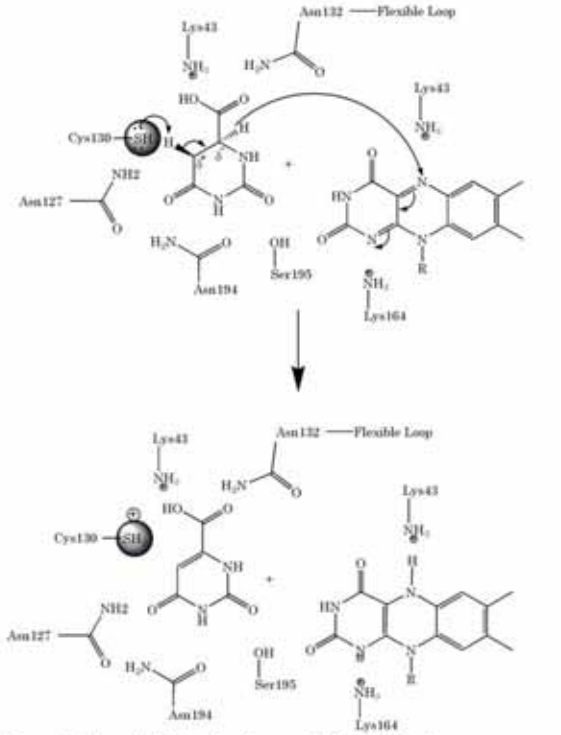

Figure 3. First-half mechanism – The L – dihydroorotate oxidation

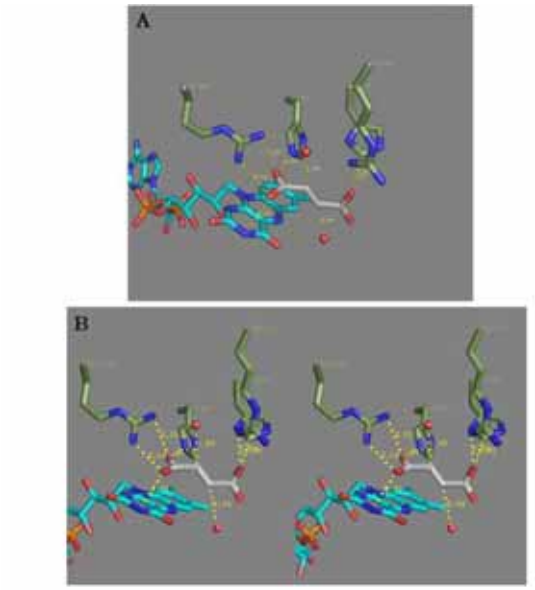

Figure 4. The Crystal structure of *W. succinogenes* FRD, PDB code, 1E7P (A) showing the amino acids interacting with fumarate in the active site. (B) Its stereo view.

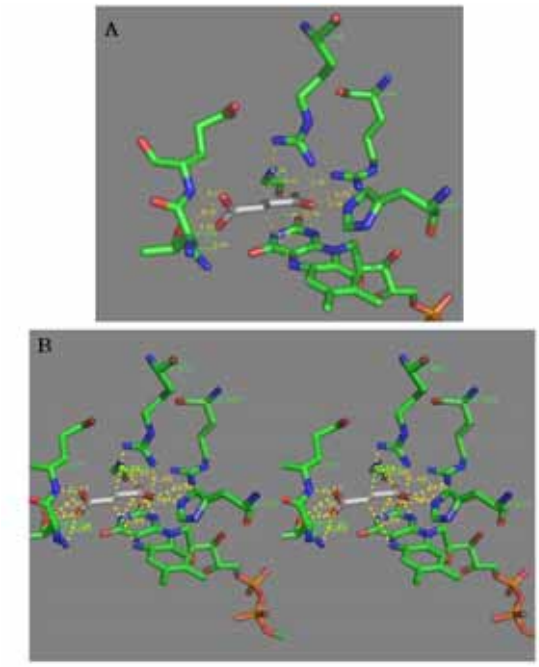

Figure 5. The Crystal structure of *S. frigidimarina* FRD, PDB code, 1D4E (A) showing the amino acids interacting with fumarate in the active site. (B) Its stereo view.

the deprotonation of the Cys130 in the active site is not necessary to start the hydride transfer from L-dihydroorotate to flavin (Fig 3).

