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Carbonic anhydrase (CA) is a zinc-containing enzyme that catalyzes the reversible hydration of CO_2. Based on the amino acid sequences, CAs are classified into three evolutionarily distinct families, designated α-, β- and γ-CAs. CA from *Chlamydomonas reinhardtii* belong to the α type enzyme and, homologous to CA from animal and eubacteria source. To reveal the structure, we have been engaged on X-ray study of *Chlamydomonas* CA. The enzyme was extracted from Cultured *Chlamydomonas* cells and highly purified by column chromatography [1]. The enzyme is composed of two subunits, small and large. Crystals were obtained in a solution containing ammonium sulfate. X-ray diffraction data were collected at 100K with synchrotron radiation at NW12 of PF-AR (Tsukuba). Diffraction patterns were processed with the program HKL2000. The crystal diffracted to a maximum resolution of 2.4Å. The unit-cell dimensions were a=b=134.6 and c=120.0Å with a space group of $P6_1$ or $P6_5$. Initial phases were derived by the molecular replacement method using the atomic coordinates of CA from *Neisseria gonorrhoeae*, which has a sequence identity of 37 % with the present CA.

[1] Yang S-Y., Tsuzuki M., Miyachi S., *Plant Cell Physiol*.,1985, **26**, 25-34.

Keywords: carbonic anhydrase, *Chlamydomonas reinhardtii*, X-ray crystal structure analysis

P.04.09.2

Acta Cryst. (2005). A**61**, C227

Crystallographic Study of Fructokinase from *Sulfolobus tokodaii* strain7

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Fructokinase (FK) (ATP:D-fructose-6-phosphotransferase, EC 2.7.1.4) catalyses the phosphorylation of fructose-6-phosphate with ATP. This enzyme is essential for removing fructose to prevent the reverse reaction, in glycolytic pathway. The crystal structures of FK were reported only for prokaryote, *Bacillus subtilis* and *Salmonella enterica*. To reveal the tertiary structure of FK from an Archaea *Sulfolobus tokodaii* strain7, we have performed X-ray analyses of the protein. *Sulfolobus* FK produced in *E. coli* was purified by column chromatography, and crystallized. X-Ray diffraction data taken at 100K show that there are two forms with $P2_12_12_1$ and $P6_122$. Their initial crystal structures were derived by molecular replacement with 2-keto-3-deoxygluconate kinase from *Thermus thermophilus*, and refined to 2.8Å and 1.85Å resolutions, respectively. The two tertiary structures are similar to each other, but quite different from that of *Bacillus* FK with a little bit high sequence identity (29%). The present structures are rather similar to *Salmonella* FK though the sequence identity is low at 27%. An interesting feature is that although the function is different from *Thermus* 2-keto-3-deoxygluconate kinase, but the two tertiary structures are similar at higher sequence identity (35%).

Keywords: fructokinase, *Sulfolobus tokodaii* strain7, X-ray crystal structure analysis

P.04.09.3

Acta Cryst. (2005). A**61**, C227

Crystal Structure of Translation Initiation Factor IF2beta-IF2gamma Complex

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IF2 is a heterotrimeric GTPase composed of alpha, beta, and gamma subunit. It has been identified in both of eukaryotes and archaea. The gamma subunit is core subunit which binds Met-$tRNA_i^{Met}$ and GTP. The beta subunit was reported to interact with eIF5 that stimulates GTP hydrolysis, and also interact with Met-$tRNA_i^{Met}$ and mRNA [1,2], while alpha subunit functions as a regulatory element of IF2.

We have determined the structures of aIF2beta-aIF2gamma and aIF2beta-aIF2gamma-GDP complex

The aIF2beta subunit was located at G-domain of aIF2gamma and was close to GTP-binding site. The detail of the complex structure will be discussed and the model of ternary complex IF2-GDP-Met-$tRNA_i^{Met}$ will be given in this presentation.

[1] Das S., Maiti T., Das K., Maitra U., *J. Biol. Chem.*, 1997, **272**, 31712. [2] Laurino J. P., Thompson G. M., Pacheco E., Castilho B. A., *Mol. Cell. Biol.*, 1999, **19**, 173.

