MS16.P16

Acta Cryst. (2011) A**67,** C292

Reversible DNA scission by type II topoisomerase from *Streptococcus pneumoniae* and its modification upon drug binding

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Type II topoisomerases are essential enzymes responsible for changing and stabilizing DNA supercoiling and are also involved in chromosome segregation in prokaryotes. They are also targeted by antibacterials such as quinolones or quinazolinediones. Topo II enzymes operate by forming a transient covalent cleavage complex with a gate-DNA (G-segment) duplex and transporting the second duplex (T-segment) though a double-stranded break in the formed protein-DNA gate thus changing the DNA supercoiling in steps of 2. Despite the biological and clinical importance of these enzymes, the current understanding of their mechanism of action and enzyme-antimicrobial drug interaction. In order to further our understanding of the topo II role in DNA supercoiling and drug interaction, we have solved several crystal structures of a DNA cleavage complex of topoisomerase IV from *S. pneumoniae* stabilised by a number of different antibacterials and also captured in-crystal two stages of the cleavage complex reversal thus providing the structural snapshots the sequential states of enzyme operation[1], [2].

Drug-arrested forms of the cleavage complex were solved in presence of quinazolinedione PD 0305970 (3.1 Å) and quinolones such as levofloxacin (2.9 Å) and clinafloxacin (3.35 Å). In all studied cases the structures revealed two drug molecules intercalated at a symmetrically cleaved B-form DNA gate and stabilized by drug-specific protein contacts. In contrast with the dione-stabilised cleavage complex the quinolone-stabilised complexes contain magnesium ions chelated by the drug molecules and involved in protein-drug interaction which explains the magnesium dependence of the quinolone activity.

Drug-free cleaved and resealed DNA complexes were obtained from the original dione-stabilised cleavage complex by subsequent dione release. In contrast with the drug-stabilised complexes the DNA gate in drug-free complexes adopts an unusual A/B-form helical conformation and the active site magnesium ion is positioned close to the scissile phosphodiester group thus allowing its coordination and promoting reversible cleavage by active-site tyrosines. These structures solved for putative reaction intermediates of a type II topoisomerase provide the structural information about different stages of a normal reaction cycle of the enzyme as well as drug-specific interaction mechanisms which is important for the rational drug design in the future.

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Keywords: topoisomerases, breakage-reunion, protein-DNA complexes

MS16.P17

Acta Cryst. (2011) A**67,** C292

Structure and molecular interactions of an anti-tumor antibody specific for EGFR

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Overexpression of the epidermal growth factor (EGF)receptor (EGFR) in cancer cellscorrelates with tumor malignancy and poor prognosis for cancer patients. For this reason, the EGFR has become one of the main targets of anti-cancer therapies. Structural data obtained in the last few years have revealed the molecular mechanism for ligand induced EGFR dimerization and subsequent signal transduction, and also how this signal is blocked by either monoclonal antibodies or small molecules.

Nimotuzumab (also known as h-R3) is a humanized antibody that targets the EGFR and has been successful in the clinics. In this work we report the crystal structure of the Fab fragment of Nimotuzumab, revealing some unique structural features in the heavy variable domain. Furthermore, competition assays show that Nimotuzumab to domain III of the extracellular region of the EGFR, within an area that overlaps with both the surface patch recognized by Cetuximab (another anti-EGFR antibody) and the binding site for EGF. A computer model of the Nimotuzumab-EGFR complex, constructed by docking and molecular dynamics simulations and supported by mutagenesis studies, unveils a completely novel mechanism of action, with Nimotuzumab blocking EGF binding while still allowing the receptor to adopt its active conformation, hence warranting a basal level of signaling.

Keywords: cancer, egfr, antibody

MS16.P18

Acta Cryst. (2011) A**67,** C292-C293

Saquinavir resistance mechanism of non-active site mutants of HIV-1 Protease

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Since virally encoded protease plays a key role in the life cycle of Human immunodeficiency virus (HIV-1), it is the prime target of drugs in chemotherapy against Acquired immunodeficiency syndrome (AIDS). Protease inhibitors are important components of Highly Active Anti Retroviral Therapy (HAART) which is a standard treatment regimen followed for the treatment of HIV/AIDS. However, emergence of drug-resistant mutations in HIV-1 protease necessitates continuous improvement of existing drugs and design of new HIV-1 protease inhibitors based on understanding of the mechanisms of drug resistance. Structurally, these mutations can be categorized into active and non-active site mutations. As all the approved anti-HIV-1 protease drugs are competitive inhibitors, active site mutations may directly affect their binding. But effect of non-active site mutations is indirect and difficult to comprehend. Saquinavir (SQV) is one of

the important protease inhibitor which is currently used in the HIV-treatment-regimen. Mutation combination L90M/C95F is observed to confer resistance against this drug. Both are non-active site mutations.

