

Structures of *Trypanosoma cruzi* dihydroorotate dehydrogenase complexed with substrates and products: Atomic resolution insights into mechanisms of dihydroorotate oxidation and fumarate reduction

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Introduction

Dihydroorotate dehydrogenase from *Trypanosoma cruzi* (TcDHOD) is a flavoenzyme that catalyzes oxidation of (*S*)-dihydroorotate to orotate, the fourth step and the only redox reaction in the *de novo* pyrimidine biosynthesis pathway. In the first half-reaction, oxidation of dihydroorotate is coupled with reduction of a flavin mononucleotide (FMN) cofactor, then TcDHOD utilize fumarate as a physiological oxidant, in conjunction with oxidation of the reduced FMN co-factor during the second half-reaction. In this study, we determined the crystal structures of TcDHOD in the native form, in complexes with all known substrates and products of the first- and second half-reactions, and in a complex with an inhibitor, oxonate, at atomic resolution. These structures, in particular those of TcDHOD complexed with dihydroorotate and fumarate, enabled us to clarify the catalytic mechanisms of dihydroorotate oxidation and fumarate reduction.

Results and Discussion

Structural insight into the first half-reaction, dihydroorotate oxidation. In the crystal structure of the TcDHOD-dihydroorotate complex, Cys130 S_γ is 3.52 Å and 3.59 Å away from dihydroorotate C₅ and water K, respectively (Fig. 1A). In addition, the calculated positions of the axial C₅ hydrogen, H_{5a}, and Cys130 S_γ hydrogen, H_S, are favorably disposed for the H₂O^K·····H_S–S_γ·····H_{5a}–C₅ interaction. The water K is linked to outside solvents through a hydrogen bond chain formed by three water molecules that are located in a hydrophilic channel connecting Cys130S_γ to the outside. Because of this exquisite disposition of Cys130 and the hydrogen bond chain, dihydroorotate H_{5a} would be abstracted by Cys130 as a proton and then relayed to an outside solvent through the hydrogen bond chain. Together with the proton abstraction from C₅, dihydroorotate H₆ is transferred to FMN N₅ as a hydride to reduce the cofactor. The structure of the TcDHOD-dihydroorotate complex shows that the reduced FMN cofactor is essentially planar, which indicates that it is anionic reduced FMN, FMNH⁻. The negative charge of the anionic reduced FMN is stabilized

by Lys43 and Lys164 (Fig. 1A). In summary, the first half-reaction proceeds as follows (Fig. 5A). After binding of dihydroorotate to TcDHOD, a hydride (or a hydride equivalent) is transferred from dihydroorotate C₆ to FMN N₅, and Cys130 S_γ completes oxidation of dihydroorotate by abstracting a proton from C₅, which is relayed to an outside solvent via the H₂O^K·····H_S–S_γ·····H_{5a}–C₅ network. The negative charge of the anionic reduced FMN is stabilized by Lys43 and Lys164.

Structural insight into the second half-reaction, fumarate reduction. The prominent feature we found in the TcDHOD-fumarate complex is that the conformation of the bound fumarate is non-planar. Twisting around the C₃–C₄ bond breaks the uniform distribution of π-electrons over the conjugated double bonds of fumarate, and partial charge separation, represented as C₂^{δ-} and C₃^{δ+}, is then induced. Together with the shorter distances of C₂^{δ-}–Cys130 S_δ (3.43 Å) and C₃^{δ+}–FMN N₅ (3.15 Å) than those of C₃^{δ+}–Cys130 S_δ (4.24 Å) and C₂^{δ-}–FMN N₅ (4.03 Å), this partial charge separation may act as a guide, leading a hydride (or hydride equivalent) from FMN N₅ to C₃^{δ+} and a proton from Cys130 S_γ to C₂^{δ-} in the thermodynamically favorable reduction of fumarate with reduced FMN. In the same manner as the TcDHOD-dihydroorotate complex, the water K acts a part of the hydrogen bond network HO^K–H·····S_γ–H_S·····C₂^{δ-}. Therefore, a proton can be relayed from an outside solvent to C₂^{δ-} through the network (Fig. 1B).

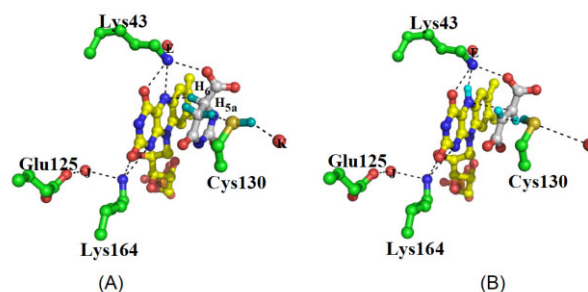


Fig. 1

References

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