Keywords: translation, translation initiation factor, IF2

P.04.09.4

Acta Cryst. (2005). A**61**, C227

Fusidic Acid Resistance and Sensitivity in Ribosomal Elongation Factor G

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Elongation factor G (EF-G) catalyzes translocation in protein synthesis on the ribosome. EF-G is inhibited by Fusidic acid (FA), a commonly used antibiotic. Structural information on FA binding to EF-G has not yet been available.

We present three crystal structures of two mutant EF-G factors; G16V, highly FA sensitive, and T84A, highly FA resistant. The crystal structures provide a first insight into the conformational changes induced by GTP binding and how this affects FA binding. These structural conformations show the general importance of the interface of domain G, III and V as a key component of the FA binding site and the specific role of Phe90 as a gatekeeper and conformational regulator. We provide an explanation on how EF-G is able to discriminate between GDP and GTP.

Keywords: protein synthesis, translation factors, antibiotic resistance

P.04.09.5

Acta Cryst. (2005). A**61**, C227

The *thrS* Messenger Path on the Ribosome

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Initiation of translation generally determines the efficiency of protein synthesis, and is the key step for the control of gene expression when fast adaptation is required. Binding of the messenger to the eubacterial ribosome involves several mRNA elements, which affect the kinetics of the initiation complex formation. These elements may include binding sites for translational regulatory proteins. *E. coli* threonyl-tRNA synthetase (ThrRS) plays such a role, as it inhibits its own synthesis by binding to *thrS* mRNA in a region located upstream from the ribosome binding site.

Here we show the path of *thrS* mRNA on the ribosome in the presence of the initiator $tRNA^{Met}$ at 5.5 Å of resolution determined by X-ray crystallography. Our data show the first visualization of a translational regulatory domain of mRNA and explain how a repressor protein can interfere with initiation of ribosome translation.

Keywords: ribosome structure and function, tRNA synthetases, regulation

P.04.09.6

Acta Cryst. (2005). A**61**, C227-C228

Productive and Non-productive Binding of Polyketides to the Ribosome Large Subunit

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Since the early days of ribosome research, the principal reaction of protein biosynthesis was localized in the large ribosomal subunit. Protein biosynthesis may be hampered by the occlusion of the exit tunnel, through which proteins emerge. This tunnel has a non-uniform diameter and contains grooves and cavities [1]. Crystal structures of complexes of the large ribosomal subunit from the eubacterium *Deinococcus radiodurans* with various polyketides (troleandomycin, telithromycin, rapamycin)[2,3] have shown that the exit tunnel is able to bind them with different fashions and that only some of those are capable to induce protein inactivation. We show that, among the three polyketides here analysed, rapamycin binds to a tunnel crevice that is located aside the typical macrolide-binding pocket and cannot occlude the exit tunnel. These structural results constitute the first example of a non-inactivating binding to the ribosome, thus suggesting that a necessary requirement for efficient antibiotic activity of macrolide-like compounds is their binding to the ribosome exit tunnel, in a manner that efficiently blocks the tunnel. Implications of polyketides binding to the ribosome large subunit will be discussed in the poster.

[1] Harms J., Schluenzen F., Zarivach R., Bashan A., Agmon I., Bartels H., Franceschi F., Yonath A., *Cell*, 2001, 679. [2] Berisio R., Harms J., Schluenzen F., Zarivach R., Hansen H., Fucini P., Yonath A., *J. Bacter.*, 2003, 4276. [3] Berisio R., Schluenzen F., Harms J., Bashan A., Auerbach T., Baram D., Yonath A., *Nat. Struct. Biol*., 2003, 366. [4] Amit M., Berisio R., Baram D., Harms J., Bashan A., Yonath A., *submitted*.

Keywords: ribosome, antibiotic, exit tunnel

P.04.09.7

Acta Cryst. (2005). A**61**, C228

Crystal Structure of a Conserved Hypothetical Protein TT1657 from *Thermus thermophilus* HB8

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Thermus thermophilus HB8 is a good model to study the structure-function relationships. Because its proteins show high thermostablility, it is easier to solve their tertiary structures and the collection of structures will give sufficient information to study the structure-function relationship comparatively.

