Design of a Synthetic Nuclease: DNA Hydrolysis by a Zinc-Binding Peptide Tethered to a Rhodium Intercalator

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The design of artificial nucleases represents an area of substantial interest.[1] While many reagents have been successfully applied to RNA hydrolysis,[2] there have been fewer successes with DNA[3,4] due to its relatively high hydrolytic stability.[5] We have approached the problem of designing a deoxyribonuclease by combining both DNA-binding and reactive moieties: (i) a rhodium intercalator which binds in the major groove with high affinity[6] and (ii) a tethered metallopeptide modeled after natural hydrolytic enzymes.[7] Previous work in our laboratory has shown that small peptides tethered to a rhodium complex bind DNA with high site specificity governed by the appended peptide.[8] Here we extend this strategy to create a synthetic endonuclease by incorporating zinc-promoted reactivity into the tethered peptide.[9]

Figure 1 shows a schematic illustration of our metal–peptide conjugate, $Rh(phi)_2bpy'$-Peptide (Rh-P). A short peptide, Asp-Pro-Asp-Glu-Leu-Glu-His-Ala-Ala-Lys-His-Glu-Ala-Ala-Ala-Lys-$CONH_2$, is tethered to $Rh(phi)_2bpy'$, a sequence-neutral intercalator, through a methylene linker derived from the modified bipyridine ligand. Complexes of rhodium(III) which contain a phenanthrenequinone diimine (phi) ligand bind in the major groove of DNA ($K_d < 10^{-6}$ M)[6] and upon photoactivation cleave DNA at the rhodium intercalation site.[10] The peptide tethered to the intercalator was designed *de novo* on the basis of the active sites of metal-containing hydrolases, well-
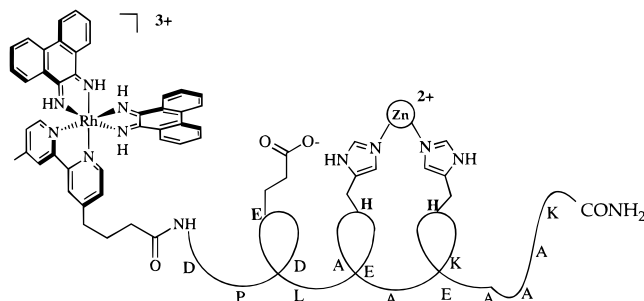

Figure 1. Metal–peptide conjugate, $Rh(phi)_2bpy'$-Peptide (Rh-P).

characterized metalloenzyme sites in which biopolymer hydrolysis occurs under mild conditions.[7] Two histidines were placed in positions 7 and 11 along the 16-residue peptide to create a zinc coordination site on one face of a putative α-helix. A glutamate was included at position 4, and glutamate–lysine salt bridges as well as space-filling alanines were added to encourage α-helicity.[11] The coupling of the metallointercalator to the peptide was carried out by methods described earlier.[12]

The ability of Rh-P to coordinate metal ions was confirmed by spectroscopy.[13] Consistent with the coordination of one metal ion to the two histidines, circular dichroism reveals that maximum helical content of Rh-P is reached with stoichiometric Zn^{2+} and decreases with additional metal ion.[14] Upfield shifts in the histidine resonances in ^1H NMR spectra with added Zn^{2+} also support coordination of one Zn^{2+} ion to the peptide.[15]

In the presence of stoichiometric Zn^{2+}, Rh-P is found to convert supercoiled pBR322 DNA (**I**) to both the nicked (**II**) and linear (**III**) forms (Figure 2). DNA cleavage is found to depend on the presence of the rhodium complex, the tethered peptide, and Zn^{2+}. The efficiency of DNA cleavage is

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(9) This synthetic flexibility contrasts our previous studies[3a] in which the systematic variations in the ligands were difficult. Moreover, the many amine ligands used for zinc coordination were problematic with respect to complex purification and the analysis of the DNA cleavage products by gel electrophoresis. See: Pustilnik, M. Ph.D. Thesis, California Institute of Technology, 1995.

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(11) Molecular modeling was carried out to verify that the intercalation of the rhodium complex could in fact deliver the active site of the peptide to an appropriate position to effect cleavage of the phosphodiester backbone.

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(14) On the basis of CD (Supporting Information), the α-helical content of Rh-P was calculated (Lehrman, S. R.; Tuls, J. L.; Lund, M. *Biochemistry* **1990**, *29*, 5590) to be 21% in the absence of Zn^{2+} and 27% with stoichiometric Zn^{2+}. These values compare favorably to metal-dependent variations seen earlier.[13b]

(15) NMR spectra (500 MHz) were obtained for the peptide alone (2.4 mM) in D_2O, pH 6.9, as a function of Zn^{2+} concentration. Gradual upfield shifts were observed with added Zn^{2+} for the protons on the $C\delta2$ carbons of the two imidazoles (6.96 and 7.02 ppm with no metal ion to 6.81 and 6.96 ppm, respectively, at 2.4 mM Zn^{2+}) and for the protons on the $C\epsilon1$ carbons (8.01 and 8.09 ppm with no metal ion to 7.89 and 7.94 ppm, respectively, at 2.4 mM Zn^{2+}).

