protein crystallized in space group $P2_1$, with unit-cell parameter a = 34.538, b = 56.772, c = 71.710 Å. The overall structure contains three alpha-helix and four beta-strands. In refined model, we can suggest that the very electrostatically positive surface of SMRD is a possible site of interaction between SMRD and DNA substrate. To define the mechanism of DNA recombination and repair, this structural insight will have to be complemented by new cell-based and complexed approaches.

Keywords: SMRD, B3BP, nicking endonuclease

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Crystal structure of the Fab fragment of antibody against *p*-bronophenylalanine

Asako Yamaguchi, Takayoshi Kinoshita, Nanako Inoue, Tomoyuki Asano, Mitsunori Kirihata, Toshiji Tada
Osaka Prefecture University, Graduate School of Science, 1-1, Gakuen-cho, Nakaku, Sakai, Osaka, 599-8531, Japan, E-mail：yamaguchi@b.s.osakafu-u.ac.jp

p-Boronophenylalanine (BPA) is used to the treatment of brain tumors as one of the boron carriers of boron neutron capture therapy (BNCT). The monoclonal antibody against BPA was prepared and named “anti-BPA”. Anti-BPA with high specificity is expected to be a potent and effective tool in order to elucidate the intra/extra cellular distribution and mode of action of BPA. The monoclonal antibody is also useful to perform the specific monitoring and analytical immunoassay system of BPA including determination of BPA concentration. We have started an analysis for the crystal structure of anti-BPA to clarify the structural features participating in antigen recognition of anti-BPA. Fab fragment of anti-BPA was prepared by papain digestion. Fab was purified by MonoQ column. The solution of Fab was concentrated to 8 mg/ml in 20 mM Tris-HCl (pH 7.5) prior to crystallization. Fab was crystallized by sitting drop vapor diffusion method at 293 K using PEG4000 as a precipitant. A data set was collected to 3.0 Å resolution from a frozen crystal using synchrotron radiation of wavelength 1.0 Å at PF. The crystal belongs to the rhombohedral space group $R3$ with unit-cell parameters a = b = 160.17, c = 306.19 Å. Molecular-replacement calculations were carried out with the program *Molrep* using structure of anti-(4-hydroxy-3-nitrophenyl) acetate antibody as a search model. Refinement and manual modifications of the model structure are currently in progress.

Keywords: antibody, boron compound, crystal structure

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Structural studies of the cytochrome c_z from the green photosynthetic bacterium *Chlorobium tepidum*

Yu Hirano[1], Makoto Higuchi[1], Hirozo Oh-oka[2], Kunio Miki[3], Zheng-Yu Wang[1]
[1]Ibaraki University, Faculty of Science, 2-1-1 Bunkyo, Mito, Ibaraki, 310-8512, Japan, [2]Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka, 560-0043, Japan, [3]Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, 606-8502, Japan, E-mail：yhirano@mx.ibaraki.ac.jp

Green sulfur photosynthetic bacteria have a reaction center (RC) with a Fe-S cluster as the terminal electron acceptor. The RC consists of five subunits: PscA containing a special pair (P840), PscB containing Fe-S clusters A and B (F_A/F_B), PscC containing a heme c (cytochrome c_z), PscD binding to the FMO, and the BChl-a protein FMO. Two molecules of cytochrome c_z bind to the RC and each of them has been reported to directly transport an electron from cytochrome bc_1 to the P840. Cytochrome c_z is supposed to consist of an N-terminal transmembrane domain and a C-terminal periplasmic domain which contains one heme c. In order to determine the crystal structure of the functional domain, we constructed a soluble variant of cytochrome c_z from the green sulfur photosynthetic bacterium *Chlorobium tepidum* (residues 111-206; C-cyt c_z). We determined the crystal structure of oxidized C-cyt c_z by the Fe-SAD method and refined to 1.3 Å resolution. The N-terminal 20 residues of C-cyt c_z are disordered and additional 8 residues form a loop structure. This feature may explain the flexibility between the transmembrane and the periplasmic domains of cytochrome c_z, which makes it possible to mediate the direct electron transfer between cytochrome bc_1 and RC. C-cyt c_z shows structural similarities with cytochrome c_{551} from *Pseudomonas aeruginosa* and cytochrome c_6 from *Monoraphidium braunii*. Despite of the overall structural similarities with the class I cytochrome proteins, the coordination pattern of the heme c iron is different between C-cyt c_z and other members in this class. On the other hand, unusual paramagnetic NMR shifts were observed for the oxidized form of C-cyt c_z. This may be attributed to the unique coordination environment of the heme c as revealed from the crystal structure.

