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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.

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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

a controlled temperature series. By comparing the results from the two crystals, effects of radiation damage could be distinguished from those of temperature, and differences in behavior of the two forms of ferritin were also examined.

As has been previously reported [2, 3], it was found that the increase in unit cell dimensions is linear with dose. Our results show that the irreversible effects of dose could be distinguished from the reversible temperature-induced effects.

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Keywords: ferritin, radiation damage, thermal cycling

P.04.10.2

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

Mitigation of Radiation Damage to Protein Crystals using a Helium Cryostream

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Experiments at APS Beamline 14BM-C (BioCARS) with D-xylose isomerase crystals tested the effect of cryogen temperature in minimizing radiation damage at synchrotron beamlines. Data were collected using cryogenic helium (Pinkerton Device) and nitrogen (Oxford Industries CryoJet). Helium data were collected at 15 K, 50 K and 100 K on similar quality crystals. Nitrogen data were collected at 100 K. Multiple crystals were used at each temperature. Initial data were collected on each crystal followed by a 10 minute exposure. Data collection and exposure cycles were completed for at least 6 cycles. Crystal statistics showed significant reduction in radiation damage at the lowest data collection temperatures (15 K helium stream). In particular, the signal-to-noise estimate, $I/\sigma(I)$, of the highest resolution shell showed progressively less deterioration as temperature of the cold stream decreased. The average decay in $I/\sigma(I)$ for the highest resolution shell was 52 % for 15 K crystals, 63 % for 50 K crystals and 75 % for 100 K crystals. The results indicate that manifestations of radiation damage appear less rapidly at lower temperatures and the effects of radiation damage can be partially mitigated at very low temperatures particularly for high resolution data.

Keywords: radiation damage, synchrotron, temperature

P.04.10.3

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

High Pressure Cooling of Protein Crystals without Cryoprotectants

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The flash cooling of protein crystals is the best known method to effectively mitigate radiation damage in macromolecular crystallography. To prevent physical damage to crystals upon cooling, suitable cyroprotectants must usually be found, a process that is time-consuming and, in certain cases unsuccessful. Recently we have developed a novel method to cryocool protein crystals without the need for penetrative cryoprotectants. In the new method, each protein crystal is pressurized up to 200 MPa (2000 atm) in He gas at 10 ˚C. The crystal is then cyrocooled under pressure and the pressure was released while the crystal is kept cooled at 77 K. Results are presented for two proteins that have been flash-cooled at ambient pressure and pressure-cooled, in all case without penetrating cryoprotectants. For glucose isomerase, the flash-cooled crystal diffracted to only 5.0 Å and mosaicity could not be estimated but the pressure-cooled one diffracted to 1.05 Å with 0.39˚ mosaicity. For thaumatin, the flash-cooled crystal diffracted to only 1.8 Å with 1.29˚ mosaicity but the pressure-cooled one diffracted to 1.15 Å with 0.11˚ mosaicity. The protein structures show that the structural perturbation by pressure is at the level of a few tenths of an angstrom, which is comparable to the typical structural changes always observed upon flash cooling at ambient pressure. A mechanism on the pressure cooling is proposed involving the dynamics of water at high pressure and high density amorphous (HDA) ice.

Keywords: high pressure cooling, cryocrystallography, crystallography of biological macromolecules

P.04.11.1

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

The Crystal Structures of HcpB and –C: Two Proteins with Sel1-like Repeat Architectures Involved in the Modulation of Innate Immune Response

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Many epsilon-proteobacteria, such as *Helicobacter pylori*, *Campylobacter jejuni* and *Wollinella succinogenes*, settle in the stomachs of higher organisms, requiring proteins to resist the harsh conditions of this ecological niche. The family of Helicobacter cysteine-rich proteins (Hcp) was identified using the method of comparative genome analysis and seems to be specific for this class of microorganisms. So far this family of secreted proteins was lacking a functional and structural description. Using recombinant HcpA, -B, -C and –E we identified high anti-Hcp antibody titers in the sera of *H. pylori* positive patients, confirming that these proteins are expressed *in* vivo [1]. The crystal structures of HcpB and –C were refined at 2 Å resolution and serve as the prototype structures for the large family of Sel1-like repeat proteins [2, 3]. The structure of HcpC reveals a peptide-binding mode that is strikingly similar to TPR proteins suggesting that Hcp proteins might be involved in protein-protein interactions. This hypothesis is corroborated by the secretion of high IFN-gamma and IL12 levels from naïve mouse splenocytes upon addition of recombinant HcpA [4], indicating an implication in the modulation of innate immune response and survival of *H. pylori* in the human stomach.

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Keywords: innate immune response, structural genomics, protein-protein interactions

P.04.11.2

Acta Cryst. (2005). A**61**, C229-C230

Structural Basis for Blue and Purple Fluorescence of Antibody-stilbene Complexes

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A panel of antibodies was generated against *trans*-stilbene[1] in order to explore the influence of protein environment on the excited electronic states of a chromophore. When irradiated by UV-light, stilbene readily undergoes photochemical *trans/cis*-isomerization and exhibits only weak fluorescence. In presence of these antibodies however, the electronically-excited stilbene affords strong fluorescence which is likely the result of precluding isomerization in the antibody pocket due to tight binding of the stilbene. Interestingly, antibody 19G2 exhibits the largest red-shift and a tenfold increase in fluorescence lifetime compared to the other purple-fluorescent antibodies.

Crystal structures of both blue (19G2) and purple (25C10) fluorescent antibodies in complex with stilbene have been determined to elucidate their different fluorescence properties and the mechanism of spectral tuning. In combination with biochemical and spectroscopic techniques, we are probing the unusually strong fluorescence of 19G2 compared to 25C10.

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