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Keywords: human aldose reductase, protein-ligand complex, interaction energies

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X-ray visualization of protein biotinylation

Bagautdin Bagautdinov, Naoki Kunishima
RIKEN SPring-8 Center, Advanced Protein Crystallography Research Group, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, E-mail：bagautdi@spring8.or.jp

Biotin-dependent carboxylases play essential roles in the metabolism of all organisms. Their activation by biotinylation is catalysed by biotin protein ligase (BPL). The crystal structures of the archaeal BPL (PhBPL), the biotinyl domain of archaeal biotin carboxyl carrier protein (PhBCCP) and the PhBPL:PhBCCP complex have been determined by X-ray diffraction. The structural feature of the intermediate (biotinyl-5'-AMP) formation was studied using the PhBPL structures liganded with biotin, ATP and biotinyl-5'-AMP [1]. The BPL:BCCP complex was obtained using two BPL mutants R48A and R48A&K111A [2]. The mutants retain functionality as an enzyme but likely with lower activity, thereby providing the complex crystals. Structural funding of the transient BPL:BCCP complexes at formation (BPL:apo-BCCP) and product (BPL:holo-BCCP) stages allow us to visualize the biotinylation process. The catalytic N- and C-terminal domains of BPL and the biotinyl domain of BCCP act as three rigid-body parts of the complex. The C-terminal domain of BPL shows large conformational motions to accommodate BCCP, suggesting its functional importance. Additionally, the C-domain has a flexible loop that must open every time when BCCP attaches to BPL, and it allows the specific lysine of BCCP to enter the active site of BPL with the intermediate biotinyl-5'-AMP. These structural details are useful to understand the protein biotinylation which is widely used in purification, quantum dots targeting as well as in the design of anti-obesity drugs.
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Keywords: biotinylation, protein-protein complex, X-ray diffraction

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Optical and electric properties depending on molecular arrangement of dehydro[12]annulene derivative

Hirofumi Senga, Yuu Sakamoto, Hajime Shigemitsu, Ichiro Hisaki, Norimitsu Tohnai, Mikiji Miyata
Graduate School of Engineering, Osaka University, Department of Material and Life Science, 2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan, E-mail：senga@molrec.mls.eng.osaka-u.ac.jp

To develop a novel π-conjugated molecule-based supramolecular assembly, we designed and synthesized trisdehydrotribenzo[12]-annulene ([12]DBA) derivative **1** which has three carboxyl groups at the periphery. Recrystallization of **1** from DMSO gave a crystal of the solvate **1**/DMSO. Crystallographic analysis revealed that a face-to-face π-stacked one-dimensional (1D) assembly of **1** was achieved and that the DMSO molecule played a significant role as a "structure-dominant element" in the crystal. This is the first example of [12]DBA to stack completely orthogonal to the columnar axis. To reveal its superstructure-dependent optical and electrical properties, **1** and its parent molecule **2**, which crystallizes in a herringbone fashion, were subjected to fluorescence spectroscopic analysis and charge-carrier mobility measurements in crystalline states. The 1D stacked structure of **1** provides a red-shifted, structureless, weakened fluorescence profile compared to **2**, due to strong interactions between the π-orbitals of the stacked molecules. The charge-carrier mobility of the single crystal of **1**/DMSO, as well as **2**, was determined by flash photolysis time-resolved microwave conductivity measurements. The single crystal of **1**/DMSO revealed significantly-anisotropic charge mobility along the columnar axis. This value is 12 times larger than that along the orthogonal axis.

Keywords: one dimensional structure, pi interactions, charge carrier mobility

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X-ray crystal structure analyses of methionine γ-lyase 1 and 2 from *Entamoeba histolytica*

Tsuyoshi Karaki[1], Dan Sato[2,3], Akira Shimizu[1], Tomoyoshi Nozaki[3], Shigeharu Harada[1]
[1]Kyoto Institute of Technology, Applied Biology, Gosyokaido-cho, Matsugasaki, Sakyo-ku, Kyoto, 606-8585, Japan, [2]Keio University, 246-2, Mizukami, Kakuganji, Tsuruoka, Yamagata, 997-0052, Japan, [3]Gunma University Graduate School of Medicine, 39-22, Showa-machi 3-chome, Maebashi, Gunma 371-8511, Japan, E-mail：karaki08@yahoo.co.jp

Methionine γ-lyase (MGL) is a member of the γ-family of pyridoxal 5-phosphate (PLP)-dependent enzymes and catalyzes α, γ or α, β - elimination of sulfur-containing amino acids such as methionine, homocysteine and cysteine to produce ammonia, α-keto acid and volatile thiol (hydrogen sulfide or methanethiol). There are two isoenzymes (MGL1 and MGL2; amino acid sequence identity of 70%) in *E. histolytica* showing somewhat different substrate specificities. Since MGL is not present in mammalian hosts, MGL1 and 2 are promising targets for structure-based drug design of amebicides. In this study, we succeeded in crystallizing MGL1 and 2, and carried out X-ray structure analyses for the enzymes. MGL1 and 2 were crystallized using ammonium sulfate and polyethylene glycol 400 as a precipitant, respectively. The structures of MGL1 and 2 were solved by the molecular replacement method using MGL from *P. putida* (PDB code 1gc2) as a search model and refined to R=17.2% (1.93 Å resolution) and R=18.2% (1.80 Å resolution), respectively, based on diffraction data collected at BL5A of Photon Factory or BL44XU at SPring-8. The refined structures of MGL1 and 2 as well as those complexed with methionine reveal that both enzymes exist as homotetramers consisting of two catalytic dimers, and that the PLPs are covalently bound to the ε-amino group of lysine residues (205/208) in the active sites of the enzymes. Furthermore, it is also indicated that tyrosine residues (108/111) play a crucial role as a general acid catalyst in γ- elimination of methionine. Currently, we are trying to prepare crystals of MGL1 and 2 complexed with compounds such as trifluoromethionine, a suicide substrate of MGL, to develop potential pro-drugs.

Keywords: methionine gamma-lyase, X-ray structure analyses, gamma-elimination