Keywords: cytochromes, photosynthesis-related proteins, paramagnetic NMR

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Isopeptide bonds stabilize Gram-positive bacterial pilus structure and assembly

Edward N. Baker, HaeJoo Kang, Fasseli Coulibaly, Fiona Clow, Thomas Proft
University of Auckland, School of Biological Sciences, Private Bag 92019, Auckland, Auckland, 1142, New Zealand, E-mail：ted.baker@auckland.ac.nz

Bacterial pili are long, slender appendages that mediate adhesion to host cells, and thus promote colonisation and infection. Gram positive organisms such as Streptococcus pyogenes have extremely thin (~30 Å) pili, formed by covalently-linked pilin subunits. We have determined the crystal structure of the major S. pyogenes pilin protein, and have derived an atomic model for pilus assembly which explains the extraordinary strength and stability of this structure. The 2.2 Å crystal structure reveals an extended structure comprising two all-beta domains. The molecules associate in columns through the crystal, providing a model for pilus assembly and for the location of the inter-subunit covalent bonds. The structure also revealed novel intramolecular crosslinks in each subunit, in the form of isopeptide bonds linking Lys and Asn side chains. Located at strategic places in the fold, these give strength and stability. Mutagenesis shows that they are generated by an intramolecular reaction involving adjacent Glu residues. Mass spectrometric analyses of purified pili confirm both the intramolecular isopeptide bonds and the intersubunit isopeptide bonds suggested by the crystal packing. Our results provide a model for the assembly of GAS pili, in which self-generated intramolecular isopeptide bonds complement the sortase-catalyzed intermolecular bonds. Database searches indicate that internal isopeptide crosslinks also exist in other proteins where mechanical strength and stability are needed, notably in other surface proteins of Gram-positive organisms.

Keywords: bacterial pili, isopeptide bonds, oligomer assembly

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Recognition of an unusual peroxisomal targeting signal 1 by the import receptor Pex5p

Krisztian Fodor, Nicole Holton, Simon Holton, Matthias Wilmanns
EMBL Hamburg Outstation, Structure Biology Unit, Notkestr. 85, Hamburg, Hamburg, 22607, Germany, E-mail：fodor@embl-hamburg.de

The structure of the peroxisomal receptor Pex5p in the presence of a small model cargo revealed a large conformational change of the receptor upon cargo binding (Stanley et al., 2006). It, however, remained unknown whether the binding was cargo-specific and only indirect methods were applicable to test cargo activity during the translocation process. In order to investigate whether the previously observed type of cargo binding is generally applicable, we have determined the structure of Pex5p in complex with alanine-glyoxylate aminotransferase (AGT). The complex reveals how the unusual C-terminal KKL receptor recognition motif can be accommodated within the previously characterized central binding cavity. The present structure, similarly to the Pex5p-mSCP2 complex, reveals a secondary interaction site. From the receptor side the motif that participates in the interaction is the very same than the one in the Pex5p-mSCP2 structure. A common feature of all the available Pex5p crystal structures is that one of the seven TPR repeats of the receptor (TPR4) is not visible and thus can be considered as a highly flexible part of the molecule. Since most TPR proteins participate in various interactions it would not be surprising if the TPR4 segment was serving as an interaction site and stabilized by a binding partner in a later step of peroxisomal translocation. Important to note that TPR4 and the secondary binding motif are located on the opposite sides of the receptor. It is intriguing to hypothesize that secondary interactions are playing a role in the correct orientation of the cargo to ensure the accessibility of the TPR4 region during translocation process. Our structural and biochemical data also consistently show that AGT remains fully active when bound to the receptor.

