P.04.15.34

Acta Cryst. (2005). A**61**, C249

X-ray Structure for an RNase H Inhibitor Bound to HIV-1 Reverse Transcriptase

Daniel M. Himmel[1], Stefan G. Sarafianos[1], Sanjeewa Dharmasena[2], Mofazzal Hossain[2], Kessler McCoy-Simandle[2], Patrick K. Clark[3], Arthur D. Clark, Jr.[1], Jennifer L. Knight[4], Ronald M. Levy[4], Stephen H. Hughes[3], Michael A. Parniak[2], Eddy Arnold[1], *[1]Center for Advanced Biotechnology and Medicine (CABM), Rutgers University, USA. [2]University of Pittsburgh, School of Medicine, USA. [3]NCI-Frederick Cancer Research and Development Center, USA. [4]Department of Chemistry and Chemical Biology and BIOMAPS Institute for Quantitative Biology, Rutgers University, USA*. E-mail: himmel@cabm.rutgers.edu

We have determined a 3.0 Å resolution X-ray crystal structure of HIV-1 reverse transcriptase (RT) complexed with DHBNH, an RNase H inhibitor (RNHI). HIV-1 RT uses two enzymatic activites, a polymerase and an RNase H, to convert the viral genomic single-stranded RNA into double-stranded DNA suitable for integration into the host genome [1]. RNase H is essential for virus replication; however, very few small molecule inhibitors targeting this function have been reported, and there are no crystal structures of HIV RT in a complex with an RNase H inhibitor. DHBNH is an N-acyl hydrazone derivative that inhibits RNase H with an IC_{50} of 0.5 μM but does not inhibit the RT polymerase (IC_{50}>20 μM). Despite this specificity, the inhibitor binds more than 40 Å away from the RNase H active site, at a novel binding site in the palm of the p66 subunit, between the primer grip and the polymerase active site. The inhibitor partially overlaps the non-nucleoside RT inhibitor (NNRTI) binding pocket. The inhibitor appears to interact with the conserved residues Asp186 and Trp229, as well as with Tyr188, Lys223, Asp224, Pro226, Phe227, and Leu228. Certain substitutions on DHBNH can enhance interactions in the NNRTI binding pocket, resulting in "dual inhibitors" that inhibit both the polymerase and RNase H activities of HIV-1 RT. Our results are consistent with the view that binding of DHBNH alters the trajectory of the nucleic acid substrate, affecting the RNase H activity. Knowledge gained from this study provides new opportunities for structure-based drug design.

[1] Coffin J. M., Hughes S. H., Varmus H. E., *Retroviruses*, Cold Spring Harbor Laboratory, Plainview, NY., 1997.

Keywords: HIV-1 reverse transcriptase, RNase H inhibitor, rational inhibitor design

P.04.15.35

Acta Cryst. (2005). A**61**, C249

Crystal Structure of the NAD Kinase from *Listeria monocytogenes*

Gilles Labesse[a], Guillaume Poncet-Montange[a], Liliane Assairi[b], Emmanuel Margeat[a], Stefan Arold[a], Anne-Marie Gilles[b], *[a]Centre de Biochimie Structurale, Montpellier, France. [b]Institut Pasteur, Paris, France.* E-mail: labesse@cbs.cnrs.fr

The NAD kinase is involved in the essential step for the biosynthesis of NADP the dinucleotidic cofactor for numerous enzymes. The NAD kinases was previously proposed to share the ATP-binding site of phosphofructokinases despite important sequence divergence [1]. The first crystal structure of a NAD kinases was recently solved [2]. We present, here, the structure of one of the two NAD kinases from *Listeria monocytogenes*, a food-borne human pathogen. The crystal structure, refined at 2.4 A resolution, reveals the conserved tetrameric structure of NAD kinases in agreeement with its oligomeric state in solution. Co-crystallization as well as complementary biophysical characterizations (SAXS, ...) in presence of the ligands are currently undertaken in order to analyse putative conformation changes. Meanwhile, the structure allows virtual screening in order to identify potential inhibitors.

