Crystal structure of dihydropyrimidinase from *Sinorhizobium meliloti*

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Dihydropyrimidinases (EC 3.5.2.2) are involved in the reductive pathway of pyrimidine degradation. They catalyse the hydrolysis of 5,6-dihydrouracil and 5,6-dihydrothymine to the corresponding N-carbamoyl-β-amino acids. They are also known as hydantoinases as this enzyme is most famous for its application in the production of optically pure amino acids starting from racemic mixtures of 5-monosubstituted hydantoins [1]. This enzymatic manufacturing process is called the "hydantoinase process", where a chemically synthesised D,L-5-monosubstituted hydantoin ring is hydrolysed by a stereoselective hydantoinase enzyme. Further hydrolysis of the resulting N-carbamoyl-α-amino acid to the free optically pure α-amino acid is catalysed by highly enantiospecific N-carbamoyl- D- or L-amino acid amidohydrolase (depending on the configuration of the desired amino acid to be obtained). At the same time as hydantoinases hydrolyse one of the isomers of the 5-monosubstituted hydantoin, the chemical and/or enzymatic racemization of the other 5-monosubstituted hydantoin isomer starts.

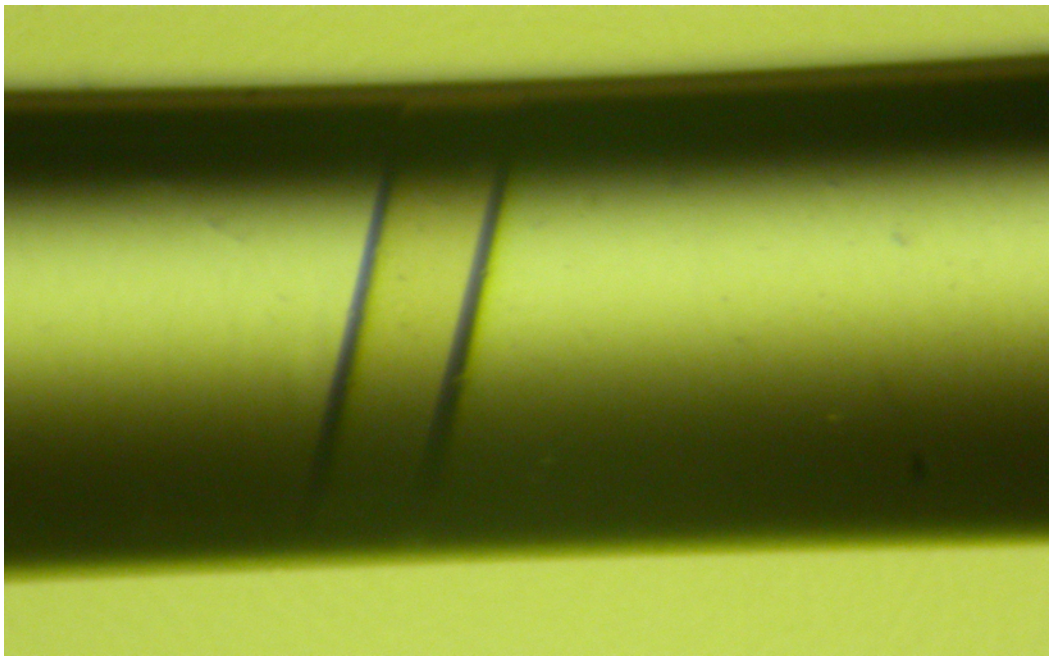

Figure 1: Crystal of SmelDhp grown in a capillary by the counter-diffusion method.

We crystallized Dihydropyrimidinase from *Sinorhizobium meliloti* is [2] and determined its three dimensional structure by X-ray diffraction. The asymmetric unit contains a dimer. The SmelDhp monomer consists of three domains: (1) a distorted TIM barrel consisting of an inner ring of eight parallel β-strands whose length decreases from the N- to C-terminus and around which eight α-

helices are wrapped. The catalytic centre is located at the C-terminal part of the β-strands as typical for this kind of fold. Inserted into the TIM domain is an all α-helical. The third domain is a β-sheet sandwich domain formed by 11 β-strands placed on the lateral lower part of the barrel. Finally there are thee additional isolated helices inserted into the TIM barrel domain and a C-terminal extension without regular secondary structure extends towards the other monomer in the dimer.

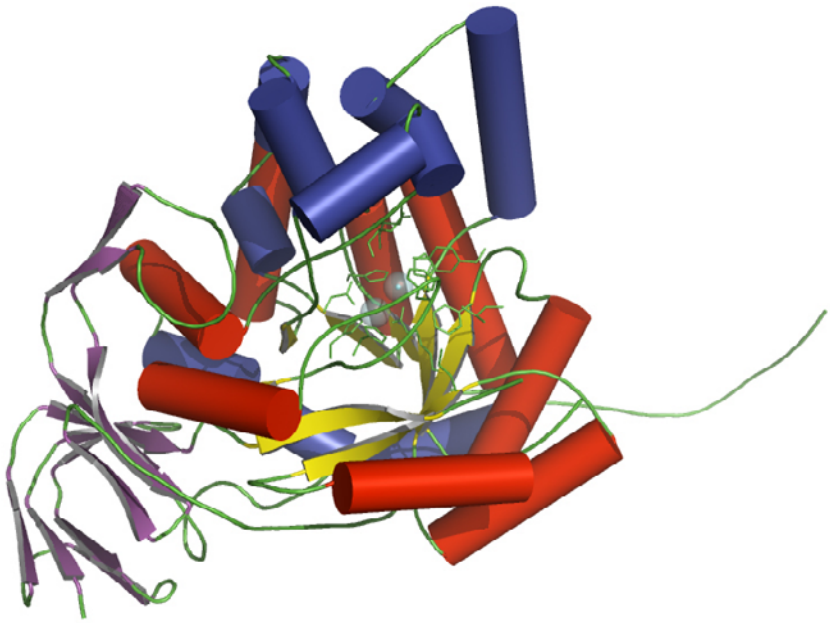

Figure 2: Ribbon diagram illustrating the domain architecture of SmelDhp. The TIM-like domain has its β-strands in yellow and its α-helixes in red. The βsandwich domain is in purple. The α-helical domain inserted into the TIM barrel domain is coloured dark blue. The three additional α-helices decorating the structure are in light blue.

Although a hydrolytic mechanism has been already proposed earlier for this enzyme [3], no structural information about the recognition of the lateral side chain of the substrates is available. This information is of crucial importance for protein engineering to obtain enzymes with a wider substrate specificity. Thus, our efforts are currently set on soaking the enzyme with different substrates. Our first attempts using short-soaking times did not reveal electron density that could be attributed to either substrate or product. As the binding site is rather buried, it is possible that the substrates cannot enter it without a conformational change in the enzyme. Attempts to co-crystallize SmelDhp in the presence of product and inhibitors are ongoing.

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References

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