street. Room# 508 Banting Bldg, Toronto, Ontario, M5G 1L5, Canada, E-mail：bumsoo.hong@utoronto.ca

Choline kinase (ChoK) catalyzes the ATP-dependent phosphorylation of choline, the first committed step in the CDP-choline pathway for the biosynthesis of phosphatidylcholine. Overexpression and increased activity of one of the human ChoK isoforms, ChoK alpha have been constitutively reported in malignant cells and tumour tissues. This suggests that the enzyme plays a relevant role in tumorigenesis. Some sets of in vitro and in vivo experiments confirmed that ChoK inhibition is one of the potential novel strateges for the development of new antiproliferative and anticancer drugs. The importance of ChoK for the regulation of cell proliferation has been studied by using an inhibitor hemicholinium-3 (HC-3), which was initially characterized as a lethal, respiratory paralytic agent. Most of the ChoK inhibitors introduced so far are the chemically modified derivatives based on the structure of HC-3. Crystal structures of HC-3 bound human ChoK alpha were determinded with and without ADP. In the crystal structures, HC-3 molecule was well accommodated between the N and C-terminal lobes of ChoK protein, and its one end was placed on the same binding site as a substrate choline, while the other end partially exposed to the solvent. The inhibitor molecule was stabilized mainly through the hydrophobic interactions contributed by the C-terminal lobe. These 3D information provides the first molecular detailed view concerning the mode of inhibitory action and expand our understanding of the factors governing selectivity.

Keywords: tumorigenesis, antiproliferative and anticancer drug, inhibitor

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Fragment screening and structure-based design of adrenaline synthesis inhibitors

Jennifer L Martin[1], Nyssa Drinkwater[2], Christine L Gee[3], Michael J McLeish[4], Gary L Grunewald[5]
[1]University of Queensland, Institute for Molecular Bioscience, 306 Carmody Road, Brisbane, Queensland, 4072, Australia, [2]University of Queensland, 306 Carmody Road, Brisbane, Queensland, 4072, Australia, [3]University of Queensland, 306 Carmody Road, Brisbane, Queensland, 4072, Australia, [4]University of Michigan, Ann Arbor, Michigan, USA, [5]Kansas University, Lawrence, Kansas, USA, E-mail：j.martin@imb.uq.edu.au

The enzyme phenylethanolamine N-methyltransferase (PNMT) catalyses the biosynthesis of adrenaline, a neurotransmitter linked to the central control of blood pressure. As part of an ongoing international collaboration to develop PNMT inhibitors, we found that the enzyme conceals a cryptic binding site (1-2). This site is revealed upon binding inhibitors that are double the size of the physiological substrate. The changes in active site size and shape are brought about by unfavourable side-chain conformations and rigid-body helix motions, at a modest estimated energetic cost of 2-3 kcal/mol. Our findings further underline the importance of incorporating protein flexibility in structure-based inhibitor design studies, and raise the question of whether such sites are accessible through moderate affinity fragment screening approaches. To address this question, we implemented fragment-based screening by X-ray crystallography for PNMT. We used the ActiveSight library of 384 compounds and found that a number of fragments bind to the PNMT active site. These will now be elaborated to develop potent and selective PNMT inhibitors.

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Keywords: enzyme inhibitor drug design, structure-based drug design, binding enzyme inhibitors

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Design of anti-allergic inhibitors for human hematopoietic prostaglandin D synthase

Hiroshi Kikuchi[1], Yuji Kado[1], Hiroyoshi Matsumura[1], Yoshifumi Fukunishi[2], Takayoshi Kinoshita[3], Yasushi Okuno[4], Isao Nakanishi[4], Seiji Minakata[1], Tsuneaki Sakata[1,5], Kohsuke Aritake[6], Yoshihiro Urade[6], Tsuyoshi Inoue[1,5]
[1]Grad. Sch. of Engineering, Osaka Univ., Applied Chemistry, 2-1 Yamada-Oka, Suita, Osaka, 565-0871, Japan, [2]National Inst. of Advanced Industrial Science and Technology (AIST), 2-41-6, Aomi, Koto-ku,135-0064, Japan, [3]Grad. Sch. of Agriculture and Life Sciences, Osaka Prefecture Univ., 1-1 Gakuen-cho, Osaka 599-8531, Japan, [4]Grad. Sch. of Pharmaceutical Sciences, Kyoto Univ., Kyoto 606-8501, Japan, [5]NPO BioGrid Center Kansai, 1-4-2 Shinsenri-Higashimachi, Osaka 560-0082, Japan, [6]Osaka Bioscience Inst.,Osaka 565-0874, Japan, E-mail：h_kikuchi@chem.eng.osaka-u.ac.jp

