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Capturing hammerhead ribozyme structures in action by modulating the rate of general base catalysis

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We have obtained pre-catalytic (enzyme-substrate complex) and post-catalytic (enzyme-product complex) crystal structures of an active full-length hammerhead ribozyme that cleaves in the crystal. Using the natural satellite tobacco ringspot virus (sTRSV) hammerhead RNA sequence, the self-cleavage reaction was modulated by substituting the general base of the ribozyme, G12, with A12, a purine variant with a much lower pKa that does not significantly perturb the ribozyme's atomic structure. The active but slowly cleaving ribozyme thus permits isolation of enzyme-substrate and enzyme-product complexes without modifying the nucleophile or leaving group of the cleavage reaction, nor any other aspect of the substrate. The pre-dissociation enzyme-product complex structure reveals RNA and metal ion interactions potentially relevant to transition-state stabilization that are absent in pre-catalytic structures.

Keywords: hammerhead ribozyme, general base catalysis, active conformation

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Ligand binding and structural rearrangements of quadruplexes containing human telomeric sequences

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Telomere maintenance is integral to the progression of human cancer and its disruption through the stabilization of novel G-quadruplex DNA structures by small molecule ligands is an attractive strategy for anti-cancer therapies. Knowledge of the biologically relevant folded topology adopted by these single-stranded telomeric sequences is critical for the design of drugs that selectively target and stabilize these structures. In an attempt to validate and design selective ligands we have used crystallographic techniques to provide a detailed understanding of the mode of ligand binding to human telomeric DNA. We will report on two classes of ligands that have co-crystallized in complex with both an intramolecular and a bimolecular quadruplex DNA of human telomeric sequence. A tetra-substituted naphthalene diimidine and the experimental anticancer drug BRACO-19, a 3,6,9-trisubstituted acridine. Both ligands have been shown to bind tightly to telomeric DNA, inhibit telomerase enzymatic activity resulting in telomere shortening. These crystal structures reveal that the quadruplex topology in both sequences is unchanged by the addition of the ligands, with the ligands binding to the external 5' and 3' planar G-tetrad surfaces. There is however, some remodelling to the previously observed TTA loop structures to provide additional sites for interaction. These new DNA/ligand structures have enabled us to identify the modes of ligand binding and apply a rational, structure based approach to design for these classes of ligands. Structural aspects of ligand interaction and design will be discussed along with future implications for selective quadruplex-binding ligands.

Keywords: anticancer drug structural study, DNA-ligand interactions, drug discovery and design

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Crystal structures of DNA-bound Co(III)·bleomycins

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Bleomycins constitute a widely studied class of complex DNA cleaving natural products that are used to treat squamous cell carcinomas, lymphomas, and testicular carcinomas. The bleomycins consist of a disaccharide-modified metal-binding domain connected to a bithiazole/C-terminal tail via a methylvalerate-Thr linker. Their mechanism of action is to induce DNA damage after oxygen activation through site-selective cleavage of duplex DNA at 5'GT/C sites. Using a host-guest crystallographic approach, we have determined the structures of two isoforms of Co(III)·bleomycin (A2 and B2) bound to 5'GT containing oligonucleotides. The host in this case is the N-terminal fragment of Moloney murine leukemia virus reverse transcriptase and the guest, a hexadecanucleotide including preferred 5'GT bleomycin binding sites. Both A2 and B2 isoforms of Co(III)·bleomycin were soaked into preformed host-guest crystals and their structures determined at 3.0 and 2.8 Å, respectively. Distinct modes of intercalation of the bithiazole/C-terminal tail domains of each isoform correlate with different orientations for the methylvalerate-Thr linker while retaining similar hydrogen bonding interactions between the linker and the DNA. Minor groove binding and base-specific hydrogen bonding of the metal binding and disaccharide domains is also retained in the two isoforms. Modeling of a hydroperoxide ligand coordinated to Co(III) suggests that the drug molecule is ideally positioned for C4'H abstraction. Our studies reveal that intercalation of the bithiazole/C-terminal domain is independent of ordered minor groove binding, that linker flexibility is necessary for the molecule to bind to the DNA, and that the disaccharide may play a more important role in DNA binding than was previously suggested.

Keywords: DNA-drug complexes, DNA-drug interactions, biological crystallography

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Molecular recognition and the DNA Holliday junction

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