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

P04.14.352

Acta Cryst. (2008). A**64**, C341

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.

references
Kedersha, N.L., Rome, L. H., (1986). J. Cell. Biol. 110, 895
Kickhoefer, V. A., Searles, R. P., Kedersha, N. L., Garber, M.E., Johnson, D. L., Rome, L. H., (1993). J. Biol. Chem. 268, 7868
Kickhoefer, V. A., Siva, A. C., Kedersha, N. L., Inman, E, M., Ruland, C., Streuli, M., Rome, L. H., (1999a). J. Cell. Biol. 146, 917
Kickhoefer, V.A., Stephen, A.G., Harrington, L., Robinson, M.O., Rome, L. H., (1999b). J. Biol. Chem. 274, 32712
Kato, K., Tanaka, H., Sumizawa, T., Yoshimura, M., Yamashita, E., Iwasaki, K. and Tsukihara, T. (2008) Acta Cryst. D (in press)

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

P04.14.353

Acta Cryst. (2008). A**64**, C341

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

P04.15.354

Acta Cryst. (2008). A**64**, C341-342

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