DNA Recognition by Peptide Complexes of Rhodium(III): Example of a Glutamate Switch

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Abstract: A family of metal–peptide complexes has been synthesized by coupling short oligopeptides (13 residues) onto the metallointercalating $[Rh(phi)_2(phen')]^{3+}$ (phi = 9,10-phenanthrenequinone diimine; phen' = 5-(amidoglutaryl)-1,10-phenanthroline). These complexes were prepared to explore whether the side-chain functionalities of small peptides may be used to augment metal complex recognition. The metal–peptide complexes bind and, with photoactivation, cleave DNA. The DNA site-specificity is seen to depend on the peptide side-chain functional groups. In particular, a single glutamate at position 10 is found to be essential in directing DNA site-recognition to the sequence 5′-CCA-3′. Methylation of the glutamate side chain or direct substitution of glutamine for glutamate abolishes the 5′-CCA-3′ selectivity, while substitutions at other likely DNA-binding residues show no appreciable change in selectivity. Significantly, the 5′-CCA-3′ selectivity is even sensitive to a highly conservative E10D substitution. DNA photocleavage of oligonucleotides by the metal–peptide complexes and HPLC analysis of DNA products provide evidence for major groove chemistry. Circular dichroism indicates significant α-helical content in the peptide, which depends upon the presence of the glutamate. A model for the glutamate-dependent site-selectivity is presented using shape-selective intercalation of the metal complex and base-specific contacts of the ancillary peptide. These monomeric metal–peptide complexes appear to serve as particularly useful mimics for larger site-specific DNA-binding proteins and may provide a basis for the design of an array of small, sequence-specific DNA-binding metal complexes.

Introduction

There has been considerable interest in DNA recognition by small molecules and large peptides to establish essential elements for site-specificity.[1,2] Our laboratory has focused on the construction of coordinatively saturated octahedral metal complexes for site-specific DNA recognition.[3-6] In particular, 9,10-phenanthrenequinone diimine (phi) complexes of rhodium(III) have been prepared which bind avidly through intercalation in the DNA major groove[4] ($K_a \geq 10^6\ M^{-1}$) and upon photoactivation promote strand scission via abstraction of the deoxyribose C3′-hydrogen atom.[5] Recently we reported the assembly of complexes which, like DNA-binding proteins, pose several functionalities for site-specific, noncovalent interaction with DNA.[6] We report here the design of metal–peptide complexes (Figure 1) based upon attachment of short oligopeptides (13 residues) onto the metallointercalating skeletal complex, $[Rh(phi)_2(phen')]^{3+}$ (Sk, phen' = 5-(amidoglutaryl)-1,10-phenanthroline). Our interest is in exploring whether the side-chain functionalities of small peptides may be used augment metal complex recognition.

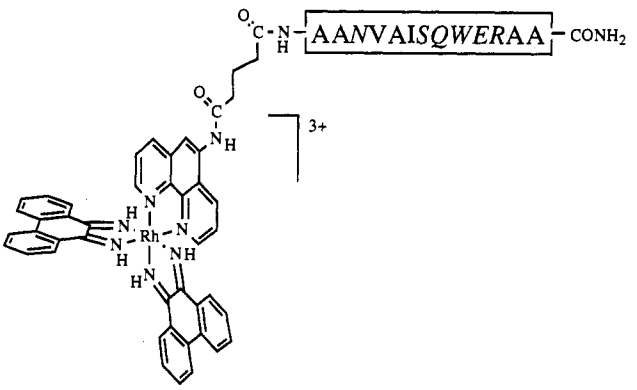

Figure 1. Representative metal–peptide complex, Sk-PA, showing linkage of the peptide to the skeletal complex. The italicized residues have been substituted in the family of metal–peptide complexes synthesized.

Studies of peptide recognition of DNA have focused mainly on dimeric DNA-binding domains of proteins. Different strategies to dimerize α-helical peptides have included the incorporation of leucine zippers or adamantane–β-cyclodextrin tethers in noncovalent dimeric assemblies[7] and disulfide or modified $Fe(trpy)_2^{3+}$ or biphenyl linkages in covalent dimers.[2] In each case, specific DNA binding has required dimerization of the peptides, likely because the monomeric peptides lack the nonspecific binding affinity that is present in the intact protein. Several examples of tethering DNA-cleaving moieties such as Fe-EDTA[2e] or $[(1,10\text{-phenanthroline})Cu]^+$,[8] onto DNA-binding proteins have also been reported in an effort to engineer new sequence-specific nucleases; this approach has been used elegantly to elucidate the binding modes and relative orientations of the DNA-binding proteins.[9] However, an important distinction in all these examples

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is that the peptide dimers or protein fragments have been large (>20 residue/monomer) and exhibit specific DNA affinity that is independent of the attached cleaving functionality.

Our rationale behind the design of small metal–peptide complexes is the application of $[Rh(phi)_2(phen)]^{3+}$ as a delivery system for an array of peptide functionalities to the major groove of DNA. This design takes advantage of the octahedral metallointercalator as a small molecular anchor in the major groove.[4,5] The skeletal complex provides DNA-binding affinity, while the attached peptide functionalities may be varied to achieve site-specific DNA recognition. By varying systematically the sequence of short, monomeric peptides, we may explore the targeting of a variety of DNA sequences. The DNA photocleavage chemistry of the skeletal complex[5] also serves as a convenient tag for assaying DNA site-selectivity. Here we have chosen to investigate the DNA recognition properties of the α_3-helix of the DNA-binding phage P_{22} repressor[10] in isolation from the rest of the protein. The helix is small (eight residues) and contains an array of side-chain functionalities; the understanding of P_{22} targeting is unclear. A family of metal–peptide complexes was therefore constructed, and their DNA-binding characteristics were examined. Remarkably, we find that a single glutamate is essential in directing site-specific recognition in these complexes.

