Keywords: nucleoside metabolism, halophilic enzymes, quaternary association of proteins

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Structure of vault purified from rat liver

Koji Kato[1], Hideaki Tanaka[1], Tomoyuki Sumizawa[2], Eiki Yamashita[1], Masato Yoshimura[3], Yong Zhou[4], Min Yao[4], Isao Tanaka[4], Kenji Iwasaki[1], Tomitake Tsukihara[1]

[1]Osaka University, Institute for Protein Research, 3-2 Yamadaoka, Suita, Osaka, 565-0871, Japan, [2]University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi, Kitakyushu, 807-8555, Japan, [3]Taiwan Beamline Office at SPring-8 1-1-1 Kouto,Sayo-cho, Sayo-gun, Hyogo, Japan, [4]Hokkaido University, Sapporo, 060-0810, Japan, E-mail：k-kato@protein.osaka-u.ac.jp

Vault is a 12.9-MDa ribonucleoprotein particle with a barrel-like shape that is highly conserved in a wide variety of eukaryotes. Multiple copies of two additional proteins, vault poly(ADP-ribose) polymerase and telomerase-associated protein 1 , as well as a small vault RNA are also associated with vaults. (Kedersha and Rome, Kickhoefer et al., Michael P. Kowalski et al.). The vault crystals belong to space group *C*2 with unit-cell parameters a = 708.0 Å, b = 385.0 Å, c = 602.9 Å, β = 124.8°. Rotational symmetry searches based on the R factor and correlation coefficient from non-crystallographic symmetry (NCS) averaging indicated that the particle has 39-fold dihedral symmetry (Kato et al.). Electron cryo-microscopy electron density was used as the starting model for phase improvement and phase extension. A model of the MVP was composed of 9 β-sheet domains, shoulder domain and cap long helix. C terminal regions form a intermolecular pseudo β-sheet ring and a intermolecular pseudo α helix ring, which may be initiation structures of oligomerization of MVPs.

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Keywords: X-ray crystallography of biological macromolecules, crystallography of biological macromolecules, large molecular assemblies

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Macromolecular X-ray powder diffraction from the *in vivo* arm photophore of firefly squid

Keiko Miura[1], Katsuaki Inoue[1], Masatsugu Seidou[2], Toshiaki Hamanaka[3]

[1]Japan Synchrotron Radiation Research Institute, Industrial Application Division, 1-1-1 Kouto, Sayo, Sayo-gun, Hyogo, 679-5198, Japan, [2]Aichi Prefecture Univ. of Fine Arts and Music,1-114 Mitsugamine,Nagakute, Aichi-gun, Aichi,480-1194, Japan, [3]Osaka Univ.Grad. School of Engineering Science,1-1 Machikaneyama, Toyonaka, Osaka, 560-0043, Japan, E-mail：miurakk@spring8.or.jp

The firefly squid, Watasenia scintillans, emits brilliant flashes of light from three tiny luminous organs which are located at the tip of each of the fourth pair of arms. The notable feature of the histology of the brachial organs is that they contain numerous rod-like bodies, which are 2.5-5 micrometer long and 1-3 micrometer thick, of protein assembly. Previously, its X-ray diffraction pattern was determined by directly irradiating a photophore at the tip of the fourth arms and showed numerous sharp reflections. It suggested that the rod-like bodies were made by micron-sized macromolecular crystal. X-ray diffraction data of the rod-like bodies extracted from the photophore were collected at room temperature at BL40B2/SPring-8 using its SAXS system with camera distance of 2111 mm and imaging plate system. The powder diffraction pattern was also observed from the extracted rod-like bodies, up to the resolution of 15 Å spacing with 5 minutes exposure. Indexing of the diffraction rings was done by using software McMaille v.3.04 (A. Le Bail,2004) and suggested that the micro-crystal belonged to an orthorhombic space group $P2_12_12_1$ with unit cell dimensions a=348 Å, b=195 Å, c=214 Å. The microscopic observation showed that the micro-crystal emitted a greenish fluorescent light by the excitation with 400-410 nm light. Since the luciferase of Watasenia is membrane-bound, the micro-crystal may be the storage of the luciferin binding protein. These results show that there is a unique macromolecular assembly in intact organs with the crystallographic manner and it is expected to be determined its crystallographic structure in the future.

