between domains. In addition, FAD formed covalent bond in His114 and Cys176. Based on the structural information obtained above, amino acid mutations of the four ionizable residues (H292, Y417, E442 and Y484) located in the vicinity of FAD is now in progress to clarify their contribution on its enzymatic function.

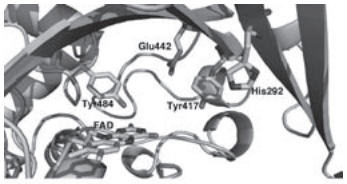

Figure. Active site of THCA synthase

Keywords: enzyme mechanics, flavoenzymes, plants

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Structure determination of hydrogenase maturation factor HypB from *Archaeoglobus fulgidus*

Kam-Bo Wong, Ting Li, Kwok-Ho Chan
The Chinese University of Hong Kong, Department of Biochemistry, Room 507B Mong Man Wai Building, Hong Kong, NA, NA, Hong Kong SAR, E-mail：kbwong@cuhk.edu.hk

The assembly of the [NiFe]-hydrogenases requires incorporation of Ni ions into the enzyme's metallocenter, which process requires the GTPase activity of hypB. To better understand the structure-function of hypB, we have solved the structure of apo-form of AfHypB by X-ray crystallography, crystals of AfHypB were grown using the hanging-drop-vapour-diffusion method. Protein (1 ul at 15 mg/ml) was mixed with 1 ul of reservoir solution (8% PEG4000, 0.1 M sodium acetate at pH 4.6), and equilibrated over 1ml of reservoir solution. Crystals were diffracted to ~2.4 Å, and belong to space group $P2_12_12$, with unit cell dimensions a=73.14, b=82.15, c=68.59 Å, $\alpha=\beta=\gamma$=90 deg. The structure was determined by molecular replacement using GTP-gamma-S-bound form of hypB from M. jannaschii as a search template, and two molecules of AfHypB were found in the asymmetric units. Structure differences between the apo-form of AfHypB and GTP-bound form of hypB from M. jannachii is discussed. This work is supported by RGC grants (CUHK4610/06M).

Keywords: hydrogenase maturation factor, nickel, protein interaction

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Production and crystallization of tomato β-galactosidase 4

Masahiro Eda[1], Megumi Ishimaru[1], Toshiji Tada[1], Takayoshi Kinoshita[1], David L. Smith[2], Kenneth C. Gross[2]
[1]Osaka Prefecture University, Graduate School of Life and Environmental Sciences, 1-1, Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, [2]USDA-ARS, 10300 Baltimore Avenue, Beltsville, Maryland 20705-2350, USA, E-mail：cfaag700@gmail.com

β-Galactosidase hydrolyzes terminal, non-reducing β-D-galactosyl residues from β-D-galactosides. Tomato β-galactosidase 4 (TBG4) is closely related to tomato fruit softening. We have started an X-ray crystal structure analysis of TBG4 to clarify the substrate recognition mechanism of this novel enzyme. The His-tagged recombinant proteins of TBG4 were expressed under the control of the alcohol oxidase promoter in *Pichia pastoris* and secreted into the culture medium. The recombinant TBG4 was purified by nickel affinity chromatography and migrated as a single 78-kDa band on SDS-PAGE. The purified enzyme showed β-galactosidase activity and strong exo-β-1,4-galactanase activity. The optimum pH was 4.0 and the K_m value against ρ-nitrophenyl-β-D-galactopyranoside was 1.67 mM. The solution of purified TBG4 was concentrated in 20 mM sodium acetate (pH 4.0) prior to crystallization. The screening of crystallization is currently under way.

Keywords: beta-galactosidase, crystallization, solanum lycopersicum

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Crystal structure of *Aquifex aeolicus* PPX/GPPA in complex with ppGpp

Ole Kristensen, Birthe Ross, Michael Gajhede
University of Copenhagen, Biostructural Research, Dept. Medicinal Chemistry, Universitetsparken 2, Copenhagen, Copenhagen, 2100, Denmark, E-mail：ok@farma.ku.dk

The crystal structure of the *Aquifex aeolicus* exopolyphosphatase/guanosine pentaphosphate phosphohydrolase in complex with the intracellular second messenger guanosine tetraphosphate was determined at 2.7 Å resolution. The hydrolytic base is identified as E119. Results from this study identify the glutamic acid residue 119 as the important hydrolytic base and establish that the dual specificity of the homologous *Escherichia coli* protein is compatible with a common active site for guanosine pentaphosphate and polyphosphate hydrolysis. This resolved a dispute originating from previous structural studies of the apo-enzymes. Distinct and different configurations between the two domains of the enzyme are associated with substrate binding. The residues R22 and R267, residing in different domains, are crucial for guanosine pentaphosphate specificity and recognition of the unique 3'-ribose phosphorylation. The orientation of the nucleotide base is somewhat different from expectations based on modeling of the related ATP-binding ASKHA protein family members.

Keywords: guanosine pentaphosphate phosphohydrolase, exopolyphosphatase, protein-ligand complex

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Inhibition by dTTP of the bifunctional dCTP deaminase:dUTPase from *Mycobacterium tuberculosis*

Pernille H Harris[1], Signe S. Helt[1], Tina Vognsen[1], Majbritt Thymark[2], Claus Aagaard[3], Jes Dietrich[3], Sine Larsen[2,4], Martin Willemoes[5]
[1]Technical University of Denmark, Department of Chemistry, Kemitorvet Building 207, Kgs. Lyngby, Denmark, DK-2800, Denmark, [2]Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark, [3]Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark, [4]European Synchrotron Radiation, BP220, F38043 Grenoble Cedex,France, [5]Department of Biology, University of Copenhagen, Ole Maaloes vej 5, DK-2200 Copenhagen, Denmark, E-mail：ph@kemi.dtu.dk

dCTP deaminase from *Mycobacterium tuberculosis* has been proven to be a bifunctional dCTP deaminase:dUTPase. dCTP deaminase:dUTPase is a homotrimeric enzyme, closely related to the dCTP deaminases and the trimeric dUTPases. The enzyme catalyses both the deamination and the triphosphate hydrolysis

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*

Masaru Tanokura[1], Kosuke Ito[1], Masayuki Nakanishi[2], Woo-Cheol Lee[1], Yuehua Zhi[1], Hiroshi Sasaki[1], Shuhei Zenno[3], Kaoru Saigo[3], Yukio Kitade[2]
[1]The University of Tokyo, Department of Applied Biological Chemistry,Graduate School of Agricultural and Life Sciences, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan, [2]Department of Biomolecular Science, Faculty of Engineering, Gifu University, [3]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, E-mail：amtanok@mail.ecc.u-tokyo.ac.jp

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

Michael Gajhede[1], Gabriel Paes[2], Michael J O'Donohue[2], Lars K Skov[1,3], Jette S Kastrup[1], Osman Mirza[1]
[1]University of Copenhagen, MEdicinal Chemistry, Universitetsparken 2, Copenhagen, Copenhagen, 2100, Denmark, [2]INRA UMR FARE 614, 8, rue Gabriel Voisin, BP 316, 51688 Reims Cedex 2, France, [3]Novozymes A/S, Krogshojvej 36, DK-2880 Bagsvaerd, Denmark, E-mail：mig@farma.ku.dk

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

Joanne I. Yeh, Unmesh Chinte, Shoucheng Du
Univ. of Pittsburgh, Structural Biology, 3501 5th Avenue; 1036 BST3, Pittsburgh, PA, 15260, USA, E-mail：jiyeh@pitt.edu

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