binding protein (SBP) component of the high-affinity manganese ABC-type transport system from the cyanobacterium *Synechocytis* sp.PCC 6803 (*Syn*) to 2.9Å by combined MAD/molecular replacement. The metal ion binding site containing Mn^{2+} has a distorted tetrahedral geometry, with Glu220 and Asp295 situated closer to the ion than His89 and His154. This geometry may be due to a disulfide bond between Cys219 and Cys268.

Sequence homology comparisons show that only putative cyanobacterial manganese SBPs contain these conserved cysteines, suggesting the MntC has a special role in manganese mobilization into the photosynthetic apparatus. We show that reduction of the disulfide bond *in vitro* releases bound manganese. We propose that *in vivo* reduction of the disulfide bond by a redox active protein, alters the position of Glu220 thereby modifying the affinity towards the bound metal. We have identified a homologous gene from the thermophilic cyanobacterium *T. vulcanus*. The final full length clone (GenBank accession code AAV65297) was sequenced and found to be 54% homologous with the *Syn mntC* and it contains the conserved cysteins. The gene was cloned into an expression vector and the expressed protein has been purified and crystallized. Preliminary ICP-MS measurements show that this protein binds Mn^{2+}, and we thus propose that this gene encodes for the MntC homolog in these species. We are now in the process of fine tuning the crystallizing conditions of this protein in order to determine its crystal structure.

Keywords: ABC transporter system, photosynthesis, redox

P.04.01.6

Acta Cryst. (2005). A**61**, C172

3D Structure Determination of the Cpn60-2 Protein from *Mycobacterium tuberculosis*

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Heat shock proteins (HSP) are a large super-family of proteins which are highly conserved throughout evolution and are necessary for the correct folding of proteins inside the cell. Cpn60-2 from *Mycobacterium tuberculosis (Mt)* belongs to the HSP60 family which is also called Chaperonins. These proteins are involved in folding of a large number of proteins in an ATP dependent manner. In addition, Cpn60-2 is one of the most immunogenic of all *Mt* proteins, eliciting a significant immune response when whole cells are used in vaccination. Due to its high immunogenity, Cpn60-2 has a medical importance.

We have isolated Cpn60-2 by over expression of the cloned gene encoding for Cpn60-2 into pQE60 vector to enable metal chelate affinity purification. The recombinant protein was shown to protect *E. coli* cells from heat shock stress. Crystals of His-Cpn60-2 grow in 2-14 days and were improved by different methodologies. The crystallization conditions are 10% 2-propanol, 20% PEG 4K, 0.1M Hepes pH 7.5. Crystallographic analysis shows the crystals to be monoclinic ($P2_1$) with unit cell parameters of a=58.460Å, b=112.209Å, c=77.5Å, β=95.482° and containing a dimer in the asymmetric unit. We have collected a complete 2.75Å data on ESRF beamline ID14-1. The structure has been solved by the molecular replacement method using a lower resolution model recently published. At present, the structure of the Cpn60-2 has been refined to R/R_{free} factors of 23.69/31.79%.

Keywords: protein crystallography chaperones, stress, bacteria

P.04.01.7

Acta Cryst. (2005). A**61**, C172

Crystal Structure of OXA-24, a Novel Class D β-lactamase with Carbapemenase Activity

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One of the main concerns in Medicine is the presence of microorganisms causing infections which harbour antibiotic resistance mechanisms. Among the different mechanisms associated with antibiotic resistance, much attention is currently being focused on the presence of β-lactamases. Oxacillinases are Ambler class D β-lactamases that possess active site serine groups like class A and class C β-lactamases. These enzymes are characterized by their hydrolytic activity for isoxazolyl, penicillins, methicillin and aztreonan significantly, sparing most extended-spectrum cephalosporins.

Six oxacillinases with carbapenem-hydrolyzing activity have been sequenced from *Acinetobacter baumannii*. OXA-24 shares 40% identity with a group of oxacillinases consisting of OXA-5, -7, -10 and -11. Despite these similarities, some interesting and differing features exist between previous oxacillinases and OXA-24. Thus, OXA-24 lacks hydrolytic activity against oxacillin, cloxacillin, and methicillin but displays a moderate level of resistance to carbapenemes. Crystals of OXA-24 from *A. baumannii* were grown using the vapour diffusion technique. They belong to space group $P4_12_12$, with cell dimensions a=b=102.2 Å, c=86.1 Å and one molecule in the asymmetric unit, which diffracted beyond 2.5 Å. It was possible to locate the position of the enzyme in the unit cell using molecular replacement with the coordinates of OXA-10 as a search model. The three dimensional structure of OXA-24 could establish the molecular basis to explain the relevance of the substitutions in its hydrolytic activity. The structure is currently undergoing refinement.

Keywords: β-lactamases, antibiotic resistance, protein crystallography

P.04.01.8

Acta Cryst. (2005). A**61**, C172

Crystallographic Studies of Human Methionine Adenosyltransferase (MAT)

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Methionine adenosyltransferase (MAT) catalyzes the formation of the key enzymatic cofactor, S-adenosylmethionine (AdoMet), from ATP and methionine. AdoMet is important because of it's involvement in various biochemical pathways including polyamine synthesis as well as the methylation of nucleic acids and lipids. MAT activity, in mammals, is regulated by a β subunit which lowers the Km of MAT for L-methionine and renders the enzyme more susceptible to feedback inhibition by AdoMet. This regulatory subunit has been modeled and is currently in crystallization trials. Furthermore, to better understand the role of the β-regulatory subunit, the three dimensional structure of the human MAT complexed to its β subunit will be determined through crystallographic studies.

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Keywords: methionine adenosyltransferase, S-adenosylmethionine, L-methionine

P.04.01.9

Acta Cryst. (2005). A**61**, C172-C173

Optimization of Crystallization of the Flavoprotein WrbA by using Additives

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Tryptophan (W)-repressor binding protein A, WrbA, is an *Escherichia coli* stationary-phase protein. Its predicted influence on the binding interaction between DNA and the tryptophan repressor (TrpR) wasn't proved [1] and thus its physiological function remains unclear. According to sequence analysis and homology modelling, WrbA was identified as the founding member of a new protein family, sharing the open, twisted α/β fold typical for flavodoxins [2]. The biochemical and biophysical studies of purified WrbA apoprotein [1]