

Structures of the ligand-free and the inhibitor complexes of *E. coli* DHOase: Consequences for loop movement in catalysis.

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Dihydroorotase (DHOase) is a zinc metalloenzyme that catalyses the reversible cyclization of *N*-carbamyl- L-aspartate (CA-asp) to L-dihydroorotate (DHO) in the *de novo* pyrimidine biosynthetic pathway. DHOase is a potential anti-malarial drug target as malarial parasites can synthesize pyrimidines only via *de novo* pathway and do not possess salvage pathway for pyrimidine synthesis. Two different conformations of a surface loop (residues 105-115) are found in the dimeric *E. coli* DHOase crystallized in the presence of L-DHO [1-2]. The loop asymmetry mirrors that of the active site contents of the two subunits: DHO is bound in the active site of one subunit and CA-asp in the other active site. In the CA-asp-bound subunit, the surface loop reaches in towards the active site and makes hydrogen bonds with the bound substrate, whereas the loop forms part of the surface of the protein in the DHO-bound subunit.

We have crystallised *E. coli* DHOase without ligand (1.7 Å resolution) and in the presence of two different inhibitors: 2-oxo-1,2,3,6-tetrahydropyrimidine-4,6-dicarboxylate (HDDP, a transition-state analogue, 2.0 Å resolution) and 5-fluoroorotic acid (FOA, a product (DHO) mimic, 2.2 Å resolution). Analysis of the structures show different combinations of loop conformations associated with the chemical structure of the entity bound at the active sites. All three structures will be presented, along with their implications for the mechanism of DHOase and ideas for future inhibitor design.

(1) Thoden, et al. (2001) *Biochem*, 40, 6989

(2) Lee, et al. (2005) *J Mol Biol*, 348, 523