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Aurora kinase A belongs to the serine/threonine subclass of kinases (the other two members are named B and C) and is involved in the regulation of mitosis. More specifically, Aurora kinase A localizes to the centrosomes and to spindle poles and it is implicated in centrosome maturation, centrosome separation and bipolar spindle assembly [1]. The human Aurora-A gene localizes to a chromosomal region (20q13.2) that is amplified in a variety of human cancers [2]. Concomitantly, Aurora-A is overexpressed in the corresponding tumours, and recent studies have identified that Aurora-A is a low-penetration tumour susceptibility gene in both mice and humans [3]. Excess Aurora-A activity has been shown to confer tumourigenic properties to cells [4] and it is plausible that this reflects the ability of overexpressed Aurora-A to produce failure of cytokinesis, leading to centrosome amplification and polyploidization [5]. Given that aberrant expression and activity of Aurora kinase A is associated in tumorigenesis the inhibition of its kinase activity is an attractive approach for the treatment of tumours. Several inhibitors of Aurora-A have been developed up to date. These inhibitors target the ATP-binding site of Aurora A, which is identical among all members of this family, so they inhibit not only Aurora-A but also Aurora-B and Aurora-C. It is desirable to develop inhibitors that can discriminate between these kinases to ensure a properly targeted therapy, to reduce the likelihood of patient side-effects, and to produce more precise tools for cell biology studies. This presents a significant challenge for structure-based drug design. In this study we present the crystal structure of Aurora-A bound to several small molecule inhibitors and we describe the design principles underpinning the production of Aurora-A selective inhibitors. These principles can be applied to other systems amenable to protein crystallography. The binding modes of the inhibitors suggest the residues involved in the ATP binding pocket that can be successfully exploited in order to design small molecules inhibitors with high potency and selectivity. Selectivity can then be introduced by iterative cycles of structural biology, hypothesis-based chemistry and validation using selectivity assays.


Keywords: aurora kinase a; inhibitors; protein crystallography

FA1-MS07-P07

Crystal Structures of Actinohivin, an Anti-HIV Protein from an Actinomycete, and its Complex with Mannobiose. Masaru Tsunoda\textsuperscript{a}, Kaoru Suzuki\textsuperscript{a}, Sagara Tsubasa\textsuperscript{a}, Atsushi Takahashi\textsuperscript{a}, Junji Inokoshi\textsuperscript{b}, Satoshi Omura\textsuperscript{b}, Takeshi Sekiguchi\textsuperscript{b}, Haruo Tanaka\textsuperscript{b}, Akio Takenaka\textsuperscript{b}, \textsuperscript{a}Iwaki Meisei University, Iwaki, Japan. \textsuperscript{b}Kitasato University, Tokyo, Japan.
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Actionohivin (AH) isolated from actinomycete Longispora albida K97-0003\textsuperscript{a} inhibits HIV viral entry to human susceptible cells. As compared with cyanovirin-N, AH binds more specifically to the high mannose-type saccharide chains (HMTG) of HIV gp120 protein. This lectin is a small protein of 114 amino acid residues, being arranged in a unique sequence repeated by three times tandemly. To reveal the structure of the specific binding, X-ray analyses of the apo-form and its complex with mannobiose Man\textsuperscript{1(2)} Man\textsuperscript{2} have been performed at 1.19 and 1.65 Å resolutions, respectively. In the apo-form crystal, the protein structure is composed of the three modules similar to each other, as speculated from the tandem repeats in the sequence. Each module consists of a β-sheet of four β-strands, a long loop and a π-helix. The three modules are associated with a pseudo three-fold symmetry, in which the three β-sheets form a triangular barrel. Inside of the barrel, hydrophobic residues form a stable core. On the outer surface, a long loop with a π-helix in each module is running from the top to the bottom of the barrel. This loop and the preceding two β-strands containing the carbohydrate-binding motifs LD-QXW for a valley with a pocket for carbohydrate-binding. In the three sites of the complex-form, three mannobiose are bound, respectively. In the first pocket of the module 1, the carbonyl group of Asp15 bridges between the two hydroxyl groups (OH\textsuperscript{1} and OH\textsuperscript{2}) attached to the C\textsuperscript{1} and C\textsuperscript{2} atoms of Man\textsuperscript{1}, through hydrogen bonds. At the same time, OH\textsuperscript{1} forms another hydrogen bond with the amino group of Asn28 and OH\textsuperscript{2} forms another hydrogen bond with the hydroxyl group of Tyr23. In addition, the mannose ring of Man\textsuperscript{1} is sandwiched between Leu25 and Tyr32 by hydrophobic interactions. To stabilize the pocket formation, Gln33 forms double hydrogen bonds with the main chain amide group at Asn28 and the carbonyl group at Pro26. In the second and the third pockets, the mannobiose are also bound in the similar ways. The structural features are completely consistent to the results of mutation experiments of these amino acid residues. The Man\textsuperscript{1} group which is largely bent by the α(1-2) bond is protruded into the solvent region. In this pocket, the mannobiose end of D1 branch of HMTG could be accommodated in a way similar to the geometry described above, and the end of D2 or D3 branch might contact with a valley. Therefore, it is possible to speculate that each pocket accepts the D1 and D2/D3 branches of HMTG. The three pockets are located to form an almost regular triangle at a distance of 17 Å between the
pockets. This separation might be possible to accept three HMTGs on a gp120 at the same time.

