P.04.25.13

Acta Cryst. (2005). A**61**, C265

Crystal Structure Analysis of Maize Glutamine Synthetase

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Glutamine synthetase (GS; EC 6.3.1.2) in higher plants has important roles in the assimilation of inorganic nitrogen into glutamine. In plants, there are mainly two types of GS isozymes and they are localized in distinct subcellular compartments, namely the cytosol (GS1) and the plastids/chloroplasts (GS2). Ammonia from the soil and derived from the fixation of dinitrogen in leguminous bacteria is assimilated into glutamine by cytosolic GS1 in roots and nodules, respectively. In maize, five genes (*GS1a*, *GS1b*, *GS1c*, *GS1d* and *GS1e*) encoding cytosolic GSs are known [1]. Isozymes of cytosolic GS are subdivided into ammonia-induced (GS1a and GS1b) and noninduced (GS1c and GS1d) enzymes, which show distinct enzymatic and physicochemical properties.

In this study, we expressed the GS1a protein in *E. coli* cells and purified the protein for crystallization. Crystals suitable for X-ray analysis were obtained by the hanging drop vapor diffusion method. The crystals belong to space group $P2_1$with cell dimensions a=95.8 Å, b=191.0 Å, c=118.1 Å, β=101.5º. We are doing further analysis to obtain atomic resolution structure.

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Keywords: crystallization, protein structure determination, enzymatic structure-activity relationship

P.04.25.14

Acta Cryst. (2005). A**61**, C265

The Sulfur Oxygenase Reductase from *Acidianus ambivalens*

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The biological oxidation of elemental sulfur and reduced inorganic sulfur compounds to sulfate is one of the major reactions in the sulfur cycle, in biotechnological processes such as bioleaching of low-grade metal ores, and for life in solfataras and hot springs of volcanic origin. Little is known about the enzyme systems involved in these oxidation pathways, especially "sulfur enzymes" from microorganisms living in acidic and/or hot environments [1]. The cytoplasmatic sulfur oxygenase reductase (SOR) [2] of *Acidianus ambivalens* is the initial enzyme in its aerobic sulfur oxidation pathway. It catalyzes the oxygen-dependent disproportionation of elemental sulfur leading to sulfite, thiosulfate and sulfide. Sulfur is simultaneously electron donor and acceptor of the reaction. The only identified redox-active site in SOR is a low-potential mononuclear non-heme iron center [2]. Three cysteines conserved in all SOR amino acid sequences are also thought to be involved due to the inhibitory effect of thiol-binding compounds [3]. The catalytic reaction is not yet elucidated, as it requires not yet well understood complex redox transitions. The enzyme is a large homo-multimer composed of identical subunits of 308 residues with a diameter of 15.5 nm [2]. SOR was crystallized and its 3D structure determined by MIRAS, showing a 24-oligomer with 432 NCS [4] and 870 kDa.

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Keywords: sulfur metabolism, non-heme iron protein, extremophile

P.04.26.1

Acta Cryst. (2005). A**61**, C265

Structural Basis for Carbohydrate Specificity of Basic Winged Bean Lectin

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Lectins, multivalent carbohydrate binding proteins, which recognize diverse sugars specifically, provide a unique system to understand protein carbohydrate interactions. One of the major concerns in the lectin research is the elucidation of the structural basis of this interaction. As part of an ongoing program on the study of structure and function of lectins, we have solved the structure of basic lectin from Winged beans (*Psophocarpus tetragonolobus*) (WBAI) in complex with four monosaccharides (Gal, GalNAc, me-O-α-GalNAc, Tn-antigen), two disaccharides (Galα(1,3)Gal and Galα(1,4)Gal) and two trisaccharides (Galα(1,3)Galβ(1,4)Gal and Galα(1,3)Galβ(1,4)Glc).

These structures reveal that among the four loops (A, B, C, D) which establish the sugar binding pocket, the first three are substantially conserved and provide the essential structural framework required for binding of monosaccharides. The D loop of this lectin is the longest observed in legume lectins, which makes the binding pocket extended. The enhanced affinity of WBAI for GalNAc, me-O-α-GalNAc and Tn-antigen can be attributed to the additional interactions made by the sugar with loops B and C. In case of disaccharides and trisaccharides the additional interactions required for the stabilization of sugar moieties at the reducing end are provided by the loop D. These observations further emphasizes the role of D loop in determining the specificity.

Keywords: legume lectin, carbohydrates, basic winged bean lectin

P.04.26.2

Acta Cryst. (2005). A**61**, C265

Experimental Tests of the Theoretical Dose Limit for Cryocooled Protein Crystals

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Various aspects of radiation damage to protein crystals at 3rd generation synchrotron sources have been investigated. Experiments have been carried out on beamline ID14-4 at the ESRF to investigate the effect of the dose, dose-rate, and the macromolecular composition on the degradation in diffraction by, and derived structure of, apoferritin and holoferritin crystals.

To compare with the theoretical dose limit of $2x10^7$ Gy [1] for half the diffraction pattern to disappear, the dose absorbed by a crystal must be calculated. This is a function of both the beam parameters and the crystal composition and can be determined using RADDOSE [2]. The elemental composition was measured using microPIXE [3].

The decay in diffraction of four holoferritin and three apoferritin crystals was analysed. Crystals were exposed to an unattenuated beam between datasets; changes in diffraction quality and crystal structure could then be analysed as a function of the cumulative absorbed dose.

A new dose limit for cryocooled protein crystals is proposed and linked to experimentally observed reductions in diffractive and structural quality.

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Keywords: radiation damage, ferritin, microPIXE