STRUCTURE FUNCTION ACTIVITY STUDIES REGARDING OF THE CRYSTAL STRUCTURES OF THE PROTEIN MABA WILD TYPE AND MUTANTS FROM *MYCOBACTERIUM TUBERCULOSIS*

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The fatty acid elongation system FAS-II is involved in the biosynthesis of mycolic acids, which are major and specific long chain fatty acids of the cell envelope of *Mycobacterium tuberculosis* and other mycobacteria, including *M. smegmatis*. The protein MabA, also named FabG1, has recently been shown to be part of FAS-II and to catalyse the NADPH-specific reduction of long chain beta-ketoacyl derivatives. This activity corresponds to the second step of a FAS-II elongation round. FAS-II is inhibited by the antituberculous drug isoniazid. Thus, the other enzymes making up this enzymatic complex represent potential targets for designing new antituberculous drugs. We first solved the crystal structure at 2.0 Å resolution of the wild-type apo-enzyme. After examination of the sequence of MabA and its homologue we performed various mutations. The structure activity relationship was studies by the way of these mutants' structures

Keywords: KETOACYL REDUCTASE ENZYMATIC ACTIVITY CONFORMATIONAL CHANGE

FIRST STRUCTURE OF 20 α-HSD REVEALS A NEW CATALYTIC ACTOR FOR THE TRANSFORMATION OF PROGESTERONE BY AN AKR

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20-α-hydroxysteroid dehydrogenase (20α-HSD, AKR1C1) catalyzes the reduction of Progesterone (Prog). It has been observed in the rat that this enzyme is induced before the initiation of parturition. This suggests that 20α-HSD is involved in the withdrawal of Prog before parturition. However, this enzyme shares 97% identity with type 33α-HSD. To understand such a remarkable enzymatic specificity within the aldo-keto reductase family we have undertaken structural studies. Here we report the 1.59 Å crystal structure of the human 20α-HSD in a ternary complex with NADP and the reduced product of Prog. To our knowledge, this is the first reported structure of an AKR family HSD in complex with its natural product. Previously reported steroid:AKR crystal structure complexes have shown a close interaction between the steroid A-cycle and the Tyr55 hydroxyl, previously identified to be a proton acceptor/donor during the oxydo-reduction reaction. In contrast, the present structure reveals with a clear density that the D cycle (holding the O20 reactive oxygen) is oriented further away from Tyr55. The steroid seems to be stabilized in this orientation by numerous hydrophobic interactions and a hydrogen bond involving the O20 of the steroid and the Nepsilon of His222 (2.95 Å), whereas Tyr55 hydroxyl is at a distance of 5.4 Å. Kinetic studies of the His222Ser mutant confirmed the importance of this residue for the efficiency of h20α-HSD activity. This, in turn, would suggest that, depending on the targeted carbonyl region, different catalytic mechanisms might be used by AKRs.

Keywords: **HYDROXYSTEROID DEHYDROGENASE PROGESTERONE ALDOKETO REDUCTASE**

STRUCTURE AND FUNCTION OF A FEW PLANT PROTEINS

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X-ray structural studies of a chymotrypsin inhibitor and a trypsin/chymotrypsin inhibitor, isolated from the seeds of winged bean, have been carried out. Winged bean is a crop of the tropical region and is rich in protein content. Structure of the chymotrypsin inhibitor has been determined at high resolution using cryocrystallography and the inhibitory function of the seed protein has been explained in terms of its structure. This protein has the unique property of being a double-headed inhibitor and the position of the second reactive site has been postulated using structural similarity. Structure-based protein engineering has been carried out with a view to understand the importance of the protein scaffolding towards the conformational rigidity of the reactive site loop. Structure-function relationship for the other inhibitor has also been established. Structures of two thiol proteases have also been determined at high resolution. These enzymes have been isolated from the latex of the medicinal plant *Ervatamia coronaria*. Conserved N-terminal amino acid residues, typical of plant thiol proteases of the papain super family, indicate that the enzymes belong to the same papain family. However, these enzymes exhibit some unique properties distinct from papain and other members of the family, which includes a difference in substrate specificity. These novel properties of the enzymes have been explained in terms of their three-dimensional structures. The amino acid sequences of the proteins have been determined by crystallographic means.

Keywords: **PLANT PROTEINS, ENZYMES, ENZYME INHIBITORS**

THE STRUCTURES OF V8 PROTEASE FROM *STAPHYLOCOCCUS AUREUS* AND ITS COMPLEX WITH 3-4-DICHLOROISOCOUMARIN

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The V8 protease, an extracellular protease of *Staphylococcus aureus*, is likely related to the mammalian serine proteases. The enzyme exhibits a unique substrate specificity as it cleaves exclusively on the carbonyl side of either aspartic or glutamic acid residues. There is no significant sequence homology with other serine proteases except in regions involving catalytically important residues. In addition, V8 protease possesses no disulfide bridges, which is a major evolutionary difference, since all other related serine proteases have at least two of these groups. The V8 protease crystallizes in the hexagonal space group $P6_522$ with a = 60.66 Å & c = 219.22 Å. X-ray diffraction data were collected to a resolution of 1.9 angstroms. The native structure was solved by MAD phasing using an Osmium derivative. A total of 62 amino acid residues were found missing from the C-terminus, suggesting autolysis. This was confirmed using mass spectrometry. The data for V8-Protease in complex with its inhibitor 3-4-dichloroisocoumarin (DCI) were collected to a resolution of 1.9 Å. In the complex the ring of DCI opens and undergoes chemical modification.
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