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Structural Analysis of *Thermus thermophilus* HB27 Mannosyl-3-Phosphoglycerate Synthase Susana Gonçalves, Nuno Borges, Ana M. Esteves, Bruno Victor, Cláudio M. Soares, Helena Santos, Pedro M. Matias. *Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal.*
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Glycosyltransferases (GTs) catalyze the synthesis of an immense diversity of oligosaccharides, polysaccharides, and glycoconjugates. Many of these nucleotide-sugar dependent transferases are grouped within two well-characterized structural folds, GT-A and GT-B [1]. GTs are classified as either retaining or inverting according to the stereochemical conservation of their sugar substrates in the reaction products. The enzymatic mechanisms proposed for GTs are based on those established for their glycoside hydrolase counterparts [1]. However, due to the lack of a consensual nucleophilic residue in the retaining GTs together with difficulties in the trapping of a covalently bound glycosyl-enzyme intermediate, the mechanistic picture for this enzyme class is still incomplete.
Mannosyl-3-phosphoglycerate synthase (MpgS; EC 2.4.1.217) belongs to the retaining GT55 family (www.cazy.org), and is involved in the synthesis of mannosylglycerate, a compatible solute that accumulates in response to salt and/or heat stresses and is widespread in hyper/thermophilic bacteria and archaea from marine environments [2-4]. The crystallographic structure of MpgS from *Thermus thermophilus* HB27 in its binary complex form, with GDP-α-D-mannose and Mg^{2+}, shows a second metal binding site about 6 Å away from the mannose moiety. Kinetic and mutagenesis studies provided evidence for the first time that this metal site plays a role in catalysis, and is most likely to be relevant for enzymatic activity in all MpgSs in the GT55 family [5]. Additionally, Asp167 in the DXD motif is found within van der Waals contact distance of the anomeric C1' atom in the mannopyranose ring, suggesting its action as a catalytic nucleophile during the glycosyl-transfer mechanism, either in the formation of a glycosyl-enzyme intermediate in light of a double-displacement S_N2-like reaction mechanism, or in the stabilization of the oxocarbenium ion-like intermediate according to the $D_N{*}A_{Nss}$ (S_Ni-like) reaction mechanism. We propose that either mechanism may occur in the retaining GTs with a GT-A fold, and based on the gathered structural information we identified an extended structural signature towards a common scaffold between the inverting and retaining GTs**.**

[1] Lairson L.L., Henrissat B., Davies G.J., Withers S.G. *Annu. Rev. Biochem*, 2008, 77, 521. [2] Empadinhas N., Marugg, J.D., Borges N., Santos,H., da Costa M.S. *J. Biol. Chem*, 2001, 276, 43580. [3] Santos H., Lamosa P., Faria T.Q., Borges N., Neves C. In: Gerday, C., and Glansdorff, N. (eds). Physiology and Biochemistry Extremophiles, ASM Press, Washington D.C. 2007. [4] Martins L.O., Empadinhas N., Marugg J.D., Miguel C., Ferreira C., da Costa M.S., Santos,H., *J. Biol. Chem*., 1999, 274, 35407. [5] Goncalves S., Borges N., Esteves, A.M.,Victor, B., Soares, C.M., Santos, H., Matias P.M. *J. Biol. Chem*, 2010, in press, doi:10.1074/jbc.M109.095976.