Here, we studied one of the *Thermus thermophilus* hypothetical proteins, TT1657, by the X-ray crystallography. The crystal structure was solved by the Se-MAD method. This protein forms dimer in the crystal and it is consistent with the result of the gel filtration experiment. Although BLAST search indicated that this protein has higher homology with some hypothetical proteins and weaker homology with phosphoesterases and phosphatases, DALI search shows high structural homology with some of phosphatases.

Keywords: phosphatases, thermostable proteins, crystal structures

P.04.09.8

Acta Cryst. (2005). A**61**, C228

Structural Characterization of Archaeal Elongation Factors

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Elongation factors (EF) are enzymes that play a major role in protein biosynthesis. However, limited structural information is available on elongation factors isolated from archaea/eukarya [1,2]. We have undertaken structural studies on elongation factors isolated form the hyperthermophilic archaeon *Sulfolobus solfataricus*. The interest for these proteins is twofold. Indeed, they represent a valuable system to investigate structure/function relationships in archaeal/ eukaryal elongation factors and to study structure/stability correlations. Our previous investigations have provided insight into the function of *Ss*EF-1αand into the role played by the magnesium in the nucleotide exchange process. Our data also provided a preliminary mechanism for the exchange process in EF-1α̃ In order to better define this mechanism, we are currently performing structural investigations on the exchange factor *Ss*EF-1β̃Native and MAD data have been collected and the solution of the structure is in progress. Furthermore, the complex between *Ss*EF-1α and ¸ *Ss*EF-1β has been prepared for crystallographic investigations. Finally, a combined analysis by CD spectroscopy and molecular modeling has contributed to highlight the structural determinants of *Ss*EF-1αthermostability.

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Keywords: elongation cycle, protein biosynthesis, thermostability

P.04.09.9

Acta Cryst. (2005). A**61**, C228

Engineering the Substrate Specificity and Catalysis from Crystal Structures of the Beta-subunit of Acyl-CoA Carboxylase

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The carboxylation of acyl-Coenzyme A is one of the key regulation checkpoints for the biosynthesis of fatty acids and polyketides. Acetyl-Co A carboxylases (ACC) and propionyl-CoA carboxylase (PCC) catalyze the carboxylation of acetyl- and propionyl-CoAs to generate malonyl- and methylmalonyl-CoA, respectively. Inhibitors of the ACCases have been identified as potential therapeutics for cancer and obesity, as well as herbicides and antibiotics. The crystal structures of the carboxyltransferase domain, AccB and PccB in *S. coelicolor*, are hexamers [1] that assemble into a ring shaped complex. The biotin-binding pocket has been identified where biotin and propionyl-CoA bind perpendicular to each other and are highly hydrophobic. Mutagenesis and kinetics studies of PccB and AccB allowed interconversion of their corresponding substrate specificity for acetyl-CoA, propionyl-CoA and butyl-CoA. The mutants structures show that dimer interaction is essential for enzyme catalysis, stability, and substrate specificity, which is highly conserved among biotin-dependent carboxyltransferases. ACCase mutants with relaxed substrate specificity can provide novel extender units, which can be fed into the polyketide biosynthesis pathway to generate "unnatural" natural products.

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Keywords: carboxylase, X-ray structure determination, fatty acid biosynthesis

P.04.10.1

Acta Cryst. (2005). A**61**, C228-C229

The Effects of Temperature and Radiation on Holo and Apo Ferritin Crystals

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It is known that both temperature and radiation dose induce expansion of the unit cell of cryocooled macromolecular crystals [1]. Dose-induced increases are not thought to be caused by temperature changes in the crystal. We have investigated the nature of the dose and temperature induced unit cell expansion.

A series of datasets were collected at SRS Daresbury and ID14-4 at ESRF Grenoble on crystals of apo and holo ferritin at 100K. Further sets were collected on a second crystal of each type, but this time over