Because there was no report about the molecular mechanism of fumarate reduction in Family1A DHODs, the TcDHOD was compared with the bacterial soluble (*Shewanella frigidimarina* and *Shewanella putrefaciens*) and membrane type (*Wolinella succinogenes*) FRDs. The fumarate binding domain is very conserved among these FRDs; however the mechanism of hydride transfer from flavin and the source of the second proton during the formation of succinate are different. In the membrane type FRD (Fig 4), one of the two molecules of water stacking over fumarate in the active site is the second proton donor while for the soluble type FRD (Fig 5) is an arginine (Arg402). In TcDHOD the second proton donor is the Cys130 (Fig 6) conserved among the Family1A and Family1B DHODs. The TcDHOD shows no sequence and structural similarity with those FRDs (Nara, *et al.*, 2000); however the twisted conformation of fumarate observed in all types of FRDs and also in TcDHOD should be important to facilitate the hydride transfer to fumarate. The TcDHOD has one molecule of water in the active site; however the role of this water is to stabilize the carbanion formed after the hydride transfer to fumarate (Fig 7).

Fumarate reductases are not present in humans and TcDHOD shows very low

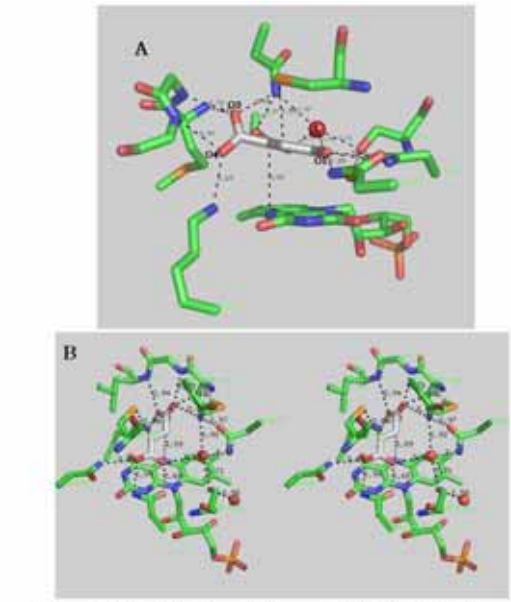

Figure 6. The side view of fumarate binding site of TcDHOD (A) showing that only the second carboxylate group of fumarate is twisted. Stereo view of fumarate interaction (B). Dashed lines indicate the distance. Fumarate are colored in white and water in red.

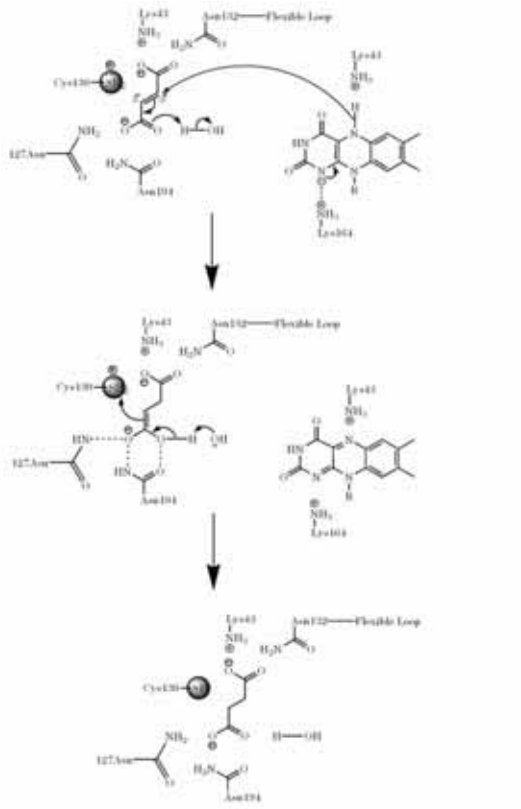

Figure 7. Second-half mechanism – The fumarate reduction

sequence and structural similarity to human DHOD (23 %). The structural analysis of TcDHOD with human DHOD indicates that there are differences in their active sites and in the molecular catalysis mechanism. These findings support that the bifunctional TcDHOD is a drug target for chemotherapy of Chagas' disease. Based in the structural data of TcDHOD obtained in this work, drug development by Structure Based Drug Design is now in progress with collaboration of Prof. Tanuma at Tokyo University of Science.

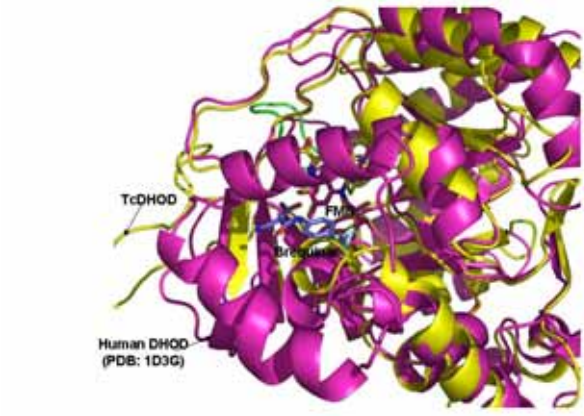

Figure 8. Superimposition of crystal structure of human DHOD (pink) and TcDHOD (yellow).