(16) With higher Zn^{2+} concentration, the peptide may coordinate more than one zinc ion, thereby disrupting the designed site.

(17) For the kinetics experiments, the reactions were carried out as described in Figure 2A except the pH was varied (in 20 mM sodium citrate). Rate constants (k_{obs}) were determined on the basis of the disappearance of I_M.

(18) The rate constant increases with decreasing pH. Gite, S. U.; Shankar, V. *Crit. Rev. Microbiol.* **1995**, *21*, 101.

(19) Preliminary mutational studies also indicate that the glutamate does not participate in the mechanism.

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(23) When the oligonucleotide is labeled instead at the 3′-end, the products contain exclusively 5′-phosphate termini.

(24) To confirm the presence of 3′-hydroxyl termini, the 5′-labeled products were enzymatically extended at the 3′-end by one dideoxynucleotide using terminal transferase.

3380   *J. Am. Chem. Soc., Vol. 119, No. 14, 1997*

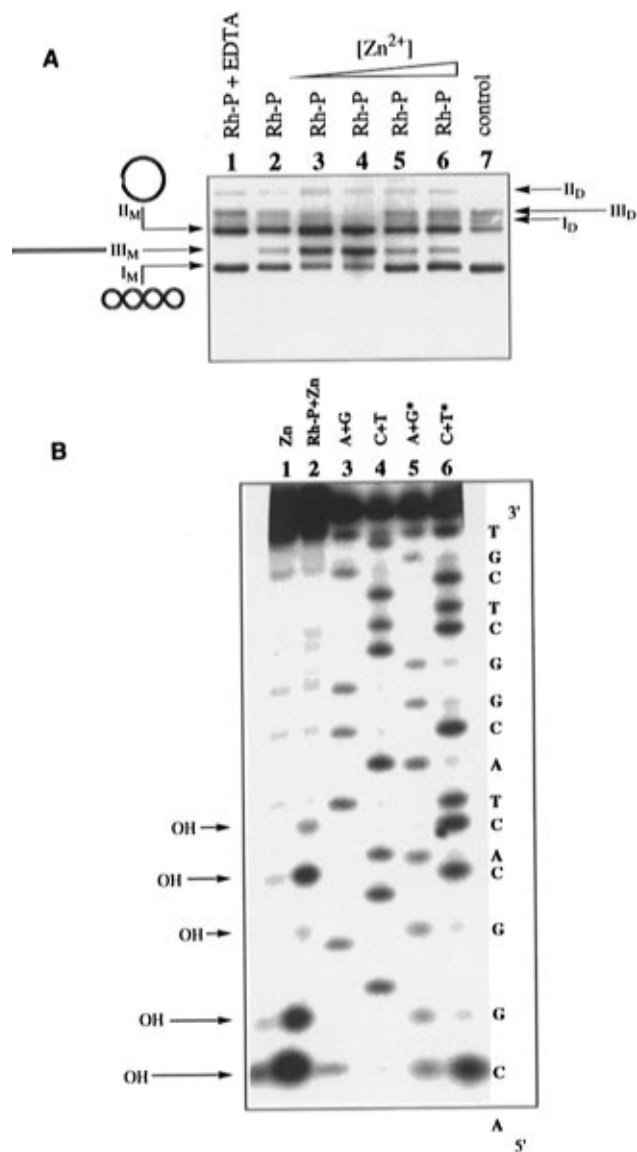*Communications to the Editor*

Figure 2. Analysis of the cleavage by Rh-P of (A) supercoiled pBR322 DNA by agarose gel electrophoresis and (B) a 17-base pair oligonucleotide by polyacryamide gel electrophoresis. (A) The reactions were carried out with pBR322 DNA (40 μM bp), Rh (5 μM), and ZnCl$_2$ in sodium borate (20 mM at pH 7.0) for 24 h at 37 °C. The reactions were quenched by the addition of a mixture of EDTA, sodium dodecyl sulfate, bromophenol blue, and xylene cyanol. The products were electrophoresed on a 1% agarose gel at 75 V for 4 h, and then stained with ethidium bromide. Lane 1: DNA + Rh-P and EDTA. Lane 2: DNA + Rh-P. Lane 3: DNA + Rh-P and 2.5 μM Zn^{2+}. Lane 4: DNA + Rh-P and 5.0 μM Zn^{2+}. Lane 5: DNA + Rh-P and 10.0 μM Zn^{2+}. Lane 6: DNA + Rh-P and 20.0 μM Zn^{2+}. Lane 7: DNA alone. Note pBR322 DNA contains dimeric supercoiled (**I$_D$**) and monomeric (**I$_M$**) forms. (B) A DNA oligonucleotide (d(ACGGCACTACGGCTCGT)) was [5'-^{32}P]-end-labeled and bound to its complement. The cleavage reactions were carried out with oligonucleotide (40 μM bp), Rh-P (5 μM), and ZnCl$_2$ (5 μM) in sodium citrate (20 mM at pH 6.5) for 24 h at 37 °C. The reactions were quenched by lyophilization, and the products were electrophoresed on a 20% polyacrylamide gel at 2000 V for 4 h. Lane 1: DNA + Zn^{2+}. Lane 2: DNA + Rh-P and Zn^{2+}. Lanes 3 and 4: The labeled strand was sequenced to give A + G and C + T products using standard Maxam–Gilbert methods. Lanes 5 and 6: T4 polynucleotide kinase was used to remove the terminal phosphates from the sequencing products to give the analogous products containing 3'-hydroxyl termini (A + G* and C + T*). The bands observed in lane 2 comigrated with the sequencing products containing 3'-hydroxyl termini, indicating that the DNA cleavage by Rh-P + Zn^{2+} yielded exclusively products which contained 3'-hydroxyl termini.

maximized at stoichiometric Zn^{2+}.[16] Little DNA cleavage is seen with Rh-P alone or with EDTA, the rhodium intercalator alone, the peptide with Zn^{2+} but no intercalator, or Zn^{2+} alone (Supporting Information). The rhodium intercalator therefore serves to deliver the reactive Zn^{2+}-coordinated peptide to the DNA helix, making efficient cleavage of DNA possible at micromolar concentrations.