Experimental Section

Materials. Sonicated calf thymus DNA was purchased from Pharmacia, plasmid pUC 18 was purchased from Boehringer-Mannheim, and all enzymes utilized were from commercial sources. [α-^{32}P]dATP and [γ-^{32}P]ATP were obtained from NEN-Dupont. Oligonucleotides were synthesized by the phosphoramidite method,[11] using 1.0 μmol columns on an ABI 391 DNA–RNA synthesizer.

Instrumentation. Absorption spectra and quantitation of the metal complexes were recorded on a Cary 219 spectrophotometer. HPLC was carried out on a Waters 600E system equipped with a Waters 484 tunable detector. All photocleavage experiments were carried out with an Oriel Model 6140 1000 W Hg/Xe lamp fitted with a monochromator and a 300 nm cutoff filter to avoid light damage to DNA. CD experiments were performed on a Jasco J-600 spectrometer. Gel electrophoresis experiments were quantified using a Molecular Dynamics phosphorimager and ImageQuant software.

Synthesis of the Metal–Peptide Complexes. The peptides were synthesized on PAM resin by automated solid-phase peptide synthesis using *N-t*-Boc-protected amino acids. The family of metal–peptide complexes was constructed individually on the solid support either by coupling of Sk onto the amino terminus of the oligopeptide or by coordination of the precoupled phen′-peptide to $[Rh(phi)_2(DMF)_2]^{3+}$. The metal–peptide complexes are stable to standard peptide deprotection and cleavage conditions. The complexes were purified by HPLC on a Vydac C-18 protein and peptide reverse-phase column using a water (0.1% TFA)–acetonitrile (0.1% TFA) gradient. A single peak corresponding to a mixture of diastereomers (Λ and Δ at the metal complex) was isolated in each case. The metal–peptide complexes display the additive spectral and chemical characteristics of Sk and the isolated peptides. Molecular ion peaks observed (and calculated) for the different complexes by mass spectroscopy are as follows: Sk 824.6 (824.7); Sk-PA 2193.6 (2193.3); Sk-PB 2150.3 (2149.2); Sk-PC 2105.1 (2107.2); Sk-PC-OMe 2119.8 (2121.2); SK-PD 2118.5 (2119.2); SkhPE 2145.8 (2147.3); Sk-PF 2204.6 (2205.6); Sk-PG 2146.1 (2148.3); Sk-PH 2105.4 (2105.5). UV–visible spectra: ϵ_{350} = 23 600 M^{-1} cm^{-1}, and at pH 3.2, ϵ_{382} = 32 600 M^{-1} cm^{-1}. Amino acid analyses for the metal–peptide complexes are as predicted on the basis of the peptide sequence and are stoichiometric with skeleton content. The details of the synthesis and characterization will be published elsewhere.[12]

Photocleavage of DNA Restriction Fragments. Plasmid pUC-18 was digested with *Eco*RI restriction endonuclease. The digested plasmid was 3′-end-labeled by treatment with Klenow fragment of the DNA polymerase I and [α-^{32}P]dATP, dTTP, dCTP, and dGTP.[13] A separate batch of the digested plasmid was treated with calf intestinal alkaline phosphatase and 5′-end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP.[13] After labeling, the DNA was digested with *Pvu*II to yield a 180 and 140 base pair fragment. The 180 base pair fragment was isolated by 8% nondenaturing preparative polyacrylamide gel electrophoresis followed by electroelution.

Cleavage reactions were carried out in 20 μL total volume contained in 1.7-mL presiliconized eppendorf tubes. The reaction mixtures contained 50 μM (nucleotide) calf thymus DNA, labeled restriction fragment (~40 000 cpm per sample) and 1.0 μM rhodium complex in 50 mM sodium cacodylate, pH 7.0. Reaction mixtures were incubated in the presence (or absence) of 5 mM $MnCl_2$ at 55 °C (or room temperature) for 5 min and then irradiated at the same temperature at 313 nm, as indicated. Controls without metal complex were also irradiated in parallel to test for light damage and cleavage by Mn^{2+}.

All samples were ethanol precipitated after irradiation by addition of 10 μL of 7.5 M NH_4OAc and 130 μL of ethanol. The precipitated DNA was dried and resuspended in 3 μL of 80% formamide loading buffer. The samples along with Maxam–Gilbert G+A and C+T sequencing reactions[13,14] were loaded onto an 8% denaturing polyacrylamide gel and electrophoresed at 1600 V for approximately 135 min. The gel was transferred to paper and dried prior to autoradiography.

HPLC Analysis of Photocleavage Products. Reaction samples (40 μL total volume) were made to contain 1 mM (in base pairs) of a 32 base pair of oligonucleotide duplex (5′-TGACTTTAAAGGTACCAATAT-TCCTAGGCAGT-3′ and the complementary strand) in 50 mM ammonium formate. The samples were incubated in the presence or absence of 50 μM Sk-PG for 5 min and irradiated at 313 nm for 10 min at ambient temperature. After irradiation, the samples were analyzed by HPLC on a Cosmosil 5 μm, 15 cm C-18 reverse-phase column under isocratic elution condition of 50 mM ammonium formate (flow rate 1.5 mL/min). The products were detected at 260 and 300 nm.[5]

Photocleavage of Oligonucleotides. A 31 base pair oligonucleotide with the sequence 5′-GCCACGAGCCACGACGAGCCACGAAC-GAGCC-3′ was 5′-end-labeled using T4 polynucleotide kinase and [γ-^{32}P]-ATP. The cold single strands corresponding to the 31 base pair duplex and the labeled strand were annealed by heating up to 90 °C followed by slow cooling to room temperature. Reaction samples contained 50 μM base pair oligonucleotide (including ~120 000 cpm of labeled oligonucleotide), 1.0 μM rhodium complex, and 5 mM $MnCl_2$ in 50 mM sodium cacodylate, pH 7.0. Samples were incubated at 55 °C for 5 min and irradiated at 55 °C for 15 min, as before. Approximately 20 000 cpm/sample was transferred into fresh eppendorf tubes, dried, and then resuspended in 3 μL of loading buffer. The samples were loaded onto a 20% denaturing polyacrylamide gel and electrophoresed at 2000 V for 3 h 15 min. The gel was then transferred to a film and stored at −70 °C during autoradiography.

