

FA1-MS07-P01**The Binding of Pyrimidinyl Phosphonucleotide Inhibitors to Bovine Seminal Ribonuclease.**

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Bovine seminal ribonuclease (BS-RNase) is a potent antitumor agent. However, the antitumor action of BS-RNase is associated with severe side effects such as aspermatogenicity, immunosuppression and embryotoxicity that render the pharmaceutical use of BS-RNase problematic. A resolving key might be the attainment of a delicate balance between effective antitumor activity and side effect actions. A potent and specific BS-RNase inhibitor could assist on that by playing the role of an on/off switch and acting as an antidote to the side effects of the antitumor action of BS-RNase. To initiate structure-assisted inhibitor design studies, we have investigated the binding of five substrate analogs, uridine 2' phosphate (U2'p), uridine 3' phosphate (U3'p), uridine 5' diphosphate (UDP), cytidine 3' phosphate (C3'p), and cytidine 5' phosphate (C5'p), to BS-RNase by kinetic experiments and X-ray crystallography. The five pyrimidinyl compounds are moderate inhibitors of the enzyme with similar K_i values. In vivo studies on the aspermatogenic effect of BS-RNase in mice after intratesticular injections of BS-RNase and inhibitors C3'p and C5'p have shown that both compounds inhibit significantly the biological action of BS-RNase in mice confirming our hypothesis that ribonucleolytic inhibitors have a potential as pharmaceuticals in attenuating the side effects of the anticancer activity of BS-RNase. ADME pharmacokinetic property predictions reveal inhibitors U2'p, U3'p and C5'p as the most promising with respect to oral bioavailability. However, calculations suggest that the number of H-bond acceptors/polar surface areas need to be reduced in future development of more "drug-like" phosphonucleotide derivatives.

Keywords: protein-ligand complexes; ribonuclease; reaction kinetics

FA1-MS07-P02**Crystal Structure of the Taz2 Domain of p300 Transcriptional Coactivator.**

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Crystals of human p300 peptide (residues 1723-1836) corresponding to the extended zinc-binding Taz2

domain were grown under paraffin oil from a mixture of ammonium sulfate and alcohols. The space group is $I4_132$ with $a=155.3$ Å. The 2.5 Å data set was collected at the SER-CAT beamline 22-ID at the single wavelength of 1.283 Å. The crystal structure was solved by SAD approach utilizing anomalous diffraction signal of the bound Zn atoms and was refined with REFMAC to an R-factor of 20.1% (R-free=22.7%). The structure comprises a helical bundle held by three Zn fingers and is very similar to the solution structures determined for the shorter peptide [1, 2] corresponding to the evolutionarily conserved Taz2 domain from CBP and p300. Residues 1813-1834 from the current construct form a helical extension of the C-terminal helix and make extensive crystal contact interactions with the peptide binding site of Taz2. The structure thus provides information relevant to the specificity of CBP/p300 interactions with transcription factors.

[1] De Guzman R.N., Liu H.Y., Martinez-Yamout M., Dyson H.J., Wright P.E. *J. Mol. Biol.* **2000**, 303, 243. [2] Feng H., Miller Jenkins L.M., Durell S.R., Hayashi R., Mazur S.J., Cherry S., Tropea J.E., Miller M., Wlodawer A., Appella E., Bai Y. *Structure* 2009, 17, **2002**.

Keywords: transcription regulation; zinc finger protein; anomalous diffraction

FA1-MS07-P03**Human MST3 in Complex with Mn-ADP: Molecular Switch by Autophosphorylation.**

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MST is a subfamily of mammalian serine/threonine kinases related to the yeast sterile-20 protein implicated in regulating cell growth and transformation. The MST3 protein contains a 300-residue catalytic domain followed by a 130-residue regulatory domain, which can be cleaved by caspase. The enzyme is then activated by autophosphorylation, and promotes apoptosis. Here we present five crystal structures of the catalytic domain of MST3, including a complex with ADP and manganese, a unique cofactor preferred by the enzyme, and another complex with adenine. Similar to other protein kinases, the catalytic domain of MST3 folds into two lobes: the smaller N-lobe forms the nucleotide-binding site, and the larger C-lobe recognizes the polypeptide substrate and also participates in catalysis. The bound ADP and Mn ions are covered by a glycine-rich loop and held in place by Asn149 and Asp162. A different orientation is observed for the ligand of the MST3-adenine complex. In the activation loop of all structures, the side chain of the key residue Thr178 has been phosphorylated and sandwiched by Arg143 and Arg176, making the loop well ordered and clearly visible. A core segment containing two prolines,