[1] Labesse G., Douguet D., Assairi L, Gilles A.M., *TiBS*, 2002, 273-5. [2] Garavaglia S., Raffaelli N., Finaurini L., Magni G., Rizzi M., *J. Biol. Chem.*, 2004, 40980-6.

Keywords: protein crystallography, rational drug design, comparative modelling

P.04.16.1

Acta Cryst. (2005). A**61**, C249

Identical Sets of Residues Produce Two Strikingly Different Dimers in the NF-κB Family of Proteins

De-bin Huang, Don Vu, Gourisankar Ghosh, *Department of Chem. & Biochem. UC-San Diego, USA*. E-mail: dehuang@chem.ucsd.edu.

The proteins of the Nuclear Factor-kappaB (NF- κB) family proteins are important transcription factors that regulate the expression of genes involved in immune and inflammatory response and apoptosis. There are five known NF-κB proteins, p50(NF-κB1), p52(NF-κB2), p65(RelA), c-Rel and RelB, that exist as homo- and heterodimers. Unlike other family members that form all possible functional combinatorial dimers, RelB forms heterodimers with only p100/p52 and p105/p50. The X-ray crystal structure of the RelB dimerization domain (DD) alone, and in complex with p52 DD have been determined. This reveals that RelB/p52 DD heterodimer forms a "regular" dimer similar to other NF-κB dimers, unlike RelB DD which forms an intertwined homodimer. We have shown that RelB forms an intertwined homodimer in solution as well. The residues that are critical in NF-κB dimer formation are invariant in RelB, however, a solvent exposed hydrophobic patch destabilizes the RelB domain fold, a feature that is also essential for its association with p52. We propose that the intertwined unstable RelB homodimer may serves as an intermediate to before converting into highly stable heterodimers with p105/p50 or p100/ p52.

Keywords: transcription factor structures, NF-kB proteins, residues

P.04.16.2

Acta Cryst. (2005). A**61**, C249

Crystal structure of the *Actinomadura* R39 DD-peptidase (PBP4)

Eric Sauvage, Paulette Charlier, *Centre d'Ingénierie des Protéines, Université de Liège, Institut de Physique B5,B-4000 Liège, Belgium*. E-mail: eric.sauvage@ulg.ac.be

Actinomadura sp. R39 produces an exocellular DD-peptidase penicillin-binding protein (PBP) whose primary structure is similar to *Escherichia coli* PBP4. It is characterized by a high beta-lactam-binding activity (second order rate constant for the acylation of the active site serine by benzylpenicillin: k_2/K = 300 mM^{-1} s^{-1}). The crystal structure of the DD-peptidase from *Actinomadura* R39 was solved at a resolution of 1.8 Å by single anomalous dispersion at the cobalt resonance wavelength. The structure is composed of three domains: a penicillin-binding domain similar to the penicillin-binding domain of *E. coli* PBP5 and two domains of unknown function. In most multimodular PBPs, additional domains are generally located at the C- or N-termini of the penicillin-binding domain. In R39, the other two domains are inserted in the penicillin-binding domain, between the SXXK and SXN motifs, in the way of "Matryoshka dolls". One of these domains is composed of a five-stranded beta-sheet with two helices on one side and the other domain is a double three-stranded beta-sheet inserted in the previous domain. Additionally, the 2.4 Å structure of the acyl-enzyme complex of R39 with nitrocefin reveals the absence of active site conformational change upon binding the beta-lactams.

Keywords: peptidoglycan biosynthesis, carboxypeptidase, penicillin-binding protein

P.04.16.3

Acta Cryst. (2005). A**61**, C249-C250

Sulfur SAD Structure of Heparin-Binding CRISP from *Naja atra* Reveals Protease and Ion Channel Blocking Domains

Chun-Jung Chen[a,b,c], Yu-Ling Wang[ab], Shao-Chen Lee[b], King-Siang Goh[b], Wei-Ning Huang[d], Wen-guey Wu[b], *[a]Biology Group, National Synchrotron Radiation Research Center. [b]Department of Life Sciences & Structural Biology Program. [c]Department of Physics, National Tsing-Hua University. [d]Department of Medical Technology, Yuanpei University, Hsinchu, Taiwan.* E-mail: cjchen@nsrrc.org.tw