Circular Dichroism Studies. Samples of the metal–peptide complexes were prepared in 10 mM sodium phosphate or 10 mM Tris-HCl, pH 7.0. CD spectra were recorded in a 0.1 cm path length cell at room temperature and at 55 °C using a temperature-controlled water-circulating bath. The effect of temperature and the presence of 5 mM Mn^{2+} was measured over the range 350–200 nm.

Results

DNA Cleavage by a Metal–Peptide Complex. Photocleavage of DNA restriction fragments by the first member of the family of metal–peptide complexes, Sk-PA, was investigated under varying reaction conditions. As shown in Figure 2, Sk-PA promotes DNA strand scission upon photoactivation similar to the skeletal complex lacking the peptide. The efficiency of photocleavage is comparable to that for other phi complexes of rhodium.[5] Importantly, highly specific cleavage is apparent at

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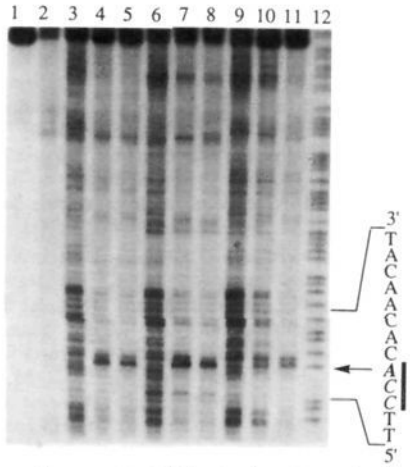

Figure 2. Autoradiogram of an 8% denaturing polyacrylamide gel showing photocleavage of the 180 base pair [5′-^{32}P]-end-labeled *Eco*RI/*Pvu*II fragment of plasmid pUC18 by Sk and Sk-PA in the presence of various reagents: untreated fragment (lane 1); fragment irradiated in the absence of rhodium complex (lane 2); fragment irradiated in the presence of 1 μM Sk and 5 mM Mg^{2+} (lane 3), 5 mM Mn^{2+} (lane 6), or 10 vol % trifluoroethanol (lane 9); fragment irradiated in the presence of 1 μM Sk-PA and 5 mM Mg^{2+} (lane 4), 10 mM Mg^{2+} (lane 5), 5 mM Mn^{2+} (lane 7), 10 mM Mn^{2+} (lane 8), 10 vol % trifluoroethanol (lane 10), or 25 vol % trifluoroethanol (lane 11); Maxam–Gilbert A+G sequencing reaction (lane 12). The samples containing Mg^{2+} or Mn^{2+} were incubated and irradiated at 55 °C, while those containing trifluoroethanol were at room temperature. Incubation time in every case was 5 min, and the samples were irradiated for 20 min.

the site 5′-CCA-3′, with cleavage at the highlighted A. Similar specific cleavage is not evident with the skeletal complex lacking the peptide. This site-specificity is enhanced upon the addition of reagents such as trifluoroethanol or in the presence of divalent cations (Mn^{2+}, Mg^{2+}) at elevated temperature (55 °C).

Site-Selectivity on DNA Restriction Fragments by the Family of Metal–Peptide Complexes. Photocleavage of DNA with Sk-PA indicates that the peptide is essential in directing the metal complex to recognize the 5′-CCA-3′ site. In order to identify the residues involved in this selectivity, we mutated residues with functional side chains to alanine individually or in pairs. The family of metal–peptide complexes thus constructed, as shown in Table 1, includes Sk-PB (N3A), Sk-PC (R11A), Sk-PD (S7A/Q8A), and Sk-PC-OMe (E10-methyl ester). We also synthesized Sk-PE, Sk-PF, and Sk-PG to ascertain the role of Glu10 and Trp9 in recognition. These three complexes have, in addition, two lysine substitutions, which we envisioned would increase the overall binding affinity of the metal–peptide complexes.

Table 1. Family of Metal–Peptide Complexes and Their DNA Recognition Sequences

metal complex	metal–peptide sequence[a,b]	DNA recognition site[c]
Sk[d]	[Sk- COO^-]$^{2+}$	
Sk-PA	[Sk- AANVAISQW**E**RAA-$CONH_2$]$^{3+}$	5′-CC**A**-3′
Sk-PB	[Sk- AAAVAISQW**E**RAA-$CONH_2$]$^{3+}$	5′-CC**A**-3′
Sk-PC	[Sk- AANVAISQW**E**AAA-$CONH_2$]$^{2+}$	5′-CC**A**-3′
Sk-PC-OMe[e]	[Sk- AANVAISQW<u>E</u>AAA-$CONH_2$]$^{3+}$	
Sk-PD	[Sk- AANVAIAAW**E**RAA-$CONH_2$]$^{3+}$	5′-CC**A**-3′
Sk-PE	[Sk- AAKVAISQKQRAA-$CONH_2$]$^{6+}$	
Sk-PF	[Sk- AAKVAISQWKRAA-$CONH_2$]$^{6+}$	
Sk-PG	[Sk- AAKVAISQK**E**RAA-$CONH_2$]$^{5+}$	5′-CC**A**-3′
Sk-PH	[Sk- AANVAIAAWDRAA-$CONH_2$]$^{3+}$	

[a] The skeletal complex has a free carboxylate. The carboxy terminus of all the peptides is present as an amide. The overall charge shown on each metal–peptide complex is that expected at pH 7.0 assuming that the imine-nitrogen atoms on the phi ligands are fully protonated. [b] When present, the essential glutamate at position 10 is indicated in bold. [c] The primary photocleavage site is indicated in bold. [d] Sk denotes the skeletal complex, $[Rh(phi)_2(phen')]^{3+}$. [e] The underlined glutamate has its side-chain carboxylate as a methyl ester.