Keywords: mannosyl-3-phosphoglycerate synthase, glycosyltransferase, mechanism

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Structural basis for a new mode of glycosyltransferase inhibition. Rene Jørgensen[a], Thomas Pesnot[b], Monica M Palcic[a], Gerd K Wagner[b]. [a]*Carlsberg Laboratory, Copenhagen, Denmark.* [b]*School of Pharmacy, University of East Anglia, Norwich, UK.*
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In all domains of life, the biosynthesis of complex glycoconjugates requires the concerted action of a multitude of glycosyltransferases (GTs)-enzymes that catalyze the transfer of a mono- or oligosaccharide from a glycosyl donor (for example, a sugar-nucleotide) to a suitable acceptor (for example, a glycan, peptide or lipid)[1]. GTs play a key role in many fundamental biological processes underpinning human health and disease, such as cell signaling, cellular adhesion, carcinogenesis and cell wall biosynthesis in human pathogens. The development of small-molecule GT inhibitors is therefore of considerable scientific interest in chemical glycobiology and drug discovery. We have developed several new, base-modified UDP-Gal derivatives with an aromatic or heteroaromatic substituent in position 5 of the uracil base as chemical tools for the investigation of glycosyltransferases and other UDP-Gal dependent glycoprocessing enzymes. The most potent of these new derivatives act toward five different GTs, as a inhibitors of glycosyl transfer, with K_i values in the low micromolar to nanomolar range. To understand the molecular basis for the enzymological behavior of these inhibitors we chose a mutant of the ABO(H) blood group A and B glycosyltransferases which catalyze the final step in the synthesis of the A and B antigens. This mutant is a cis-AB mutant capable of transferring both Gal and GalNAc to the H-antigen with equal efficiency. We have solved high-resolution crystal structures of several of these inhibitors bound to the cis-AB mutant. Surprisingly, the inhibitors block the closure of a flexible loop in the active site by preventing the stacking of two amino acid residues where one is placed in the flexible loop and one in the C-terminus. This is a new mode of inhibition for GTs that, given the strong mechanistic similarities between many GTs, will probably also be applicable to other enzymes in this class.

[1] Weadge, J.T. & Palcic, M.M. in Wiley Encyclopedia of Chemical Biology Vol. 2 (ed. Begley, T.P.) 198–211 (Wiley, New York, 2009).
[2]Pesnot T., Jørgensen R., Palcic M.M. & Wagner G.K. Nature Chemical Biology. 2010 April 4. [Epub ahead of print]

Keywords: Glycosyltransferase, inhibitor binding, human enzymes.

FA1-MS11-P03

Chemokine Binding Protein from Orf Virus Modulates Immune Function- a new twist on an old motif Kurt L. Krause[a], Rafael Counago[a] Stephen Fleming[b], Andy Mercer[b], [a]*Biochemistry, University of Otago, Dunedin. New Zealand,* [b]*Microbiology and Immunology, University of Otago, Dunedin, New Zealand*
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Chemokine binding proteins (CBPs) are viral proteins that modulate inflammation by interfering with host chemokine signaling. CBPs bind to their cognate partner with picomolar affinity via an extended beta sandwich structure. Here we

describe the structure of a new class of CBP from the parapoxvirus, Orf virus. The crystals of this protein were challenging to produce and optimized significantly through the use of somewhat surprising additives. Crystals occupy Space Group $P6_522$ with unit cell parameters of a = b = 75.62, c = 282.49 Å, α = 90, β = 90, γ = 120°. The structure was phased using MAD methodologies and currently the 2.1Å structure is undergoing refinement. Early analysis indicates that it is a member of the β-sandwich family but it is quite distinct from other family members when superimposed. Additionally the crystal structure is consistent with a physiologic dimer and displays a very broad β sheet on its surface containing contributions from more than 10 β strands. The dimeric nature of this CBP appears to be a unique property of its class and may be key in explaining how it is able to bind different chemokines from at least two distinct chemokine classes.

Keywords: protein crystallography, chemokine , virology

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How to minimize X-ray dose used for in-house data collection on protein crystals? Vernon Smith[a], Marianna Biadene[a], Matt Benning[b]. [a]*Bruker AXS GmbH, Karlsruhe, Germany.* [b]*Bruker AXS Inc, Madison, WI, USA.*
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Datasets for *de-novo* structure solution are typically collected at synchrotron beamlines. However, with increasingly bright rotating anode generators, a significant number of datasets are collected in-house. Any exposure to X-ray radiation create free radicals inside the crystal, which can lead to decreased resolution, decreased $\langle I/\sigma(I)\rangle$, increased mosaicity and increased B-factors. It is clear that radiation damage does occur during longer data collections on rotating anode sources, even at 100 K. Unnecessary radiation damage can be avoided by using a system which maximizes detection of diffracted X-ray photons enabling more conservative incident X-ray doses to be inflicted.
The latest generation of microfocus sealed-tube sources deliver an incident X-ray beam of greater intensity than traditional rotating anode generators, but with much better beam properties and stability. Coupling with a high-sensitivity, low-noise detector creates a system (Figure 1) capable of measuring high-quality datasets while minimizing data deterioration through radiation damage.
An example will be presented comparing data from the newly developed solution with data obtained using a 'classical' rotating anode-imaging plate combination. Special attention will be paid on the X-ray dose the investigated sample receives during the data collection.