Various cysteine-rich secretory proteins (CRISP) have been identified in diverse organisms with conserved sequences, including

16 of their cysteines. Although no clear evidence exists for a physiological function of mammalian CRISP found mainly in the epididymis and salivary glands, snake venom CRISP are known to inhibit smooth muscle contraction and cyclic nucleotide-gated (CNG) ion channels. The structure of CRISP-*a* from *Naja atra* is determined at 1.58-Å resolution using the sulfur-SAD method and consists of unique disulfide patterns and two distinct structural domains: a protease sandwich fold (N-terminal) and an ion channel-blocking BgK toxin fold (C-terminal). With one positively charged cluster found at water accessible helix regions next to the Ser-His-Glu triad of the protease domain, heparin binding plays a role in regulating CRISP-*a* activity. As important residues identified to block CNG and K^+ channels of other toxin homologues are located at one face of the ion channel-blocking domain, the structure provides a basis for rational design of a peptide blocker of the CNG channel. The ion channel-blocking domain and heparin-binding site of CRISP-*a* are suggested to play a chaperone role in leading it to the site of protease action for remodeling of the extracellular matrix in mammalian cells.

Keywords: sulfur-SAD phasing, toxin CRISP structure, heparin

P.04.16.4

Acta Cryst. (2005). A**61**, C250

Structure of Parasporin-1, a Novel Bacterial Cytotoxin against Human Cancer Cells

Toshihiko Akiba[a], Tokio Ichimatsu[b], Hideki Katayama[b], Tetsuyuki Akao[b], Osamu Nakamura[b], Eiichi Mizuki[b], Michio Ohba[c], Kazuaki Harata[a], *[a]BIRC, AIST, Tsukuba. [b]BFRI, FITC, Kurume. [c]Grad. Sch. Agric., Kyushu Univ., Fukuoka, Japan.* E-mail: k-harata@aist.go.jp

The crystal structure of parasporin-1 from *Bacillus thuringiensis* strain A1190 has been determined at 1.76 Å resolution. Parasporin-1 belongs to the Cry protein family, which includes insecticidal pore-forming toxins successfully utilized in agriculture; however, the protein is not insecticidal but specifically toxic to particular types of cultured human carcinoma cells. This strict selectivity suggests its potential use as an anti-cancer drug.

Parasporin-1 has a three-domain architecture common to available structures of other insecticidal Cry proteins; the main chain of each domain is superimposed reasonably well with their counterparts in spite of low sequence homology. Significant deviations are found in a few limited regions. Of particular interest is the N-terminal extension upstream of domain 1, which clamps the domain to domain 2 and which presumably disable the transformation of the domain necessary for pore formation. Among the available Cry protein structures, only the inactive Cry2Aa protoxin has an analogous structure. These observations along with biochemical results [1] suggest that parasporin-1 may act as a simple ligand to activate an unidentified signaling pathway leading to malfunction of membrane channels rather than as a pore-forming toxin.

[1] Katayama H., et al., *J. Biochem.*, 2005, **137**, 17.

Keywords: pore-forming toxins, anticancer biochemistry, receptor recognition

P.04.16.5

Acta Cryst. (2005). A**61**, C250

Structure of Diol Dehydratase Reactivating Factor – A Novel Molecular Chaperone

Naoki Shibata[a], Koichi Mori[b], Naoki Hieda[b], Mamoru Yamanishi[b], Yoshiki Higuchi[a], Tetsuo Toraya[b], *[a]Graduate School of Science, University of Hyogo, Japan. [b]Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Japan.* E-mail: shibach@sci.u-hyogo.ac.jp

Diol dehydratase and glycerol dehydratase are adenosylcobalamin-dependent enzymes that catalyze the conversion of 1,2-propanediol, 1,2-ethanediol and glycerol to the corresponding aldehydes. Glycerol, a physiological substrate for the enzyme, inactivates the enzyme in an irreversible manner. Diol dehydratase reactivating factor is a molecular chaperone, reactivating the inactivated diol- and glycerol dehydratases in the presence of AdoCbl, ATP and Mg^{2+}. Here we report the crystal structures of ADP-bound and nucleotide-free forms of diol dehydratase reactivating factor.

