The Crystal Structures of Dihydropyrimidinases Reaffirm the Close Relationship Between Cyclic Amidohydrolases and Explain Their Substrate Specificity

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In eukaryotes, dihydropyrimidinase catalyzes the second step of the reductive pyrimidine degradation, the reversible hydrolytic ring opening of dihydropyrimidines. Here we describe the three-dimensional structures of dihydropyrimidinase from two eukaryotes, the yeast *Saccharomyces kluyveri* and the slime mold *Dictyostelium discoideum*, determined and refined to 2.4 Å and 2.05 Å, respectively. Both enzymes have a $(\beta/\alpha)_8$-barrel structural core embedding the catalytic di-zinc centre, which is accompanied by a smaller β-sandwich domain. Despite loop-forming insertions in the sequence of the yeast enzyme, the overall structures and architectures of the active sites of the dihydropyrimidinases are strikingly similar to each other, as well as to those of hydantoinases, dihydroorotase and other members of the amidohydrolase superfamily of enzymes. However, formation of the physiologically relevant tetramer shows subtle but nonetheless significant differences. The extension of one of the sheets of the β-sandwich domain across a subunit-subunit interface in yeast dihydropyrimidinase underlines its closer evolutionary relationship to hydantoinases, while the slime mold enzyme shows higher similarity to the non-catalytic collapsin-response mediator proteins involved in neuron development. Catalysis is expected to follow a dihydroorotase-like mechanism, but in the opposite direction and with a different substrate. Complexes with dihydrouracil and N-carbamyl-β-alanine obtained for the yeast dihydropyrimidinase reveal the mode of substrate and product binding and allow conclusions about what determines substrate specificity, stereoselectivity, and the reaction direction among cyclic amidohydrolases.

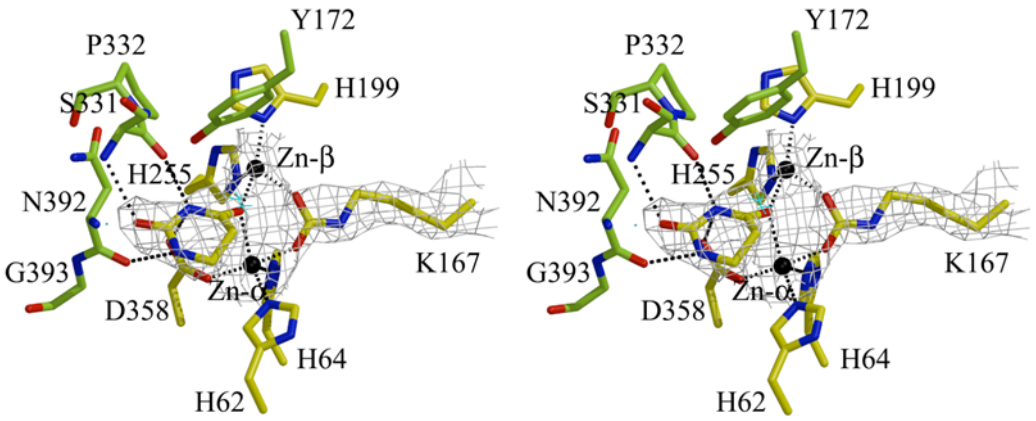

Figure 1: Substrate binding to *S. kluyveri* dihydropyrimidinase

References

[1] Lohkamp B, Andersen B, Piskur J, Dobritzsch D. (2006) J Biol Chem. 281(19):13762-76. Epub 2006 Mar 3. PMID: 16517602

[2] Lohkamp B, Andersen B, Piskur J, Dobritzsch D. (2006) Acta Crystallogr Sect F Struct Biol Cryst Commun. 62(Pt 1):36-8. Epub 2005 Dec 16. PMID: 16511257