The initial rate constant, k_{obs}, for the cleavage of pBR322 DNA by Rh-P at pH 6.0 is found to be $(2.5 \pm 0.2) \times 10^{-5}$ s^{-1}.[17-19] This value is comparable to those recently determined using complexes of Eu(III) and Co(III)[4] and all represent rate enhancements of approximately 10^{11} over that for uncatalyzed[20] DNA hydrolysis. Unlike the Eu(III) and Co(III) complexes, however, here efficient DNA cleavage is obtained with micromolar concentration of Rh-P, owing to the strong binding affinity of the intercalating moiety.

To provide direct evidence for hydrolytic chemistry at the phosphodiester linkage by Rh-P, the termini of oligonucleotide cleavage products were examined. DNA hydrolysis yields only phosphate and hydroxyl termini.[21] The products of DNA oligonucleotide cleavage containing hydroxyl termini can be distinguished using high-resolution polyacrylamide gel electrophoresis in which band migrations are compared with Maxam–Gilbert sequencing products containing either phosphate or hydroxyl termini.[22] Product analysis was therefore carried out for the cleavage of a 17-base-pair oligonucleotide duplex by Rh-P with Zn^{2+}. Again the rhodium intercalator, the peptide, and Zn^{2+} are all found to be necessary for DNA cleavage. Figure 2 shows that the products of cleavage of the 5'-end-labeled oligonucleotide contain fragments of DNA with 3'-hydroxyl termini.[23,24] This observation provides direct evidence for a hydrolytic cleavage reaction.

Several aspects of this cleavage are noteworthy. First 3'-hydroxyl but not 3'-phosphate termini are observed which indicates a stereospecific reaction by Rh-P. This stereospecificity may be the result of hydrolytic attack from the major groove of DNA. Moreover, a quantitative comparison of the reactions on pBR322 DNA and the oligonucleotide indicates that cleavage of linear DNA by Rh-P is equally efficient as the cleavage of supercoiled plasmid. It can therefore be concluded that the strain on the helix which results from supercoiling is not necessary for cleavage by Rh-P.[25]

These results indicate directly that the hydrolysis of DNA, indeed linear DNA duplexes, can be accomplished under mild conditions with a synthetic complex at low concentration. Hence, the construction of metal–peptide conjugates combining DNA-binding and reactive moieties for the hydrolysis of DNA represents a new strategy for the design of artificial restriction enzymes.

Supporting Information Available: Synthetic details for the preparation of Rh(phi)$_2$bpy′-Peptide and its spectral characterization, circular dichroism of Rh-P as a function of Zn^{2+}, and table of pBR322 cleavage (6 pages). See any current masthead page for ordering and Internet access instructions.

JA9633981

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(25) Strain on the DNA helix caused by the intercalation of the rhodium complex cannot be ruled out, however.