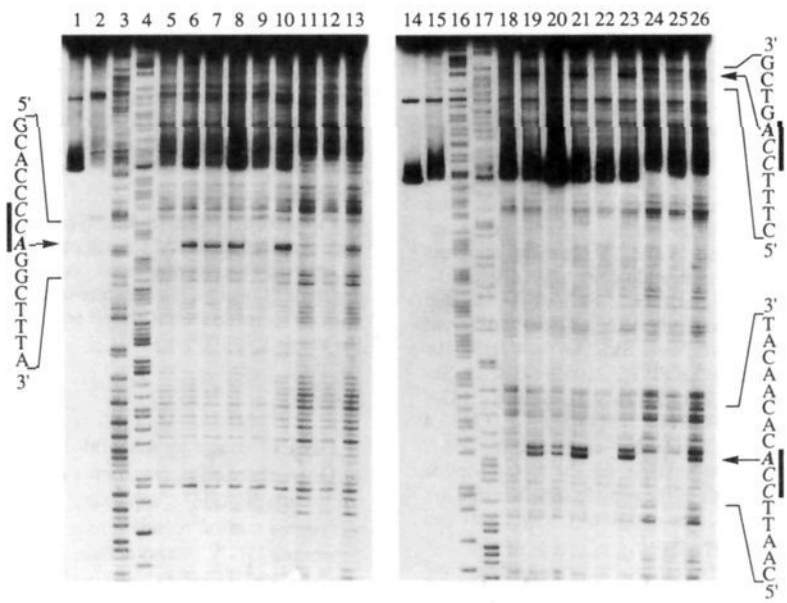

Figure 3. DNA photocleavage by the family of metal–peptide complexes: autoradiograms showing cleavage reactions on the 180 base pair fragment [3′-^{32}P]-end-labeled (lanes 1–13) or [5′-^{32}P]-end-labeled (lanes 14–26) at the *Eco*RI site. The reaction samples were incubated in the presence of 5 mM $MnCl_2$ for 5 min and irradiated (313 nm) for 10 min at 55 °C: lanes 1 and 14, DNA control; lanes 2 and 15, fragment irradiated in the absence of rhodium complex; lanes 3 and 16, Maxam–Gilbert A+G sequencing reaction; lanes 4 and 17, Maxam–Gilbert C+T sequencing reaction; lanes 5–13 and 18–26, fragment irradiated in the presence of 1 μM Sk, Sk-PA, Sk-PB, Sk-PC, Sk-PC-OMe, Sk-PD, Sk-PE, Sk-PF, and Sk-PG, respectively. The DNA recognition sequence is shown in italics. The cleavage site is in bold and indicated by an arrow. Note that the 5′-CCA-3′ recognition sequence is preferentially cleaved by the family of metal–peptide complexes except by Sk-PC-OMe, Sk-PE, and Sk-PF, all of which lack a free carboxylate at position 10.

All the metal–peptide complexes promote DNA cleavage when photoactivated, as shown in Figure 3. A subset of the complexes show high specificity for 5′-CCA-3′ sites, with cleavage at the highlighted adenosine. The cleavage is also severely canted to one strand, with no specific cleavage being detected across the site on the complementary strand. Strong specific cleavage at any other 5′-pyr-pyr-pur-3′ sequence, for example, 5′-TCA-3′, 5′-CCG-3′, or 5′-TCG-3′, is not apparent. Importantly, as illustrated in Table 1, *the site-specificity requires glutamic acid at position 10 in the peptide*. Transposing the glutamate to position 6 in the context of a different metal–peptide complex does not afford selectivity for 5′-CCA-3′ (data not shown). Methylation of the glutamate side chain, as with Sk-PC-OMe, or a direct E10Q substitution, as in Sk-PG and SK-PE, abolishes the 5′-CCA-3′ selectivity. Additionally, the skeletal complex, which also has a free terminal carboxylate, does not target 5′-CCA-3′, implying that a carboxylate in the vicinity of the DNA helix is not sufficient to impart specificity.

Substitutions at other residues with functional side chains that may bind to DNA[15] do not alter the site-selectivity appreciably. However, these substitutions perturb the intensity of cleavage at the 5′-CCA-3′ site, which may correlate with structural variations that arise with these mutations. Of particular note is Trp9, which does enhance specificity. Sk-PG, which lacks the tryptophan but contains the glutamate, targets 5′-CCA-3′, but more moderately. However, tryptophan alone, without the neighboring glutamate (as with Sk-PF), is not sufficient for weak recognition of the target sequence. These peptide complexes therefore share a distinct DNA recognition motif, governed by a single specificity determinant, but otherwise capable of tolerating a variety of substitutions.

Differences between Aspartate and Glutamate at Position 10. To investigate further the role of the glutamate at position 10, we synthesized an E10D mutant of Sk-PD, Sk-PH. Sk-PD shows the strongest recognition of the 5′-CCA-3′ sequence amongst this family of metal–peptide complexes, and hence we believed that this complex would be the most tolerant of minor variations in peptide sequence. Figure 4 shows a direct comparison of Sk, Sk-PD, and Sk-PH (Sk-PD-E10D) under different reaction conditions. The autoradiogram clearly shows the recognition to be contingent on the presence of a glutamate. Even a very conservative substitution to an aspartate is not tolerated.

