

s8a.m1.p49 Crystallographic and biochemical investigations of the catalytic mechanism of an NADP-dependent aldehyde dehydrogenase from *Streptococcus mutans*. D. Cobessi¹, F. Tête-Favier¹, S. Marchal², G. Branlant² and A. Aubry¹. ¹LCM3B and ²MAEM Faculté des Sciences, BP 239, 54506 Vandoeuvre-lès-Nancy, France.

Keywords: ALDH, catalytic mechanism, oxyanion hole.

NAD(P) dependent aldehyde dehydrogenases (ALDH) are enzymes involved in the oxidation of the aldehydes to carboxylic acids [1]. The ALDH catalytic mechanism proceeds in two steps: acylation and deacylation. Its high catalytic efficiency at neutral pH implies prerequisites relative to the chemical mechanism. First, the catalytic Cys284 should be accessible and in a thiolate form at physiological pH to attack efficiently the aldehydic group of the substrate. Second, the hydride transfer from the hemithioacetal intermediate toward the nicotinamide ring of NADP should be efficient. Third, the nucleophilic character of the water molecule involved in the deacylation should be strongly increased. Moreover, the different complexes formed during the catalytic process should be stabilised. Comparison of apoenzyme and holoenzyme crystal structures [2] shows a Cys284 side-chain rotation of 110°, upon cofactor binding, which is probably responsible for its pKa decrease. In a second apoenzyme structure an oxygen atom of a sulphate anion interacts by hydrogen bonds with the NH2 group of a conserved asparagine (Asn154 in Sm-ALDH) and the Cys284 NH group. In the ternary complex, the oxygen atom of the aldehydic carbonyl group of the substrate interacts with the Ser284 NH group and the Asn154 NH2 group. A substrate isotope effect on acylation is observed for both the wild type and the N154A and N154T mutants. All these results suggest the involvement of Asn154 in an oxyanion hole in order to stabilise the tetrahedral intermediate and likely the other intermediates of the reaction. In the ternary complex, the electron density of the nicotinamide mononucleotide part is poorly defined in comparison with the C284S holoenzyme or wild-type holoenzyme structures. It is likely due to the peculiar binding mode of NAD(P) binding to the Rossmann fold (*i.e.* non perpendicular to the plane of the β sheet), likely favoured by a characteristic loop of the Rossmann fold, longer in ALDHs than in other dehydrogenases, whose orientation could be constrained by a conserved proline. In the ternary and C284S holoenzyme structures, as well as in the Apo2 structure, the Glu250 side chain is situated less than 4 Å from Cys284 or Ser284 instead of 7 Å in the crystal structure of the wild-type holoenzyme. It is now positioned in a hydrophobic environment [3].

[1] Moore, *et al.*, (1998). *Structure*, **6**, 1541-1551.

[2] Cobessi *et al.*, (1999). *J. Mol. Biol.*, **290**, 161-173.

[3] Cobessi *et al.*, (2000). *J. Mol. Biol.*, *in press*.

s8a.m1.p50 The structure of heterodimeric imidazole glycerole phosphate synthase (tHisH/tHisF) reveals an substrate transfer channel through a $(\alpha/\beta)_8$ barrel. M. Walker*, S. Beismann-Driemeyer^d, R. Sterner^d, M. Wilmanns* ^{EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany} ^{Institute for Biochemistry, University of Cologne, Otto-Fischer-Strasse 12-14, D-50674 Köln, Germany}.

Keywords: histidine biosynthesis, imidazole glycerole phosphate synthase, substrate transfer channel.

Histidine biosynthesis plays a pivotal role in cellular metabolism for a number of reasons. First of all generation of an amino acid essential for many living systems, secondly the heterodimeric imidazolglycerol phosphate synthase in this pathway is interconnected to the nitrogen metabolism and the *de novo* biosynthesis of purines. Eight enzymes are required for the synthesis of the amino acid histidine from ATP and 5'-phosphoribosyl- α -1-pyrophosphate¹. So far only two of these proteins have been structurally characterized. tHisA and tHisF both show $(\alpha/\beta)_8$ barrels².

We have determined the crystal structure of the imidazole glycerole phosphate synthase, a heterodimer, consisting of a glutamine-amidotransferase (*hish* gene product, 23 kDa, 201 aa) and a cyclase (*hisp* gene product, 27.7 kDa, 253 aa) subunit from the hyper-thermophile *Thermotoga maritima*³ at 2.4 Å resolution. The structure was solved by molecular replacement using tHisF as model and three-fold ncs averaging.

tHisH shows an α/β hydrolase fold and interacts with the N-terminal face of tHisF. Remarkably this protein subunit does not dock at the C-terminal face of tHisF, where the active side of the latter is. The structure reveals a channel for substrate transfer through the centre of the tHisF $(\alpha/\beta)_8$ barrel from the N- to C-terminal face. This result adds a novel function to the $(\alpha/\beta)_8$ barrel scaffold.

[1] Winkler, M.E. (1996) in : *Escherichia coli and Salmonella : Cellular and Molecular Biology*, Vol. I pp 485-505 (Neidhardt, F.C.C.I., R.; Ingraham, J.L.; Lin, E.C.C.; Low, K.B.; Magasanik, B.; Renikoff, W.S.; Riley, M.; Schaechter, M., Umbarger, H.E. Ed.) ASM Press Washington D.C.

[2] Lang, D., Thoma, R., Henn-Sax, M., Sterner, R., Wilmanns, M. (2000) submitted

[3] Thoma, R., Schwander, M., Liebl, W., Kirschner, K., Sterner, R. (1998) *Extremophiles* **2**, 379-389.