reaction of dCTP - hereby directly producing dUMP from dCTP. In this way the organism avoids to accumulate the toxic intermediate dUTP. dCTP deaminase:dUTPase was first discovered in the archaea *Methanocaldococcus jannaschii* and the *M. tuberculosis* enzyme is the second bifunctional dCTP deaminase:dUTPase to be characterized. Steady state kinetics has shown that the *M. tuberculosis* enzyme has a similar affinity for the two substrates dCTP and dUTP, while dTTP is shown to bind as an inhibitor. Structural comparison between the crystal structures of the enzyme complexed with dTTP and the enzyme alone made us able to demonstrate how the bifunctional enzyme may bind dTTP without hydrolyzing this regulatory molecule [1]. Further studies on selected mutant enzymes have currently been initiated and will be presented.

[1]Helt S.S., Thymark M., Harris P., Aagaard C., Dietrich J., Larsen S., Willemoes M., *J. Mol. Biol.*, 2008, **376**, 554-569.

Keywords: deoxy-ribonucleotide metabolism, deamination, enzyme regulation

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Expansion of substrate specificity and the structural basis of AzoR from *Escherichia coli*

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AzoR is an oxidoreductase isolated from *E. coli* as a protein responsible for the degradation of azo compounds. AzoR exists as a homodimer composed of 23 kDa subunits. The reaction follows a ping-pong mechanism requiring 2 mol of NADH to reduce 1 mol of methyl red, a typical azo dye, into 2-aminobenzoic acid and N,N' -dimethyl-p-phenylenediamine. To clarify the reduction mechanism of azo compounds and to expand the substrate specificity for the development of biodegradation technologies, we have carried out the X-ray crystallographic and enzymatic analyses of AzoR. The overall structures revealed that AzoR has a flavodoxin-like structure in spite of the explicit amino acid sequence homology, and act as a homodimeric FMN-containing enzyme. Superposition of the structures of oxidized AzoR from different crystal forms revealed the regions that participate in the conformational change of the active site, which would be a mechanism to accommodate substrates of different size. The structure of AzoR in the reduced form revealed a twisted butterfly bend of the FMN cofactor's isoalloxazine ring and a rearrangement of solvent molecules. The structure of the enzyme in complex with an inhibitor dicoumarol and the enzymological analysis, combined with the structures of oxidized AzoR, indicate that the formation of a hydrophobic part around the isoalloxazine ring is important for substrate binding, and an electrostatic interaction between Arg-59 and the carboxyl group of the azo compound determines a substrate preference. Based on these results, we succeeded in expanding the substrate specificity by the substitution of Arg-59 with Ala, and we built an authentic model of the AzoR-methyl red complex. This is the first structure of FMN-dependent NADH-azoreductase.

Keywords: azoreductase, substrate specificty, oxidoreductase

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The complex between a branched pentasaccharide and *Thermobacillus xylanilyticus* arabinofuranosidase

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The crystal structure of the family GH-51 a-L-arabinofuranosidase from *T. xylanilyticus* has been solved as a seleno-methionyl derivative and as an inactive mutant Glu176Gln in complex with a branched pentasaccharide. The overall structure shows the two characteristic GH-51 domains: a catalytic domain that is folded into a (α/β)8-barrel and a C-terminal domain that displays jelly-roll architecture. The pentasaccharide is bound in a groove on the surface of the enzyme, with the mono arabinosyl-branch entering a tight pocket harbouring the catalytic dyad. Detailed analyses of both structures and comparisons with the two previously determined structures from *G. stearothermophilus* and *C. thermocellum* reveal important details unique to the *T. xylanilyticus* enzyme. In the absence of substrate, the enzyme adopts an open conformation. In the substrate-bound form, the loop connecting β-strand 2 to α-helix 2 closes the active site and interacts with the substrate through residues His98 and Trp99. The results of kinetic and fluorescence titration studies using mutants underline the importance of this loop, and support the notion of an interaction between Trp99 and the bound substrate. We suggest that the changes in loop conformation are an integral part of the *T. xylanilyticus* a-L-arabinofuranosidase reaction mechanism, and ensure efficient binding and release of substrate.

Keywords: arabinofuranosidase, enzyme, crystal structure

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Structure of glycerol-3-phosphate dehydrogenase, an essential monotopic membrane enzyme

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Sn-glycerol-3-phosphate dehydrogenase (GlpD) is monotopic membrane enzyme, functioning at the central junction of respiration, glycolysis, and phospholipid biosynthesis. Its critical role is indicated by multi-tiered regulatory mechanisms. A key flavin-linked primary dehydrogenases of the respiratory electron transport chain, the activity of the enzyme is regulated through its interaction with the inner membrane in *E. coli*. We have determined several new structures of the fully active GlpD, up to 1.75 Å resolution. In addition to elucidating the structure of the native enzyme, we have determined the structures of GlpD complexed with various substrate analogues and product. These structural results reveal conformational states of the enzyme, delineating the residues involved in substrate binding and catalysis at the glycerol-3-phosphate site. Two probable mechanisms for catalyzing the dehydrogenation of glycerol-3-phosphate are envisioned, based on the conformational states of the complexes. To further correlate catalytic dehydrogenation to respiration, we have additionally determined the structures of GlpD bound with ubiquinone analogues, identifying a hydrophobic plateau that is likely to be the ubiquinone binding site. These structural results