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.

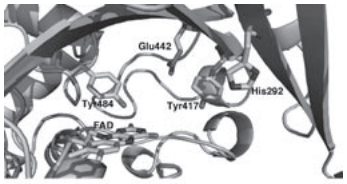

Figure. Active site of THCA synthase

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