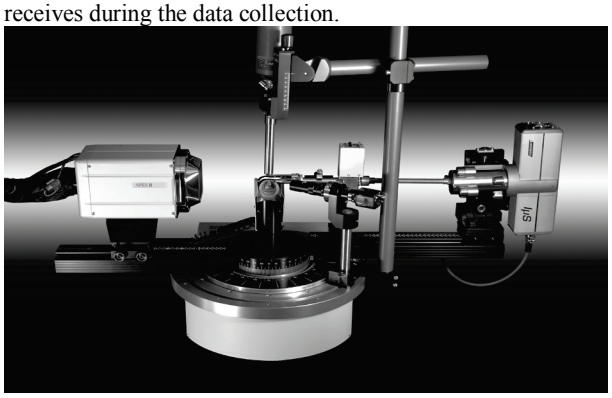

Figure 1: In-house system for X-ray dose minimization: X8 PROSPECTOR

Keywords: X-ray dose, microfocus source, crystal damage

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Crystal structure of $SppB_{TK}$, a putative signal peptide peptidase from *Thermococcus kodakaraensis.*
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Proteins secreted by the Sec-dependent pathway contain N-terminal signal peptides that are cleaved by signal peptidases following transport. The remnant signal peptides are then degraded by membrane-bound signal peptide peptidases (Spp). The crystal structure of the soluble domain of SppA from *E. coli* ($SppA_{EC}$, 67 kDa) showed that this protein consists of two domains with nearly identical structures, which assemble into a tetrameric ring [1]. *Thermococcus kodakaraensis* is a hyperthermophilic archaeon that possesses a Spp gene ($SppA_{TK}$) with approximately half the size of $SppA_{EC}$ (36 kDa) and is most homologous to the C-terminal half of $SppA_{EC}$ [2]. In addition, it also possesses another putative Spp gene (Tk0130), encoding a protein ($SppB_{TK}$) with 18% homology to $SppA_{TK}$. Biochemical data suggest that this protein functions as a signal peptide peptidase. Here, we present the crystal structure of the soluble domain of $SppB_{TK}$ in the free and substrate-bound forms. $SppB_{TK}$ structure is homologous to ATP-dependent protease ClpP and the C-terminal half of $SppA_{EC}$. It is an oligomeric protease assembled into an octameric ring. The active site of $SppB_{TK}$ consists of Ser_{130}-His_{226}-Asp_{154} triad, different from the Ser-Lys dyad of $SppA_{TK}$ and $SppA_{EC}$. Co-crystallization of S130A-$SppB_{TK}$ with a tetrapeptide substrate revealed the substrate binding mechanism of the protein. Based on these results, we discuss about the possible role of $SppB_{TK}$ in signal peptide degradation in archaea.

[1] Kim A.C., Oliver, D.C., Paetzel, M., *J. Mol. Biol.*, 2007, 376, 352.
[2] Matsumi R., Atomi H., Imanaka T., *J. Bacteriol.* 2005, 187, 7072.

Keywords: signal peptide peptidase, oligomeric proteases, structure-function proteases

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Crystal structure of AsaP1 metalloendopeptidase in complex with its propeptide. Xenia Bogdanović[a], Rajesh K. Singh[a*], Johanna Hentschke[c], Bjarnheidur K. Gudmundsdóttir[c], Winfried Hinrichs[a]. [a]*Institute for Biochemistry, Ernst-Moritz-Arndt University Greifswald, Germany* [b]*Institute for Experimental Pathology, University of Iceland, Iceland.*
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