It should be pointed out here that Sk-PD shows modest 5′-CCA-3′ selectivity even at room temperature in the absence of 5 mM Mn^{2+} in contrast to Sk-PA. Temperature and Mn^{2+} ions increase the cleavage individually, while having both present makes the selectivity the strongest. This observation suggests that the peptide structure, dictated by the sequence, is important for recognition.

It is noteworthy also that free peptide, untethered to the metal complex, does not bind comparably, even at much higher concentrations than the metal–peptide complex. In competition experiments with free peptide, it is found that 100 μM PD does not inhibit binding of 1.0 μM Sk-PD (Figure 4, lane 1). Also, in control experiments, PD in the presence of 5 mM Mn^{2+} did not cleave DNA upon irradiation.

Photocleavage of Oligonucleotides by Metal–Peptide Complexes. HPLC analysis of the photocleavage of an oligonucleotide duplex substrate by Sk-PG shows the production of base propenoic acid and free nucleic acid base products. Peaks eluting at 1.4 min (base propenoic acids), 2.0 min (cytosine), 4.0 min (guanine), and 4.7 min (thymine) under the HPLC conditions were observed. The production of the base propenoic acids (evidence for major groove chemistry)[5] was substantiated by monitoring the HPLC elution trace at 260 and 300 nm. Increasing the detection wavelength from 260 to 300 nm increases the ratio of the height of the propenoic acids peak to the nucleic acid base peaks.

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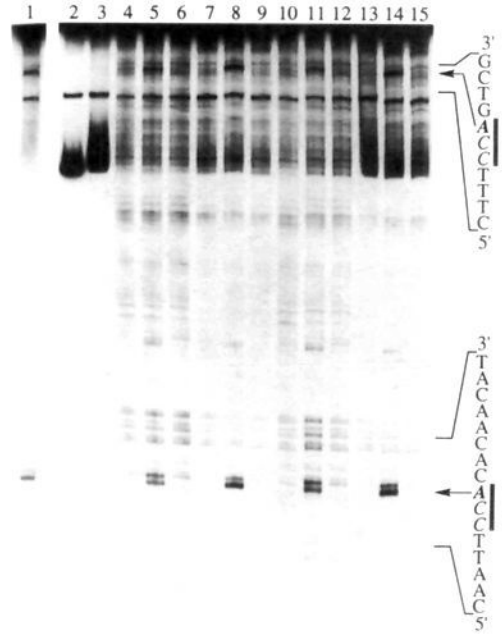

Figure 4. Photocleavage of the [5′-^{32}P]-end-labeled 180-mer DNA restriction fragment by Sk, Sk-PD, and Sk-PH comparing E^{10} and D^{10} in the presence or absence of 5 mM $MnCl_2$ and elevated temperature. Lanes 1–3, 7–9, and 13–15 contained 5 mM $MnCl_2$. Lanes 1–3 and 10–15 were incubated and irradiated at 55 °C, while lanes 4–9 were treated at room temperature. The reaction samples were incubated for 5 min and irradiated (313 nm) for 8 min at the appropriate temperature in the presence of 1.0 μM Sk (lanes 4, 7, 10, 13); 1.0 μM Sk-PD (lanes 1, 5, 8, 11, 14); or 1.0 μM Sk-PH (lanes 6, 9, 12, 15). Untreated fragment is shown in lane 2, and fragment irradiated in the absence of rhodium complex is shown in lane 3. Lane 1 shows cleavage by Sk-PD in the presence of 100 mM PD (free peptide), which serves as a competitor for Sk-PD binding to DNA. The 5′-CCA-3′ cleavage sites are indicated on the side.

Photocleavage of restriction fragments by the metal–peptide complexes is observed at the adenosine of 5′-CCAN-3′. This cleavage is consistent with intercalation in the 5′-AN-3′ base step from the major groove by analogy to cleavage observed with other related phi complexes of rhodium.[5] However, the 5′-end-labeled DNA cleavage experiments (Figures 2, 3, and 4) also reveal a second cleavage band migrating approximately with the base to the 3′-side of the cleavage site. The relative intensity of this secondary band was found to vary across the series of metal–peptide complexes. Since the resolution of these gels does not allow one to discriminate whether the band results from cleavage at an adjacent site or may correspond to a slower migrating product with a different 3′-terminus, a 31 base pair oligonucleotide duplex was synthesized containing three 5′-CCA-3′ sites to distinguish between these possibilities. Photocleavage experiments were then carried out with Sk, Sk-PD, and Sk-PH and compared to cleavage by $[Rh(phi)_2(bpy)]^{3+}$ (Figure 5). Again, only Sk-PD showed strong cleavage at the 5′-CCA-3′ sites. The four complexes all showed cleavage bands corresponding to 3′-phosphate and 3′-phosphoglycaldehyde termini on a 20% denaturing polyacrylamide gel following photocleavage of 5′-end-labeled oligonucleotides.[5] The formation of the 3′-phosphoglycaldehyde terminus results from an oxygen-dependent strand scission pathway and is sensitive to the accessibility of dioxygen to the cleavage site. Consistent with this proposal is the observation that $[Rh(phi)_2(bpy)]^{3+}$ yields a greater amount of the 3′-phosphoglycaldehyde terminus (relative to the 3′-phosphate) compared to $[Rh(phi)_2(phen')]^{3+}$. Sk-PD shows the least amount of the 3′-phosphoglycaldehyde product, suggesting that the incipient C3′ radical produced is better shielded from dioxygen by the attached peptide. In the case of Sk-PD, moderately strong cleavage was also observed at the base 3′ to

