s8a.m1.p15 **Structural analysis of substrate binding by the glucan synthesizing enzyme amylosucrase.** O. Mirza[1], L.K. Skov[1], A. Henriksen[1], G. Potocki De Montalk[2], R. M. Willemot[2], M. Remaud-Simeon[2], P. Sarcabal[2], P. Monsan[2], M. Gajhede[1]. [1]*Protein Structure Group, Dept. of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*. [2]*Institut National des Sciences Appliquèes, Avenue de Rangueil, F-31077 Toulouse Cedex 4, France.*
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Amylosucrase is a hexosyltransferase (E.C. 2.4.1.4) found in *Neisseria polysaccharea*. It catalyses the synthesis of an amylose-like polysaccharide with only α-(1→4)-glucosidic linkages using sucrose as substrate. Based on amino-acid sequence similarities, amylosucrase has been placed in the retaining glycoside hydrolase family 13: also known as the α-amylase family. Fully active recombinant amylosucrase has been expressed in *E. coli* making the production of large quantities of protein and mutational studies possible [1]. The enzyme has been crystallised and preliminary X-ray studies have been published [2]. The structure of amylosucrase has been solved using the MAD method on a Se-Met-substituted enzyme, and phase extension techniques. Data on the native enzyme was collected to 1.4 Å resolution and refinement is still in progress. The structure contains the predicted $(\beta/\alpha)_8$ barrel and also a N-terminal and a C-terminal domain. Information about the active site has been obtained from the structures of amylosucrase with glucose bound to the presumed active site (1.7 Å), and of the active site mutant E328Q in complex with sucrose (2.0 Å).

These data will give some insight into the reaction mechanism, by pinpointing the catalytic residues, and the residues shaping the specificity of this potentially very useful enzyme.

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s8a.m1.p16 **Crystal structure of glucosamine-6 phosphate N-acetyltransferase 1: a novel member of the GNAT superfamily**. C. Peneff[1], D. Mengin-Lecreulx[2] & Y. Bourne[1], [1]*CNRS, AFMB, 31 Ch. J. Aiguier, F13402 Marseille Cedex 20.* [2]*CNRS, UMR8619, Université Paris-Sud, Bat. 430, F91405 Orsay Cedex.*
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UDP-GlcNAc, the nucleoside-activated form of N-acetylglucosamine (GlcNAc), is an essential metobolite constituting the GlcNAc moiety for N-linked glycosylation and the GPI-anchor of cellular proteins.

Glucosamine-6 phosphate acetyltransferase 1 (GNA1)[1] is the third enzyme, amongst four, in the biosynthesis pathway of UDP-GlcNAc and catalyzes the formation of GlcNAc-6P from GlcN-6P and AcCoA. GNA1, whose gene disruption is lethal in yeast, has been shown to control multiple processes in cell cycle progression[2]. GNA1 belongs to the large family of GNAT acetyltransferases which all possess a common structural core composed of a four-stranded β-sheet flanked by α-helices for AcCoA binding[3]. Large variations are found within the substrate binding site which can accommodate different ligands, e.g. aminoglycosides, histone's tails, arylalkylamines.

We have overexpressed *S. cerevisiae* GNA1 in *E. coli* and obtained two different crystal forms, an orthorhombic and monoclinic for the apo and AcCoA-bound enzyme, respectively. The two structures have been solved independently applying MAD techniques on the selenomethionine-substituted enzyme and refined to 2.4 and 1.3 Å resolution respectively. For the high resolution crystal form the model has been built automatically using the warpNtrace procedure. The current models of apo and AcCoA-bound enzyme have crystallographic R-factors of 22.1% and 21.3% (R-free of 28.7% and 22.5%), respectively.

GNA1 is a dimer in solution and both crystal structures reveal an original domain swap in which the C-terminal β-strand is exchanged between the two molecules, a feature also found in other oligomeric proteins[4]. Crystals of CoA-complexed protein were obtained and soaked with the product GlcNAc-6P. This structure, which has been refined to 1.8 Å resolution (R-factor and R-free values of 18.4% and 22.3%), along with the apo and AcCoA bound structures, provides deeper insight into the GNA1 catalytic machinery.

Details of these structures together with a structural comparison with other members of the GNAT superfamily will be presented.

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