Initial electron density map of the selenomethionine-substituted ADP-bound form was obtained from the MAD diffraction data collected at the BL38B1 beam line, SPring-8, Japan. Diffraction data sets for native ADP-bound and nucleotide-free crystals were collected at the BL41XU beam line, SPring-8, Japan.

Structure of nucleotide-free diol dehydratase reactivating factor is similar to that of nucleotide-free glycerol dehydratase reactivating factor reported by Liao *et al*. [1]. The ADP-bound form of diol dehydratase reactivating factor shows rearrangement of domains with respect to its nucleotide-free form.

[1] Liao, et al., *Structure*, 2003, **11**, 109.

Keywords: diol dehydratase reactivating factor, molecular chaperone, crystal structure

P.04.16.6

Acta Cryst. (2005). A**61**, C250

Structural and Functional Analysis of PDI–related Proteins

Madhumati Sevvana[a], Qingjun Ma[a], Kathrin Barnewitz[b], Chaoshe Guo[b], Hans-Dieter Söling[b], David M. Ferrari[b], George M. Sheldrick[a], *[a]Lehrstuhl für Strukturchemie, University of Göttingen. Department of Neurobiology, [b]MPI, Göttingen, Germany.* E-mail: msevvana@shelx.uni-ac.gwdg.de

Protein Disulfide Isomerase[PDI]–related proteins are residents of the endoplasmic reticulum and are involved in several functions, some of which include redox and chaperone activities. Their function involves several non-covalent weak interactions with specific epitopes on substrate proteins. The molecular basis of these interactions has not been understood until recently [2].

We recently elucidated the first crystal structure of such a eukaryotic PDI–related chaperone, Wind from Drosophila [1]. It has been identified that Wind binds Pipe (a 2-*O*-sulfotransferase) *in vitro*. A putative peptide binding site has been mapped on the b'-domain for substrate binding with the requirement of the integrity of a surface on the d'-domain. Crystal structures of several Wind–mutants and their complexes with the peptides mimicking the Pipe binding site were elucidated giving some clues about the binding mechanism. Further, the structure of a mammalian orthologue of Wind, Erp28 has been solved, suggesting a functional role for the structural conservation between the proteins.

[1] Ma Q., Guo C., Barnewitz K., Sheldrick G. M., Söling H. D., Uson I., Ferrari D. M., *JBC*, 2003, **278**, 44600. [2] Barnewitz K., Guo C., Sevvana M., Ma Q., Sheldrick G. M., Söling H. D., Ferrari D. M., *JBC*, 2004, **279**, 39829.

Keywords: chaperone, protein disulfide isomerase, Wind

P.04.16.7

Acta Cryst. (2005). A**61**, C250-C251

TPR Repeat Domain of O-linked GlcNAc Transferase: Similarities to Importin Alpha

Martin Jinek[a], Jan Rehwinkel[a], Brooke Lazarus[b], Elisa Izaurralde[a], John A. Hanover[b], Elena Conti[a]. *[a]European Molecular Biology Laboratory, Heidelberg, Germany. [b]National Institute of Diabetes and Digestive and Kidney Disease, NIH, Bethesda, USA.* E-mail: jinek@embl.de

Addition of N-acetylglucosamine (GlcNAc) is a ubiquitous form of intracellular glycosylation, catalyzed by the conserved O-linked GlcNAc transferase (OGT). OGT contains an N-terminal domain of tetratricopeptide (TPR) repeats that mediates the recognition of a broad range of target proteins. Nuclear pore complex components are major OGT targets, as OGT depletion by RNAi results in the loss of GlcNAc modification at the nuclear envelope. To gain insight into the mechanism of target recognition, we solved the crystal structure of the homodimeric TPR domain of human OGT, containing 11.5 TPR repeats[1]. The repeats form an elongated superhelix. The concave surface of the superhelix is lined by absolutely conserved asparagine residues, in a manner reminiscent of the peptide-binding site of importin α. Based on this structural similarity, we propose that OGT employs an analogous molecular mechanism to recognize its targets.