INVESTIGATIONS INTO THE GENERALITY OF METALLOINSERTION AT DNA DEFECTS

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ABSTRACT

Metalloinsertors are substitutionally inert, octahedral transition metal complexes that bind to thermodynamically destabilized mismatched sites in duplex DNA with high affinity and selectivity. The complexes approach DNA from the minor groove, eject the mismatched bases into the major groove, and replace the displaced bases in the helical π-stack with their own sterically expansive ligands. Herein, we describe a series of five investigations aimed at elucidating the generality of metalloinsertion at DNA defects.

In an effort to develop a diagnostic for mismatched DNA, a bifunctional, mismatch-specific conjugate with rhodium metalloinsertor and fluorophore subunits has been constructed. A proof-of-concept conjugate was successfully produced that displays an almost fourfold fluorescence enhancement in the presence of mismatched versus matched DNA.

To investigate the range of metal complexes capable of mismatch-specific metalloinsertion, a ruthenium bisdipyridyl complex bearing the heptacyclic eilatin ligand has been synthesized and characterized. Electrophoresis competition experiments illustrate that the complex does display mismatch-preferential, though not necessarily mismatch-selective, binding.

To probe the generality of metalloinsertion at other common thermodynamically destabilized DNA defects, the binding of rhodium metalloinsertors at abasic sites and single base bulges has been studied. It was determined that metalloinsertors bind abasic sites with high affinity and specificity, without regard to the identity of the unpaired base and with little dependence on the sequence context of the defect. Single base bulge

recognition proved more elusive, with both the identity of the unpaired base and the sequence context influencing recognition.

To determine the structural generality of metalloinsertion, single crystal X-ray diffraction was employed to determine the structure of Δ-$Rh(bpy)_2(chrysi)^{3+}$ bound to an oligonucleotide duplex containing two A•A mismatches. Two structures were obtained at < 2 Å resolution, and each provides an archetypical picture of metalloinsertion: the bulky rhodium complex inserts into the mismatched site from the minor groove, ejecting the mismatched bases and replacing the displaced base pair with its own sterically expansive ligand.

Finally, two mismatch-specific conjugates have been designed for chemotherapeutic applications: a metalloinsertor-oxaliplatin conjugate for the selective delivery of platinum chemotherapeutics to mismatch repair deficient cells and a metalloinsertor-Auger electron emitter conjugate for the selective irradiation of mismatch-containing DNA.

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