Structure-based drug design (SBDD) is not certainly major process in the pharmaceutical company, however, the cost for drug discovery is huge and gradually increased, therefore, the importance of SBDD is thought to be greater and greater. The novel in-silico screening methods of Multiple Target Screening1 (MTS) and Docking score index2 (DSI) using the matrix on the interaction between the protein structures and chemical compounds were developed. To examine the effect of these methods, we selected human hematopoietic prostaglandin D synthase (H-PGDS) as a target. H-PGDS catalyzes the isomerization of PGH2, a common intermediate of various prostanoids, to PGD2, an inflammatory mediator, in the presence of glutathione (GSH). Oral administration of the H-PGDS inhibitor of HQL-79 suppressed antigen-induced eosinophilic accumulation in the lung of wild-type mice and human H-PGDS-overexpressing mice, gliosis and demyelination in twitcher mice, and musclar distrophy in mdx mice4. The optimizing of the known inhibitor4 as well as the screening of a novel lead compound for human H-PGDS by using in silico method are now in progress.
References

Keywords: complex compounds crystal structure, structure-based drug design, antiallergic drugs

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Structure-based drug design in HIV protease- and tRNA-guanine transglycosylase inhibitor development

Andreas Heine[1], Jark Boettcher[1], Tina Ritschel[1], Andreas Blum[1], Benedikt Sammet[1], Simone Hoertner[2], Philipp Kohler[2], Francois Diederich[2], Wibke E. Diederich[1], Gerhard Klebe[1]
[1]Philipps-University Marburg, Department of Pharmaceutical Chemistry, Marbacher Weg 6, Marburg, Hessen, 35032, Germany, [2]ETH-Zurich, Hoenggerberg HCI, 8093 Zurich, Switzerland, E-mail：heinea@mailer.

uni-marburg.de

Protein crystallography is a versatile technique in the investigation of detailed protein-ligand interactions, and hence, an important tool in structure-based drug design. Here, we will focus on two recent examples in structure-based drug design from our laboratory: HIV-1 protease and tRNA-guanine transglycosylase. The design and structural investigation of HIV-1 protease inhibitors using linear achiral oligoamines is a starting point for the development of non-peptidic inhibitors. Interestingly, initial compounds showed affinity in the low micromolar range not only for HIV-1 protease but also to other aspartic proteases such as plasmepsins and renin. Therefore, a compound series was generated that forms the basis to a general route for lead structure identification of aspartic proteases. tRNA- guanine transglycosylase (TGT) is an essential enzyme in the infection pathway of Shigella. The enzyme catalyzes the exchange of guanine in the wobble position of tRNAHis,Tyr,Asn,Asp against the modified base preQ1. Z. mobilis TGT is used for structural studies of potent ligands which are based on the lin-benzoguanine scaffold. Here, a series of different side chains leading to the modified 2-amino-lin-benzoguanines resulted in effective inhibitors in the low nanomolar range. The gain of affinity is achieved by additional charge-assisted hydrogen bonds for the protein-ligand complex.

Keywords: crystal structures, anti-HIV drug design, TGT complexes

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Crystal structure of complexes of peptidoglycan recognition protein with carbohydrates

Punit Kaur, Pradeep Sharma, Nagendra Singh, Mau Sinha, Rishi Jain, Vikram Gopalakrishnapillai, Amandeep Kaur, Sujata Sharma, Srinivasan Alagiri, Tej P. Singh
All India Institute of Medical Sciences, Biophysics, Ansari Nagar, New Delhi, Delhi, 110029, India, E-mail：kaurpunit@gmail.com