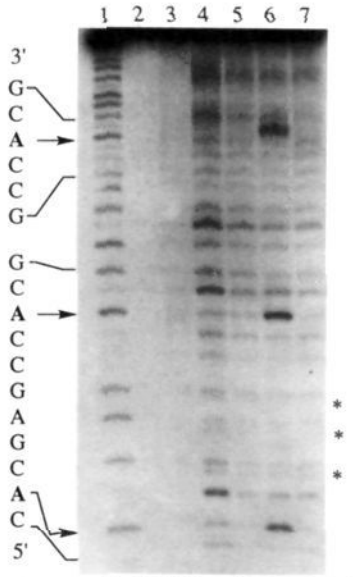

Figure 5. Photocleavage of a [5′-^{32}P]-end-labeled 31 base pair oligonucleotide duplex with $[Rh(phi)_2(bpy)]^{3+}$, Sk, Sk-PD, and Sk-PH to compare 3′-terminii produced: Maxam–Gilbert A+G sequencing reaction (lane 1); untreated oligonucleotide duplex (lane 2); oligonucleotide duplex irradiated in the absence of rhodium complex (lane 3); duplex irradiated in the presence of 1.0 μM $[Rh(phi)_2(bpy)]^{3+}$ (lane 4), Sk (lane 5), Sk-PD (lane 6), and Sk-PH (lane 7). The major cleavage products are 3′-phosphate termini which comigrate with the Maxam–Gilbert sequencing products, while the secondary products corresponding to 3′-phosphoglycaldehyde termini are indicated by the asterisks. The arrows represent cleavage at the three 5′-CCA-3′ sites present on the duplex.

the 5′-CCA-3′ cleavage site that appeared to comigrate with the corresponding 3′-phosphate of the oligonucleotide containing an additional nucleotide. This observation may be the result of intercalation of the metal complex in two neighboring base steps, both of which allow for proper orientation of the tethered peptide along its target site.

Circular Dichrosim of Metal–Peptide Complexes. Circular dichroic spectra of Sk-PD (Figure 6a), which specifically targets 5′-CCA-3′, and Sk-PH (Figure 6b), which does not, were recorded in the presence and absence of 5 mM Mn^{2+} and heat to explore correlations between site-recognition and peptide folding. Both the metal–peptide complexes show slight increases in helicity (value of θ_{222}) upon addition of 5 mM Mn^{2+} and heating to 55 °C. However, the striking difference between the two complexes lies in their inherent helicity.[16] Sk-PD is 72% helical in buffer at room temperature (89% in the presence of Mn^{2+} and heat), while Sk-PH is calculated to be only 8% helical (increasing to 18%) under identical conditions. This conformational difference between the two complexes may account for the observed sensitivity of the glutamate in DNA recognition.

CD spectra were also recorded of the free peptides PD (H_2N-AANVAIAAWERAA-$CONH_2$) and PH (H_2N-AANVAIAAW-DRAA-$CONH_2$) in 10 mM Tris-HCl, pH 7.0, at ambient temperature with and without 5 mM Mn^{2+} (Figure 6c). PH appears to adopt a random conformation under these conditions, while PD may show some helical content. It is noteworthy that coupling the skeleton onto the peptide enhances the helicity of the peptide (perhaps by virtue of capping the N-terminus, despite the positive charge of the skeleton).

A related series of spectra taken with Sk-PG and Sk-PE (Sk-PG-E10Q) at 5 μM concentration indicated only modest helicity that increased slightly upon addition of Mn^{2+} and heat (data not shown). No differences were detected between the two metal–peptide complexes. This result is consistent with Sk-PG showing weaker cleavage at the 5′-CCA-3′ site compared to Sk-PD, even though both contain E10, while Sk-PE does not cleave the site at all.

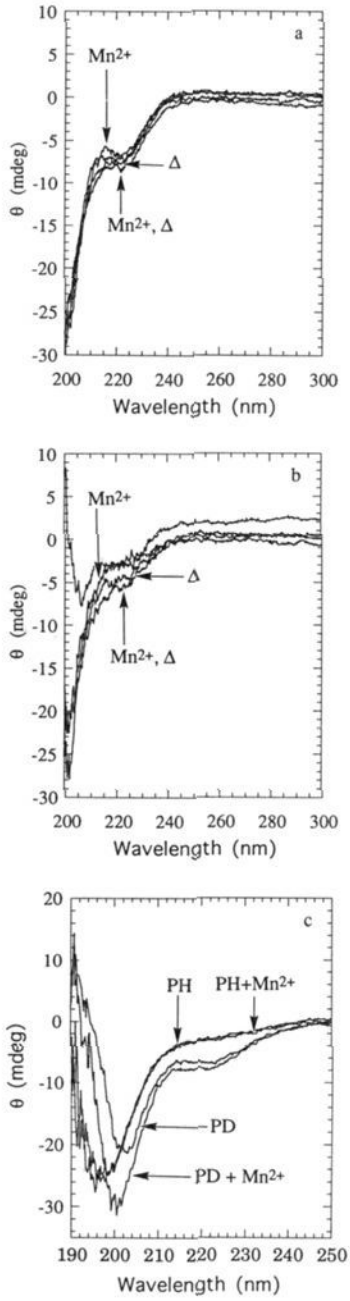

Figure 6. Circular dichroism of Sk-PD, Sk-PH, PD, and PH in 10 mM Tris-HCl, pH 7.0. The spectra of the metal–peptide complexes were recorded over the 350–200-nm range in buffer at room temperature, with the addition of 5 mM $MnCl_2$ at room temperature (Mn^{2+}), in buffer at 55 °C (Δ) or with the addition of 5 mM $MnCl_2$ at 55 °C (Mn^{2+}, Δ): (a) Sk-PD (23.0 μM); (b) Sk-PH (79.5 μM); (c) CD spectra of PD (182.0 μM) in buffer at room temperature (PD) or with the addition of 5 mM $MnCl_2$ (PD + Mn^{2+}); PH (112.3 μM) in buffer at room temperature (PH) or with the addition of 5 mM $MnCl_2$ (PH + Mn^{2+}). The spectra of the free peptides were recorded over the 250–190-nm range.

