P.04.02.11
Acta Cryst. (2005). A**61**, C183

***M. Tuberculosis* Rv0216: Unknown, Now Known?**

Alina Castell, Patrik Johansson; Kristina Bäckbro, Torsten Unge, Alwyn Jones, *Department of Cell and Molecular Biology, Uppsala University, Sweden.* E-mail: alina@xray.bmc.uu.se

Only about half of the *M. tuberculosis* genes identified as being important for *in vivo* growth have any biochemical function assigned to them. The structure of one of the unknown proteins, Rv0216, was recently determined to a resolution of 1.9Å using multiple-wavelength anomalous dispersion from a SeMet-substituted protein.

Rv0216 features two almost identical subunits packed to form a double hotdog fold with a large 10-stranded beta sheet, remotely similar to e.g. the functional dimers of bacterial and eukaryotic hydratases and dehydratase/isomerases.

A deep crevice between the two subunits is lined with residues that are highly conserved in homologous proteins within the Mycobacteriaceae family. This potential active site also shares some structural similarity to the enoyl-CoA hydratases. The counterpart to the catalytic dyad of the hydratases, consisting of a histidine and an aspartate, is in the Rv0216 case a histidine and an asparagine, suggesting a different enzymatic activity. Modelling studies indicate that Rv0216 might have a substrate similar to the crotonyl-CoA of the enoyl hydratases.

Keywords: tuberculosis, X-ray structure, hotdog fold

P.04.02.13
Acta Cryst. (2005). A**61**, C183

***E. Coli* Dihydroorotase: Loop Movement and Cooperativity**

Mihwa Lee, Camillar W. Chan, Richard I. Christopherson, J. Mitchell Guss, Megan J. Maher, *School of Molecular and Microbial Biosciences, University of Sydney, Australia.* E-mail: m.lee@mmb.usyd.edu.au

Dihydroorotase (DHOase) is a zinc metalloenzyme that catalyses the reversible cyclization of *N*-carbamyl-L-aspartate (CA-asp) to dihydroorotate (DHO) in the *de novo* pyrimidine biosynthesis. The first structure of a DHOase (from *E. coli*) has been reported to a resolution of 1.7 Å with one homodimer per asymmetric unit [1].

We have collected data from crystals of *E. coli* DHOase grown in the presence of product, DHO and refined the structure to 1.9 Å resolution [2]. As in the original report [1], we find the product DHO bound in the active site of one subunit and CA-asp in the active site of the other. Importantly, we have resolved the conformations of residues B109-112, which were disordered in the reported structure. These residues comprise a loop which takes on different orientations in the two subunits, depending on whether DHO or CA-asp is present in the active site. This is accompanied by movements of residues A/B256-258 which seem to 'communicate' between subunits the respective contents of the active sites. Subsequent kinetic analysis at low DHO concentrations shows positive cooperativity [2].

Aspects of this structure of DHOase will be discussed. In addition, the structures of inhibitor complexes and site-directed mutagenesis that allow us to understand more about this loop movement will be described in relation to the enzyme mechanism.

[1] Thoden J. B. et al., *Biochemistry*, 2001, **40**, 6989. [2] Lee M. et al., *J. Mol. Biol.*, 2005, *in press.*

Keywords: dihydroorotase, loop movement, cooperativity

P.04.02.14
Acta Cryst. (2005). A**61**, C183

Crystal Structure of a Glu-tRNA^{Gln} Amidotransferase at 2.7Å

Akiyoshi Nakamura , M. Yao, N. Sakai, Y. Tanaka, I. Tanaka, *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan.* E-mail: nakamura@castor.sci.hokudai.ac.jp

Aminoacyl-tRNA plays an important role in protein biosynthesis. The aminoacylation of tRNA is performed with 20 amino acids and the corresponding aminoacyl-tRNA synthetases (aaRSs), and aaRSs catalyze the formation of 20 aminoacyl-tRNAs in a direct amino-acylation pathway in most organisms. However, an indirect pathway is present in organisms that lack Gln-tRNA synthetase. To synthesis Gln-tRNA^{Gln} in the organisms, non-discriminating Glu-tRNA synthetase charges glutamic acid on both tRNA^{Glu} and tRNA^{Gln}, and then the mischaged Glu-tRNA^{Gln} is transamidated to Gln-tRNA^{Gln} by Glu-tRNA^{Gln} amidotransferase (Glu-AdT).[1]

Glu-AdT forms a heterotrimer composed of A, B, and C subunit (encoded by the *gatCAB* operon) in some of eubacteria and eukaryotic organelles, while archaea has a heterodimeric Glu-AdT composed of D and E subunit (encoded by the *gatD* and *gatE* genes)[2]. The detailed mechanism of the enzymes has not been clear yet, because the three-dimensional structures of the enzymes is still unknown.

We have determined the crystal structure of GatCAB complex from Gram-positive eubacteria at 2.7 Å resolution, and will discuss the moleculer mechanism of this enzymatic reaction.

[1] Alan W. *et al.*, *Proc. Natl. Acad. Sci USA*, 1997, **94**, 11819. [2] Tumbula D.L. *et al.*, *Nature*, 2000, **407**, 106.

Keywords: tRNA, aminoacyl tRNA synthetase, amidotransferase

P.04.02.15
Acta Cryst. (2005). A**61**, C183

The 1.4 Å Structure of Dianthin 30 Indicates a Role of Surface Potential at the Active Site of Type 1 Ribosome Inactivating Proteins

Simona Fermani[a], Giuseppe Falini[a], Letizia Polito[b], Andrea Bolognesi[b], [a]*Departimento di Chimica,* [b]*Dipartimento di Patologia Sperimentale, University of Bologna, Italy.* E-mail: simona.fermani@unibo.it

Ribosome inactivating proteins inhibit protein synthesis through a unique RNA *N*-glycosidase activity that depurinates major RNA. The cleaved *N*-glycosidic bond corresponds to adenine$_{4324}$ residue of 28S eukaryotic mammalian rRNA or adenine$_{2660}$ of 23S *Escherichia coli* rRNA, both located in a loop containing a GAGA sequence, highly conserved in rRNAs from bacteria, plants, and animals. This suggests that RIPs recognize this specific structure. Ribosomes invariably damaged by RIPs, cannot interact properly with elongation factors 2 with subsequent cell death (apoptosis). Although RIPs show similar chemical–physical properties and identical enzymatic activity, they act differently on ribosomes from various plants, protozoa, and animals. The three-dimensional structure of dianthin 30, a type 1 (single-chain) RIP of *Dianthus caryophyllus* (leaves), is here presented at 1.4 Å, a resolution never achieved before for any RIP. The fold typical of RIPs is conserved, despite some differences in the loop regions. The general structure comparison by superimposed α-carbon (249 atoms) and the sequence alignment by structure for dianthin 30 and saporin-S6 give a root mean square deviation of 0.625 Å. Despite the differences reported for the biological activities of the two RIPs, their structures fit quite well and both show a protein segment containing strands β7, β8, and β9 shorter than other RIPs. However, the surface electrostatic potential in the active site region neatly distinguishes dianthin 30 from saporin-S6. The possible relationship between the charge distribution and the behavior of the proteins toward different substrates is discussed. [1]

[1] Fermani S., Falini G., Ripamonti A., Polito L., Stirpe F., Bolognesi A., *Journal of Structural Biology*, 2005, **149(2)**, 204.

Keywords: ribosome-inactivating proteins, crystal structure determination, surface characterization