Dihydroorotase From *Methanococcus Jannaschii* with Substrate and Product Bound

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Dihydroorotase (DHOase) catalyzes the reversible cyclization of N-carbamoyl-L-aspartate (CA) to L-dihydroorotate (DHO) in the third step of *de novo* pyrimidine biosynthesis. Here we report the x-ray structural analysis of DHOase from *Methanococcus jannaschii* co- crystallized with DHO at pH 6.5. The crystals are isomorphous with the crystals of the apoenzyme (Vitali et al, 2023) with space group $P3_221$ and a = b = 111.4 Å, c = 101.2 Å. The structure was refined to R = 0.159 and Rfree = 0.176 at a resolution of 1.87 Å.

The electron density in the active site corresponds to the average of the substrate (CA) and product (DHO) superimposed, and this is consistent with the reversibility of the reaction.

The flexible loop (residues 140-151) displays two alternate, partially overlapping conformations. One conformation is in the open form and is very similar to the flexible loop observed in the apoenzyme structure (Vitali et al, 2023). The second conformation is in the closed form and has weaker electron density. Both CA and DHO interact with the enzyme in a manner similar to *E. coli* (Lee et al, 2007) and human (Grande-Garcia et al, 2014) DHOases. These interactions are shown in Fig. 1. For both, the interactions with the protein involve invariant residues R60, H306, N89 and the main chain of S320 and N275.

In addition, CA interacts with the two Zn ions in the active site and the invariant D302 that abstracts a proton from its amide N during the reaction. Finally, S143 in the closed form of the flexible loop interacts with CA.

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References

{1} Grande-García, A., Lallous, N., Díaz-Tejada, C. and Ramón-Maiques, S. (2014). Structure ***22****,185-198.*

{2} Lee, M., Maher, M. J., Christopherson, R. I. and Guss, J. M. (2007). Biochemistry ***46****, 10538- 10550.*

{3} Vitali, J., Nix, J. C., Newman, H. E. and Colaneri, M. J. (2023). Proteins ***91****, 91-98. Fig. 1. Interactions of CA and DHO in the active site with the protein.*

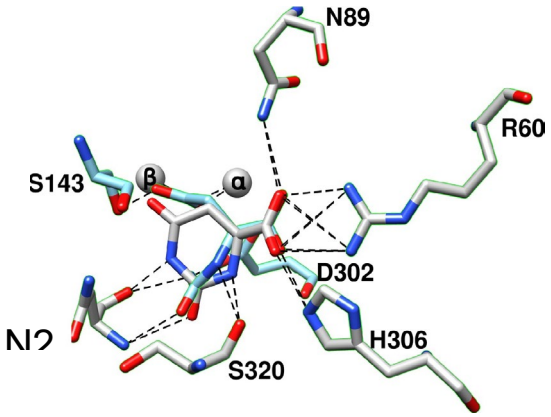

Figure 1