

HOW MYO-INOSITOL IS MADE IN EVERY LIVING CELL. THE STRUCTURES AND INSIGHTS INTO CATALYSIS OF INOSITOL-1-PHOSPHATE SYNTHASE (IPS) AND INOSITOL-1-PHOSPHATE PHOSPHATASE (IMPase/FBPase) FROM ARCHAEoglobus FULGIDUS

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In all organisms the only known *ab initio* pathway leading to synthesis of myo-inositol is conversion of D-glucose-6-phosphate to L-myo-inositol-1-phosphate (via inositol-1-phosphate synthase, IPS), followed by specific dephosphorylation via inositol monophosphatase (IMPase). We have investigated both enzymes involved in synthesis of myo-inositol in the hyperthermophilic sulfate reducer *Archaeoglobus fulgidus*. The structures of both enzymes were obtained to better than 2 Å resolution. The structure solution process and the resulting structures of both enzymes will be described in detail and compared with corresponding enzymes from other species. Insights into catalysis and possible evolutionary changes will be discussed. In particular, we will focus on the use of metal ions in the IPS catalytic cycle and the basis for dual activity of the IMPase.

Keywords: INOSITOL BIOSYNTHESIS INOSITOL-PHOSPHATE SYNTHASE INOSITOL-1-PHOSPHATE PHOSPHATASE

CRYSTAL STRUCTURE OF TABTOXIN RESISTANCE PROTEIN COMPLEXED WITH ACETYL Coenzyme A

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Tabtoxin resistance protein (TTR) is an enzyme that renders Tabtoxin-producing pathogens, such as *Pseudomonas syringae*, tolerant to their own phytotoxins. Here we report the crystal structure of TTR complexed with its natural substrate, acetyl coenzyme A (AcCoA), to 1.55-Å resolution. The binary complex forms a characteristic V shape for substrate binding and contains the four motifs conserved in the GCN5-related N-acetyltransferase (GNAT) superfamily. Our structure provides new insight into the understanding of the role of three critical residues that are conserved in the catalytic pocket of many GNAT superfamily members, including the histone acetyltransferases. Based on our results we propose a single-step mechanism through which TTR catalyses the inactivation of Tabtoxin by the transfer of an acetyl group to the toxin. We further report that TTR possess histone acetyltransferase activity, therefore, suggest a general catalytic mechanism for acetylation of beta-lactam and protein.

Keywords: TABTOXIN RESISTANCE PROTEIN

THE CRYSTAL STRUCTURE OF A NOVEL MAMMALIAN LECTIN Ym1 SUGGESTS A SACCHARIDE BINDING SITE

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Ym1, a secretory protein synthesized by activated murine peritoneal macrophages, is a novel mammalian lectin with a binding specificity to glucosamine (GlcN). Lectins are responsible for carbohydrate recognition and mediating cell-cell and cell-extracellular matrix interactions in microbes, plants and animals. Glycosaminoglycan heparin/heparan sulfate binding ability was also detected in Ym1. We report here the three-dimensional structure of Ym1 at 2.5 Å resolution by X-ray crystallography. The crystal structure of Ym1 consists of two globular domains, a β/α TIM barrel domain and a small α+β folding domain. A notable electron density of sugar is detected in the Ym1 crystal structure. The saccharide is located inside the TIM domain at the C-terminal of β-strands barrel. Both hydrophilic and hydrophobic interactions are noted in the sugar-binding site in Ym1. Despite the fact that Ym1 is not a chitinase, structurally, Ym1 shares significant homology with chitinase A of *Serratia Marcescens*. Both Ym1 and chitinase A have similar carbohydrate binding cleft. This study provides new structure information, which will lead to better understanding of the biological significance of Ym1 and its putative gene members.

Keywords: LECTIN SACCHARIDE GLYCOSAMINOGLYCAN

CRYSTAL STRUCTURE OF COAGULATION FACTOR IX-BINDING PROTEIN AT pH = 6.5

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Coagulation factor IX-binding protein (IX-bp) is an anticoagulant, contained in the venom of habu snake (*Trimeresurus flavoviridis*), and consists of homologous subunit A (129 amino acid residues, 16.8 kDa) and subunit B (123 amino acid residues, 15.7 kDa). Each subunit has a Ca(II) binding site, but with a different affinity. The biochemical study showed that the lower affinity Ca(II) binding site releases its Ca(II) at pH 6.5 involving the decrease in the ability to bind blood coagulation factor IX. In order to elucidate the effect of this Ca(II) release, we conducted the X-ray crystallographic analysis at pH = 6.5 and compared with the known crystal structure at pH = 7.8. The structure was solved at 1.8 Å resolution by molecular replacement methods. Overall structures are very similar to each other between at pH = 6.5 and at pH = 7.8. A significant structural difference was observed only in the Ca(II) binding site of subunit A, although the Ca(II) still exists there. The distance between the carboxyl oxygen of Glu43 and the Ca increases from to 3.8 Å from 2.4 Å in the case at pH 7.8, and thus Ca(II) coordination from Glu43 is apparently disrupted at pH 6.5. This finding suggests that the lower Ca(II)-binding site is located in subunit A. Further crystallographic study of the same protein at pH = 6.0 is in progress for better understanding of Ca(II) releasing mechanism.

Keywords: COAGULATION FACTOR, ANTICOAGULANT, CALCIUM BINDING SITE