To study the structural effects of these mutations, mutant clones L90M/C95F and C95F of tethered HIV-1 protease, where two sub-units of protease are covalently linked by a five residue linker, were prepared in our laboratory using site directed mutagenesis technique. Mutant proteins were expressed, purified and co-crystallized with SQV. X-ray diffraction data were collected on the FIP-beamline at European synchrotron research facility (ESRF). Crystal structures were solved by difference Fourier method using native coordinates. Comparisons of SQV bound mutant structures in the present study with that of native enzyme have shown reshaping of the active site cavity in the mutant structures. This is the result of altered packing in the core of the enzyme. SQV and in fact most of the other anti HIV-1 protease drugs are designed as competitive inhibitors so that they bind to the enzyme more tightly than its natural substrate. Hence, any change in the shape of active site cavity may affect the binding of these drugs more than that of natural substrates. Small changes in the conformation of SQV were also seen in response to those in the active site. Conversely, flexible substrates could adapt to these changes comparatively easily. Presence of these mutations may limit the structural flexibility of the active site loop which is required to accommodate the incoming inhibitor. These mutants have lower dime stability as compared to wild type enzyme as shown by reduction in the transition temperatures (T_m) as determined by using circular dichroism spectroscopy. This can be another cause of drug resistance.

Keywords: HIV-1 protease, saquinavir, drug resistance

MS16.P19

Acta Cryst. (2011) A**67,** C293

Toluene-sulfonic acid derivatives of 6-deoxyclitoriacetal, Biological Properties

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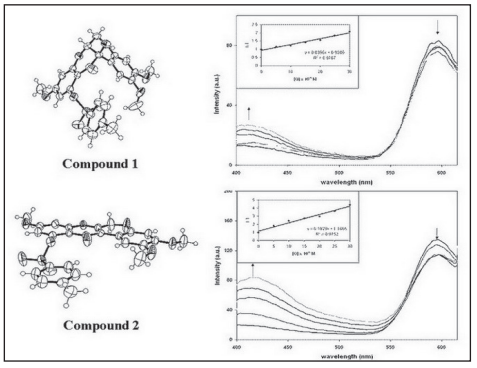

The compounds of toluene-4-sulfonic acid derivatives of 6-deoxyclitoriacetal have been synthesized and characterized. The crystal structures are different. One is in the bent shape (compound **1**) and another one is planar (compound **2**). In addition, the cytotoxic activities of both compounds are evaluated with KB cell line (epidermoid carcinoma of oral cavity), MCF-7 cell line (breast adenocarcinoma), and NCL-H187 (small cell lung carcinoma). Interestingly, the bent shaped (compound **1**) exhibits the potent significant cytotoxicity against KB (0.033 μM) and NCL-H187 (0.035 μM) but not active to MCF-7, while the planar shaped (compound **2**) is inactive for all tested cell lines. The molecular shape might effect on the cytotoxic activities of these tested cell lines. Therefore, the binding affinity with calf thymus DNA (CT-DNA) has been calculated by using UV-Vis and fluorescence spectroscopy. Moreover, the melting temperature (T_m) of CT-DNA when added with compound **1** and **2** are determined.

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Keywords: 6-deoxyclitoriacetal, binding affinity, cytotoxic activities

MS16.P20

Acta Cryst. (2011) A**67,** C293

Structural basis for inhibition of human and bacterial uridine phosphorylases by 2.2'-anhydrouridine, a modulator of 5-fluorouracil activity

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Uridine phosphorylase (Uph) is a key enzyme in the metabolism of pyrimidine based drugs. These drugs, primarily 5-fluorouracil (5-FU), remain the major components of many therapeutic regimens, particularly, in gastrointestinal malignancies. The inhibitors of Uph can potentiate the efficacy of 5-FU. Furthermore, Uph inhibition is lethal for some pathogenic parasites. Design of new clinical Uph antagonists requires a detailed investigation into the spacious organization of complexes of human and bacterial Uph at the atomic resolution. We used X-ray analysis to resolve for the first time the structure of Salmonella typhimurium Uph in a non-liganded state as well as in complexes with physiological ligands such as the phosphate anion and the potassium cation. We also determined the structure of complexes of Uph with 2.2'-anhydrouridine (ANU), 5-FU and their combinations at the atomic resolution. The structure of human Uph-ANU and Uph-phosphate ion complexes was determined by molecular modeling. Our experiments and literature data demonstrated that ANU is a reversible and competitive Uph inhibitor. Using molecular dynamics we revealed a role for K+ in stabilization of the structure of S.typhimurium Uph. The amino acid residues in the L9 loop of the active center and in the initial portion of the H8 helix were the most flexible. In the presence of K+ the intersubunit contacts in dimers became more dense. Molecular dynamics simulations showed that the L9 loop changed its position and conformation. Importantly, these data allowed us to perform an in silico design of high affinity Uph inhibitors based on ANU. These drug candidates were 5- or 6-substituted ANU derivatives containing an aliphatic saturated chain ended with an aromatic group. For 5-substituted ANU derivatives the pyridine cycle and the imidazole ring in the terminal aromatic moiety are preferred for inhibiting S.typhymurium and human Uph, respectively. This difference is attributed to variabilities in amino acid residues that form active centers of human and bacterial enzymes. For 6-substituted ANU derivatives the length of the carbohydrate chain is the critical prerequisite for the optimal inhibitor. Together, our data provide the ultrastructural basis for design of selective and efficient antitumor and anti-infective drugs.

The work was supported by basic funding from Russian Academy of Sciences.

Keywords: biomacromolecule, cancer, drug