Keywords: peroxisome, transport, Pex5p

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Crystal structure of L-CKS from *Haemophilus influenzae* in complex with KDO

Hye-Jin Yoon[1], Min-Je Ku[1], Bunzo Mikami[2], Se Won Suh[1]
[1]Department of Chemistry, Seoul National University, Gwanak-gu,, Seoul, 151-742, Korea (S), [2]Graduate School of Agriculture, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan, E-mail：yoonhj@snu.ac.kr

The enzyme 3-deoxy-manno-octulosonate cytidylyltransferase (CMP-KDO synthetase; CKS) catalyzes the activation of 3-deoxy-D-manno-octulosonate (or 2-keto-3-deoxy-manno-octonic acid, KDO) by forming L-CKS. In order to determine the structure of L-CKS from *H. influenzae*, we have crystallized it by hanging drop vapour-diffusion method at 296 K. The crystal of L-CKS is orthorhombic, belonging to the space group $P2_12_12_1$ with unit cell parameters of a = 48.42, b = 82.61, c = 115.71 Å. The presence of two monomers in the asymmetric unit gives a reasonable V_M of 2.05 $Å^3$ Da^{-1}, with a solvent content of 40.0%. We determined the crystal structure of L-CKS from *H. influenzae* in complex with KDO at 2.30 Å resolution by the multiwavelength anomalous diffraction method. The overall protein structure is similar to that of K-CKS from *E. coli*. The C-terminal alpha-helix (Ala230-Asn254) of monomer A has a unique conformation. The structure of L-CKS from *H. influenzae* in complex with KDO will be useful in structure-based inhibitor design.

[1] Ku et al. (2003) *Acta Crystallog Sect D* 59, 180-182.
[2] Jelakovic et al. (2001) *J Mol Biol* 312, 143-155.

Keywords: antibacterial target, CKS, KDO

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Towards the structural basis for bacterial two-partner secretion

Hye-Jeong Yeo, Takeshi Yokoyama
University of Houston, Biology and Biochemistry, 369 Science & Research Bldg 2, Houston, TX, 77204, USA, E-mail：hyeo@uh.edu

In pathogenic Gram-negative bacteria, many virulence factors are secreted via the two-partner secretion (TPS) pathway, which consists of an exoprotein called TpsA and a cognate outer membrane translocator called TpsB. The HMW1 and HMW2 adhesins are major virulence factors in nontypeable Haemophilus influenzae and are prototype TPS pathway exoproteins. A key step in the delivery of HMW1 and HMW2 to the bacterial surface involves targeting to the HMW1B and HMW2B outer membrane translocators by an N-terminal region called the secretion domain. To understand the structural basis for bacterial TPS, we aim to determine the crystal structures of the component proteins in the H. influenzae TPS pathway. The crystal structure of the HMW1 pro-piece (HMW1-PP), a region that contains the HMW1 secretion domain, reveals a large right-handed beta-helix fold. Comparison of HMW1-PP and the Bordetella pertussis FHA secretion domain (Fha30) displays limited amino acid homology but shared structural features, suggesting that diverse TpsA proteins have a common structural domain required for targeting to cognate TpsB proteins. Our progress on the project will be presented and discussed.

Keywords: two-partner secretion, beta-helix, HMW1 adhesin

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Structure and function of the human histone chaperone CIA complexed with the bromodomain from TFIID

Yusuke Akai[1,2], Naruhiko Adachi[1], Yohei Hayashi[3], Masamitsu Eitoku[3], Norihiko Sano[3], Norio Kudo[2], Masaru Tanokura[2], Masami Horikoshi[3], Toshiya Senda[4]
[1]JBIRC, JBIC, Aomi 2-42, Koto-ku, Tokyo, 135-0064, Japan, [2]Grad. Sch. of Agri. and Life Sciences, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-8657, Japan, [3]IMCB, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-0032, Japan, [4]BIRC, AIST, Aomi 2-42, Koto-ku, Tokyo, 135-0064, Japan, E-mail：y-akai@aist.go.jp

Histone modifications frequently function as a mark to induce nucleosome structure changes in a site-specific manner. Although many domains that specifically recognize histone modification have been identified, the molecular mechanism of the change in the nucleosome structure induced by histone modification remains elusive. We have therefore studied the functional interaction between the histone chaperone CIA, which has histone-$(H3\text{-}H4)_2$-tetramer disrupting activity, and an acetylated histone-recognizing