Keywords: powder X-ray diffraction, protein crystallography applications, macromolecular assemblies

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Designing selective inhibitors to target NagZ a family 3 glycoside hydrolase

Misty D Balcewich[1], Terry James[1], Keith Stubbs[2], David Vocadlo[2], Brian Mark[1]

[1]University of Manitoba, Microbiology, 79 Freedman Cres, Winnipeg, Manitoba, R2T2N2, Canada, [2]Simon Frasier University, 8888 University Drive, Burnaby,BC, Canada, V5A 1S6, E-mail：m_balcewich@umanitoba.ca

NagZ is a family 3 beta-glucosaminidase involved in remodeling of the bacterial cell wall. It removes terminal N-acetylglucosamine residues from internalized cell wall degradation intermediates that are subsequently recycled back into the cell wall during biosynthesis. In the presence of beta-lactam antibiotics however, the NagZ product 1,6-anhydroMurNAc-oligopeptide accumulates in the bacterial cytosol to levels sufficient to induce the expression of the ampC beta-lactamase gene through direct activation of the AmpR transcriptional activator. Previously, the crystal structure of Vibrio cholerae NagZ in complex with PUGNAc, a potent and fairly selective inhibitor was determined to 1.7Å. This structure revealed a large open pocket beneath the 2-acetamido methyl group of the inhibitor. This is in contrast with the architecture of the human family 20 hexosaminidases and family 84 O-GlcNAcases. Family 20 and 84 enzymes form a tight envelope around the 2-acetamido group, holding it in position to participate as a nucleophile during catalysis. This structural difference suggests that extensions off the 2-acetamido group of PUGNAc would confer high inhibitor selectivity toward NagZ. Subsequent synthesis of derivatives two N-butylPUGNAc and Valeryl-PUGNAc possessing modifications to this region have been chosen for crystallographic analysis. These derivatives are potent and selective for NagZ. We present a detailed structural comparison of the family 3 NagZ:N-butylPUGNAc complex (to 2.5Å) with human family 20 and 84 glycosidase crystal

structures and discuss further design strategies for highly selective family 3 glycoside hydrolase inhibitors.

Keywords: glycosyl hydrolases, antibiotic resistance, structure-based drug design

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The crystal structure of AKR1C1 in complex with an active-site inhibitor

Urmi Dhagat[1], Satoshi Endo[2], Akira Hara[2], Ossama El-Kabbani[1]
[1]Monash University, Medicinal Chemistry, 381 Royal Parade, Parkville, Melbourne, Victoria, 3052, Australia, [2]Laboratory of Biochemistry, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan, E-mail：urmi.dhagat@vcp.monash.edu.au

Hydroxysteroid dehydrogenases (HSDs) regulate a wide range of physiological processes including reproduction, development and homeostasis. AKR1C1, is a 20α-HSD involved in the conversion of progesterone to 20-hydroxyprogesterone. Increased activity of AKR1C1 in the endometrium and in breast tissues leads to the formation of tumor-promoting metabolites and to the development of endometriosis, breast cancer and endometrial cancer. At present, there are few known inhibitors that specifically bind and inhibit the adverse actions of AKR1C1. Here we present the first crystal structure of AKR1C1 in complex with potent inhibitor 3,5-dichlorosalicylic acid (IC_{50} = 44 nM). The crystal structure was solved at a resolution of 1.8 Å, with clear electron density corresponding to the inhibitor bound in the active site. The details of the enzyme-inhibitor interactions and selectivity against members of the AKR1C subfamily will also be discussed. The structural information obtained from this study will help speed up the drug design process for the development of more selective and potent compounds that can be used in the treatment of endometriosis and cancer.

