Structure assisted inhibitor design on bovine seminal ribonuclease: The binding of uridilyl nucleotide inhibitors

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Bovine seminal ribonuclease (BS-RNase), a 27 kDa homodimeric RNase with 83 % sequence identity to RNase A is a potent antitumor agent [1]. The antitumor action of BS-RNase has been attributed to its ability to permeate only malignant cells and to reach the cytosol [2] where it degrades ribosomal RNA and blocks protein synthesis, causing cell death. BS-RNase is a promising antitumor agent and experiments have shown that administration of BS-RNase to animals with tumors has led to severe inhibition of neoplastic growth [3-6]. BS-RNase has a potential for therapeutic applications against cancer since it permeates only malignant cells, evades the potent proteinaceous cytoplasmic RNase inhibitor (RI) mainly due to its homodimeric structure [7] reaching the cytosol where it degrades ribosomal RNA blocking protein synthesis and thus causing cell death. It has also been shown that BS-RNase selectively kills human multidrug-resistant neuroblastoma cells via induction of apoptosis [8]. However, the antitumor action of BS-RNase is associated with severe side effects such as aspermatogenicity, immunosuppression and embryotoxicity [1, 9-14] that render the pharmaceutical use of BS-RNase problematic. A potential solution 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 this 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.

BS-RNase is the only member of the bovine pancreatic RNase A superfamily with a quaternary structure and a potential allosteric site [15]. Native BS-RNase is a dimer with two subunits linked by a disulfide bridge between cysteines 31 and 32. The dimer of BS-RNase is a mixture of two quaternary forms, which differ in the position of the N-terminal α-helix. In the first form (denoted MxM) the N-terminal α-helix (residues 1-15) is swapped between the two subunits generating two composite active sites consisting of residues that belong to different subunits. In the second form, (denoted M=M) there is no exchange of the helices and each monomer conforms an independent entity. The two forms exist in a dynamic equilibrium in the solution with a ratio of MxM/M=M of 2.

Although the crystal structure of BS-RNase has been determined more than a decade ago [16] structure-assisted inhibitor design studies have not been reported yet. The crystal structure of BS-RNase in complex with uridylyl-2',5'-adenosine has been reported [17]. The initiation of a structure-assisted inhibitor design effort requires a number of BS-RNase – inhibitor complex structures which could be used as the starting template for further design and optimization. Therefore we have studied by kinetic experiments and X-ray crystallography the binding of uridine 2' phosphate (2'UMP), uridine 3' phosphate (3'UMP), and uridine 5' diphosphate (UDP) at high resolution.

The three uridine compounds are moderate inhibitors of the enzyme with IC_{50} values of 1.6 mM, 2.2 mM and 2.9 mM respectively. The three inhibitors bind with the uracyl at subsite B_1 packed against the phenyl ring of Phe120 participating mainly in van der Waals interactions with Phe120 and hydrogen bond interactions with the side chain atoms of Thr45. The binding mode of 2'UMP differs in the two molecules of the BS-RNase homodimer with the position of the uracyl 120° apart in the two conformations. In molecule II the ribose and the phosphate group bind at subsites R_1 and P_1 respectively while in molecule I are oriented in the opposite direction towards subsite P_0. Despite

the different orientation of the uracyl ring it forms hydrogen bond interactions with the side chain of Thr45 but with different atoms in each case. 3'UMP binds similarly in both BS-RNase molecules with the ribose and the 3' phosphate group at subsites R_1 and P_1 respectively. In both protein molecules the interactions of the phosphate group include hydrogen bond interactions with the side chain atoms of residues Gln11, His12, Lys41 and His119. The inhibitor UDP also binds similarly in both BS-RNase molecules with the ribose at subsite R_1 but the pyrophosphate group away from P_1 in a position close to the protein surface (Fig.1).

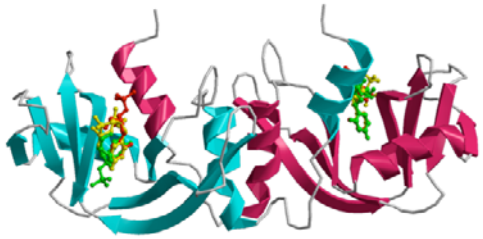

Figure 1: A schematic diagram of the BS-RNase homodimer with the three inhibitors bound at the active site.

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