(16) The percent helicity is calculated from the mean residue ellipticity ($[\theta]_{222}$) by assuming that a 100% helical peptide has $[\theta]_{222} = -31\,500$ deg cm^2 dmol^{-1}.[17a] The mean residue ellipticity is given by the relationship

$$[\theta]_{222} = 100\theta_{222}/cnl$$

where θ_{222} is the ellipticity (mdeg) measured from the CD spectrum, c is the peptide concentration (mM), n is the number of amino acids in the peptide, and l is the path length (cm) of the CD cell.[17b]

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It should also be noted that these CD spectra were taken in the absence of DNA. It is possible that minor structural differences in the native CD of the different metal–peptide complexes become accentuated in the presence of cognate DNA sites, resulting in differences in the targeting of the 5′-CCA-3′ site. High absorptivity of the skeletal complex in the region of interest (250–190 nm) made the experiment in the presence of DNA difficult to accomplish.

Discussion

DNA Binding by the Metal–Peptide Complexes. On the basis of the comparison to other phi complexes of rhodium,[4-6] the metal–peptide complexes appear also to bind DNA by intercalation of one of the phi ligands in the major groove. Like $[Rh(phi)_2(bpy)]^{3+}$, HPLC analysis of DNA cleavage products yields base propenoic acids and free bases with a similar total photoefficiency.[5] The similar pattern of photocleavage observed for the metal–peptide complexes compared to the skeletal complex at sites other than the 5′-CCA-3′ sequences also lends support to this notion.

Binding affinity for a site is a function both of the metal complex and of peptide moieties. The nonspecific binding affinity of these metal–peptide complexes is estimated to be $\geq 10^6$ M^{-1} and largely derived from intercalation of the metal complex, while the contribution of the peptide alone to binding is estimated to be on the order of 10^3 M^{-1}.[18] Indeed, free peptide at 100 times the concentration does not compete with metal–peptide binding to its specific site. We may, therefore, consider the side-chain functionalities on the peptide as augmenting the DNA-binding properties of the skeletal complex so as to achieve site-selectivity. This means of targeting a site resembles that of the larger DNA-binding proteins, where nonspecific electrostatic and hydrogen-bonding interactions contribute substantial binding affinity in the major groove, and site-selectivity is derived primarily from functional group interactions of a peptide recognition domain.

Site-Specificity of the Metal–Peptide Complexes. The specificity in DNA recognition by the family of peptide complexes is governed by several factors. First and foremost, the specificity is seen to be exquisitely sensitive to the presence of a glutamate at position 10. Conservative changes such as methylation of the carboxylate side chain or substitution with glutamine (isostere) or aspartate (lower homologue) are not tolerated. The overall peptide conformation appears important in order to present the glutamate side chain to the DNA. This suggestion is based upon the variation in intensity of specific cleavage seen with changes in some of the other amino acids, in particular, tryptophan, in the metal–peptide sequence. The high overall cleavage by Sk-PG indicates that increasing the net positive charge on the peptide may raise the overall binding affinity of the metal–peptide complex but does not appear to increase the binding specificity.

How can a single carboxylate be responsible for recognition of a three base pair (5′-CCA-3′) sequence? Peptide conformation certainly appears to affect recognition, as judged by the sensitivity of site-recognition to the reaction conditions as well as sequence. Specificity is seen to be enhanced with the addition of reagents such as trifluoroethanol or in the presence of divalent cations (Mn^{2+}, Mg^{2+}) at elevated temperature (55 °C). Nonetheless, the absolute requirement of the glutamate for recognition gives rise to two possibilities: (i) the glutamate is required indirectly in folding the metal–peptide complexes into a unique conformation under the appropriate conditions; or (ii) the glutamate participates directly in interaction with the DNA base(s) in a structure shared by all the complexes when bound to DNA. The results presented here suggest that the glutamate may be serving both these roles. The differences in intensity of specific cleavage amongst the different metal–peptide complexes suggest that the glutamate plays a direct role in DNA recognition, while the CD data ascribe a structural role to the glutamate. That glutamate is essential in both respects may be the source of the glutamate switch.

(18) Cleavage by Sk-PD at the 5′-CCA-3′ site is approximately 6.5-fold stronger than that by Sk at 1.0 μM metal concentration, which translates into a $\Delta\Delta G$ of ≥1 kcal/mol contribution of the peptide to specific binding over the skeletal complex.