Keywords: aldo-keto reductases, enzyme inhibitors, drug design

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Eimeria tenella lactate dehydrogenase as a target for anti-parasitics

Victoria Fairweather[1,2], Robert Schwarzenbacher[2], Leo Brady[1]
[1]University of Bristol, Department of Biochemistry, School of Medical Sciences, University Walk, Bristol, Bristol, BS8 1TD, UK, [2]University of Salzburg, Department of Molecular Biology, Billrothstr. 11, 5020 Salzburg, Austria, E-mail：v.fairweather@bristol.ac.uk

Eimeria species are parasitic Apicomplexa protozoans that cause gastrointestinal coccidiosis infections in birds, and are associated with general morbidity and intestinal lesions. These parasitic infections lead to major losses within the broiler industry, a situation that is deteriorating due to emerging resistance to available therapeutics. By analogy with other Apicomplexa obligate parasites, in the intracellular stages of its lifecycle the Eimeria parasite relies heavily on glycolysis for ATP production, and hence on homolactic fermentation - the action of lactate dehydrogenase (LDH) - to restore the NADH/NAD^+ balance. These parasites are therefore extremely sensitive to LDH inhibition. In common with the LDH of the malaria causing parasite Plasmodium falciparum (PfLDH), Eimeria tenella LDH (EtLDH) has a characteristic five amino acid insert in a loop directly adjacent to the active site. As a consequence, we reasoned that compounds we have previously designed to specifically inhibit PfLDH should cross-react with EtLDH. We are therefore undertaking crystallisation and structural analysis of EtLDH and its inhibitory complexes in order to explore the possibility of targeting EtLDH for novel veterinary therapeutics.

Keywords: lactate dehydrogenase, Eimeria tenella, enzyme inhibitors

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Crystal structure and structure based drug design of HU (histone like protein) from *M.tuberculosis*

Ramakumar Suryanarayanarao[1], Tuhin Bhowmick[1], Soumitra Ghosh[2], Ramagopal A Udupi[3], Nagaraja Valakunja[2]
[1]Indian Institute of Science, Physics, Raman Avenue, Bangalore, Karnataka, 560012, India, [2]Microbiology and Cell Biology Department, Indian Institute of Science, Bangalore-560012, India, [3]Albert Einstein College of Medicine, Bronx, New York, 10461, USA, E-mail：ramak@physics.iisc.ernet.in

HU is an architectural protein for bacterial chromosome compaction and organization.HU is one of the most ubiquitous proteins in the bacterial cell.It is a small basic protein, binding non-specifically throughout the nucleoid. While the sequence of HU is highly conserved through most of the bacterial species, the HU in M.tuberculosis has a sequence longer than other HUs whose crystal structures have been elucidated (Anabaena (1P71): 94 residues). It has a sequence length of 214 amino acid residues suggesting the presence of an extra domain.As the protein has a possible role in overall gene architectural modification and a global control of gene expression, it makes this HU an important candidate for structural study. For the first time a complete functional N-terminal Domain of HU from M.tuberculosis [H37Rv] (1st 100 amino acid residues) containing the sub-domains for DNA binding and dimerization was crystallized,the crystals diffracted to 2.04Å. Crystal unit cell contains biological dimer of N-terminal region of HU protein. The structure was solved by molecular replacement method. The final *R*cryst and *R*free are 20.6% and 25.0%. As the sequence is highly conserved among the other important mycobacterium species, like M.leprae, M.bovis, M.smegmatis etc., this structure is a good representative HU structure for the whole class and serves as an attractive target for drug design. Two types of drug molecules, one which can interfere with DNA-binding and other with HU dimerization, were designed computationally, utilizing the solved crystal structure of HU. The designed compounds interact with HU with high binding energies, as estimated computationally and could serve as lead molecule for drug design. Details of the 3-D models of HU-drug interactions will be discussed.

Keywords: Mycobacteria, histone like protein, structure aided drug design

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Structure-based design of anticancer prodrug PABA/NO

Xinhua Ji[1], Ajai Pal[2], Ravi Kalathur[1], Xun Hu[2], Yijun Gu[1,2], Joseph Saavedra[1], Gregory Buzard[1], Aloka Srinivasan[1],