**Keywords:** lectin proteins; HIV drug design; protein structure analysis

**FA1-MS07-P08**

Conformational Change of Adenosine Deaminase During Ligand-Exchange in Crystal State.

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Adenosine deaminase (ADA) perpetuates chronic inflammation by degrading extracellular adenosine which is toxic for lymphocytes. ADA has two distinct conformations: open form and closed form [1]. From the crystal structures with various ligands, the non-nucleoside type inhibitors bind to the active site occupying the critical water-reading position and sustain the open form of apo-ADA. In contrast, substrate mimics do not occupy the critical position, and induce the large conformational change to the closed form. However, it is difficult to predict the binding of (+)-erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), as it possesses characteristic parts of both the substrate and the non-nucleoside inhibitors. The crystal structure shows that EHNA binds to the open form through a novel recognition of the adenine base accompanying conformational change from the open form of the PR-ADA complex in crystalline state [2]. The open form crystal structure of the EHNA-ADA complex supports our hypothesis that the occupancy at the trigger-water-position is critical for determining the open/closed conformational alternation, rather than the nucleoside framework binding. We believe that the structural penetration of the EHNA-ADA complex and structural comparison of the other inhibitor-ADA complexes will support the discovery of novel ADA inhibitors by structure-based drug design.


**Keywords:** adenosinedeaminase; EHNA; conformational change

**FA1-MS07-P09**

The Structure of the Ca²⁺-ATPase Bound to Cyclopiazonic Acid Reveals a Complexed Divalent Ion. 

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We have determined the structure of the sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) in an E2.Pi-like form stabilised as a complex with MgF₂, an ATP analogue (AMPPCP), and cyclopiazonic acid (CPA). The structure determined at 2.5 Å resolution leads to a significantly revised model of CPA binding compared to earlier reports [1,2] showing that a divalent metal ion is required for CPA binding through coordination of the tetrameric acid moiety at a characteristic kink of the M1 helix found in all P-type ATPase structures which is expected to be part of the cytoplasmic cation access pathway. Our model is consistent with the biochemical data on CPA [3] function and provides new measures in structure based drug design targeting Ca²⁺-ATPase from e.g. pathogens. We also present an extended structural basis of ATP modulation pinpointing key residues at or near the ATP binding site. A structural comparison to the Na⁺,K⁺-ATPase reveals that a Phe93 side chain occupies the equivalent binding pocket of the CPA site in SERCA suggesting an important role of this residue in stabilisation of the potassium-occluded E2 state of Na⁺,K⁺-ATPase.


**Keywords:** drug design; ATPase; membrane channel transport

**FA1-MS07-P10**

An Approach for Producing a CK2alpha Inhibitor Using X-ray, Calculation and ITC. Yusuke Sekiguchi, Harumi Fukada, Tetsuko Nakaniwa, Takayoshi Kinoshita, Shinya Nakamura, Isao Nakanishi, Kazuo Kitaura, Hiroaki Ohnou, Yamato Suzuki, Akira Hirassawa, Gozoh Tsujimoto, Toshiji Tada. Graduate School of Sciences. Graduate School of Life and Environmental Sciences, Osaka Prefecture University. Department of Pharmaceutical Sciences, Kinki University. E-mail: sekiguchi07@b.s.osakafu-u.ac.jp

Protein kinase CK2alpha is a highly pleiotropic serine/threonine protein kinase. CK2alpha plays important roles in cell growth, proliferation, and survival, while it is highly expressed in a wide variety of tumors. Furthermore, CK2alpha is a target protein for glomerulonephritis (GN) therapy, because an administration of either antisense oligodeoxynucleotide against CK2alpha or low molecular weight CK2alpha-specific inhibitors effectively prevents the progression of renal pathology in the rat GN models.

To design a novel and potent CK2alpha inhibitor, we determined four X-ray crystal structures of CK2alpha-inhibitor complexes (cc-04791, cc-04820, apigenin, ellagic acid), and measured enzyme kinetic parameters using ITC (Isothermal Titration Calorimetry) for the respective