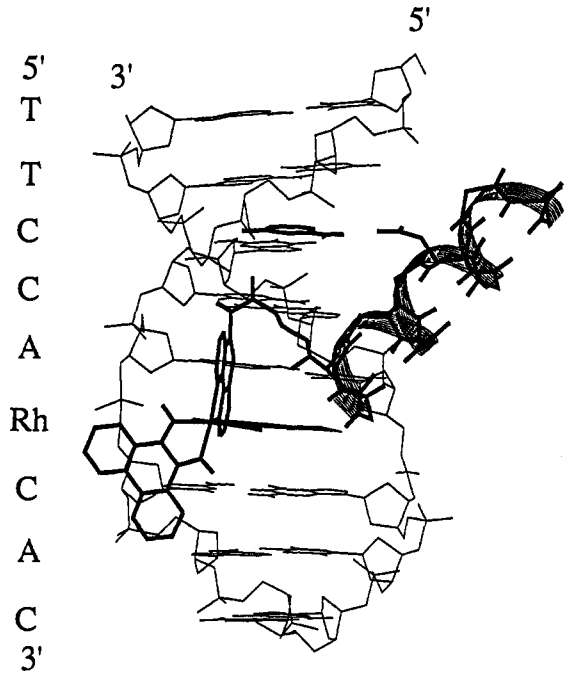

Figure 7. Model for DNA recognition by metal–peptide complexes. The model shows the rhodium complex intercalated in the major groove of DNA at the 5′-AC-3′ base step and canted toward the 5′-strand. Also shown is the glutaryl linker and the peptide backbone in a likely α-helical conformation. All the side chains on the peptide have been omitted for clarity except for the essential glutamic acid residue. The glutamate side chain is shown in a position to make a hydrogen bond with the 4-amino group on the cytosine. The model was built by docking the metal–peptide complex to an 8-mer DNA duplex (5′-TTCCA-CAC-3′) containing an intercalating site between A_5 and C_6 using Macromodel. Care was taken to avoid all steric clashes between the various nonbonded atoms in the model. The additional cleavage band resulting from intercalation between C_6 and A_7 in the figure can also be modeled to account for the same 5′-CCA-3′ recognition if the linker were to adopt a slightly more extended form away from the metal complex.

As illustrated schematically in Figure 7, we can model the 5′-CCA-3′ recognition by the metal–peptide complexes using a combination of base-specific contacts and shape-selection. We propose that the peptide folds across the intercalated metal complex (likely as a helix), necessitating an enlarged major groove; there is evidence that 5′-pyr-pyr-pur-3′ sequences are somewhat opened in the major groove.[19] The folding of the peptide then presents the glutamate near the carboxy terminus in position for specific interaction with the 5′-cytosine (Figure 7). There is precedence for glutamate side chains being involved in the recognition of cytosines.[20] Photocleavage experiments also show that the metal–peptide complex is bound asymmetrically in the intercalation site and is canted substantially toward one strand. This strand asymmetry provides room in the major groove for the peptide to interact with DNA, as our model illustrates. The added cleavage observed at the 3′-base flanking the 5′-CCA-3′ site can be modeled by positioning the linker in a more extended conformation. Further structural studies are, however, needed to validate this model.

The construction of the metal–peptide family was based upon the α_3-helix of the phage P_{22} repressor protein. The solution

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(20) (a) Schultz, S. C.; Shields, G. C.; Steitz, T. A. *Science* **1991**, *253*, 1001. (b) Raskin, C. A.; Diaz, G.; Joho, K.; McAllister, W. T. *J. Mol. Biol.* **1992**, *228*, 506.

structure of the protein has been recently solved,[10] but the exact nature of its interactions with DNA can only be inferred on the basis of analogy to the cocrystal structure of the closely related phage 434 repressor protein with its operator sequence.[21] The putative operator sequence for protein $P_{22}R$ is 5′-ANTNAAG-3′. The fact that these metal–peptide complexes do not preferentially target this sequence[22] may arise because the protein serves to position the α-helix in the major groove of DNA through contacts with a different face of the peptide than in the metal–peptide complex or the shape of the protein complements the shape of the DNA around the operator sequence so as to orient the recognition helix differently. Furthermore, in the metal–peptide complexes, which obviously lack stabilizing interactions with the rest of the protein, the peptide conformation may be directed instead by the metal center and by solvent. The solution structure of the DNA-binding domain of the P_{22} repressor indicates that I^{35} and W^{38} (I^6 and W^9, respectively, in our system) form the core of the hydrophobic interior of the protein and that E^{39} (E^{10} in our system) is solvent inaccessible (and presumably also inaccessible to DNA) as a result of hydrogen bonding to Nϵ of R^{11}/R^{14} in the interior of the protein.[10] Thus, intraprotein interactions involving these three side chains rigidly set the orientation of α_3 in the P_{22} repressor. In our system, the corresponding Ile and Trp side chains may reorient themselves to point into the major groove and away from solvent, while the glutamate side chain of E^{10} is no longer tied down and is available to make functional group interactions with the DNA.

Glutamate Switch in DNA Recognition. The recognition characteristics of this family of metal–peptide complexes provide an example of a glutamate switch in site-specific DNA recognition; if glutamate is present, 5′-CCA-3′ recognition is achieved. The sensitivity of recognition to the presence of a unique residue has been documented in other natural proteins. A single glutamic acid residue was shown to be significant for transcriptional activation by the λ repressor; a mutant containing Asp at this position activated transcription only 2-fold above the basal level.[23a] Similarly, a double substitution of VE/DQ in the HIV type I integrase enzyme rendered the integrase protein inactive for all its functions.[23b] Mutant enzymes D190E and D192E, of the rat DNA polymerase β, in which aspartic acid residues at positions 190 and 192, respectively, were replaced by glutamic acid residues, showed only about 0.1% activity of the wild type enzymes.[23c] Hence, *this small metal–peptide complex may serve as a particularly useful mimic for site-specific DNA-binding proteins.*

In general, these metal–peptide complexes appear to share an array of characteristics with DNA-binding proteins. Both may be viewed as consisting of a DNA recognition element that augments high-affinity DNA binding by the rest of the molecule through sequence-specific contacts. Both show sequence-specificity that is highly sensitive to functional group placement. Indeed, the specificity and affinity of these metal–peptide complexes exceed that of most single zinc-finger domains of transcription factors.[24] What distinguishes these metal–peptide complexes is their small molecular size and, perhaps, the simplicity of the recognition switch.

Therefore, these metal–peptide complexes may be viewed as small molecular models for larger DNA-binding proteins. The metallointercalator may be used to anchor functionality in the DNA major groove, and the peptide functionality may be used sensitively to control site recognition. The construction of these molecules represents a new strategy to create an array of monomeric metal–peptide complexes with differing specificities for double-helical DNA.

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