DNA Cross-Linking with Metallointercalator-Peptide Conjugates†

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Abstract:

Short peptides have been tethered to a DNA-intercalating ruthenium complex to create a photoactivated cross-linking reagent. The ruthenium complex, $[Ru(phen)(bpy')(dppz)]^{2+}$ (phen = 1,10-phenanthroline, bpy' = 4-(butyric acid)-4'-methyl-2,2'-bipyridine, and dppz = dipyridophenazine), delivers the peptide to DNA and initiates the cross-linking reaction by oxidizing DNA upon irradiation in the presence of an oxidative quencher. The tethered peptide, only five to six residues in length, forms cross-links with the oxidized site in DNA. Cross-linking was detected and studied by gel electrophoresis and through spectroscopic measurements. The ruthenium-peptide complex is luminescent when bound to DNA, and the binding constants for several intercalator-peptide conjugates were determined by luminescence titration. The composition of the peptide affects both binding affinity and the extent of cross-linking. The greatest amounts of cross-linking were observed with tethered peptides that contained positively charged residues, either lysine or arginine. To test the impact of individual residues on cross-linking, the central residue in a 5-mer peptide was substituted with seven different amino acids. Though mutation of this position had only a small effect on the extent of cross-linking, it was discovered that peptides containing Trp or Tyr gave a distinctive pattern of products in gels. In experiments using the untethered peptide and ruthenium complex, it was determined that delivery of the peptide by the ruthenium intercalator is not essential for cross-linking; peptide attachment to the metal complex can constrain cross-linking. Importantly, the cross-linking adducts produced with ruthenium-peptide conjugates are luminescent and thus provide a luminescent cross-linking probe for DNA.