Peptidoglycan Recognition Protein (PGRP) is a pattern recognition molecule which interacts with pathogen associated molecular patterns which are expressed by the pathogenic bacteria. The PGRP-S kill the bacteria by interacting with their cell walls and interfering with the peptidoglycan (PGN) biosynthesis of both gram positive and gram negative bacteria. The PGNs are essential components of bacteria which are absent in mammals. Thus, PGRP contributes to the host defense against bacterial infections. The first PGRP protein was isolated from mammary gland secretions from animals infected by mastitis. It was crystallized in the native state as well as with the carbohydrates, n-acetyl glucosamine, n-acetyl galactosamine, disaccharide and rhamnose. The isomorphous crystals of these complexes belong to space group *I*222 with cell dimensions, $a = 89.3$ Å, $b = 102.6$ Å and $c = 164.0$ Å. There were four crystallographically independent molecules in the asymmetric unit which form two types of dimers. The single PGRP has two binding sites, one for binding to PGN and the second for binding to non-PGN molecules. In one dimer, there are two exposed PGN binding sites while in the second the two non-PGN binding sites are exposed. In all the complexes, the carbohydrates bind at the PGN binding sites of one complex. The PGRP-S residues, His37, Thr152 and Ser154 are involved in the recognition of carbohydrate with side chains of all these residues involved in the formation of hydrogen bonds with carbohydrate residue/residues. The non-PGN binding site was empty in the second complex indicating a different preference for the ligand binding.

Keywords: peptidoglycan recognition protein, carbohydrate, antibacterial

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Crystal structure of human CK2 alpha in complex with ellagic acid

Yusuke Sekiguchi[1], Tetsuko Nakaniwa[1], Takayoshi Kinoshita[1], Isao Nakanishi[2], Kazuo Kitaura[2], Akira Hirasawa[2], Gozoh Tsujimoto[2], Toshiji Tada[1]
[1]osaka prefecture univ., science, nakai gakuencho1-1, sakai, osaka, 599-8531, Japan, [2]Graduate school of Pharmaceutical sciences, Kyoto University, E-mail：sekiguchi07@b.s.osakafu-u.ac.jp

Casein kinase 2 (CK2) is a highly pleiotropic serine/threonine protein kinase, composed of two catalytic (CK2alpha) and two regulatory (CK2beta) subunits. CK2 is a target protein for glomerulonephritis therapy. We have determined the crystal structure, at 2.35Å resolutions, of human CK2alpha in complex with ellagic acid, which is an ATP-competitive inhibitor. The structure reveals that ellagic acid binds to the active residue Lys68. Ellagic acid interestingly binds to the hinge region connecting the N- and C-lobes through a water molecule. The structural information of the complex including the indirect interaction would be a great help to design unique and potent CK2 inhibitor.

Keywords: X-ray, ck2, inhibitor

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Rapid and precise protein 3D-model preparation for SBDD

Takao Matsuzaki[1], Isao Ishizaka[2]
[1]Independent Drug Design Advisor, 2007-1-404 Uchikoshimachi, Hachioji, Tokyo, 192-0911, Japan, [2]Autofact Inc., 4-1-14 Higashikoigakubo, Kokubunjishi, Tokyo, 185-0014, Japan, E-mail：tak-matsuzaki-45@cb3.so-net.ne.jp

Though protein 3D-models have been very useful in SBDD (Structure Based Drug Design), there was no simple and practical method to make them. Light solidification of acrylic polymers and simple mechanical movements have enabled a protein of 50 Å to be shaped in 1.5 hours into a model of ca.10 cm with a precision of 0.05Å. The left photo is a 3D-model builder with a control PC. Protein 3D-models become very important when our drug target is a PPI (Protein-Protein Interaction) system. Drug target sites are not obvious in a PPI system, unlike enzyme cases where catalytic sites or substrate binding sites are mostly drug target sites. Protein 3D-models can tell potential drug target sites at a glance, making them a 'Must' tool for drug designers. The right photo shows the complicated PPI model of hexameric assembly of IL-6, receptor alpha and gp-130. Enlarged models with 1 Å = 4 mm scale for protein and ligand can show atomic level interactions and stimulate medicinal chemists how to optimize ligand structures.

Keywords: model, protein, SBDD