A New Co^{II} Complex as a Bulge-Specific Probe for DNA **

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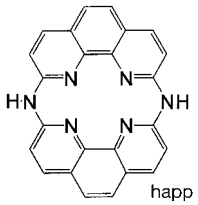
Bulge structures in nucleic acids are crucial motifs in the recognition of DNA by proteins that bind nucleic acids in biological systems.[1] Despite their importance, the structural details of only a few DNA bulges have been reported.[2] These structures are expected to be less stable. The melting temperature of DNA containing a single-base DNA bulge is destabilized by 2.0 – 3.6 °C relative to that of the corresponding totally Watson – Crick base-paired structure.[3]

Although many synthetic chemical nucleases have been developed to explore a variety of DNA/RNA structures,[4] probes specific for the DNA bulge site are still rare. Recently, two types of DNA-targeting antitumor drugs, neocarzinostatin chromophore (NCS-C) and bleomycin (BLM), were shown to have a preference for a DNA nick site near the bulge.[5] Likewise, DNA intercalators, such as ethidium bromide[6] and $[Pt(terpy)(het)]^+$ (terpy = 2,2′:6′,2″-terpyridine, het = "2-hydroxyethylenethiol" = 2-sulfanylethanol),[7] are also known to target specifically to the bulge site of nucleic acids.[6] However, these molecules bind to the double-stranded DNA domains so strongly that it is inevitable that they induce an alteration in the DNA conformation in the locale where they bind. Here, we report the novel octahedral Co^{II} complex $[Co^{II}(tfa)_2(happ)]$ (tfa = trifluoroacetate), which can serve as a specific probe for DNA bulges through its ability toward specific oxidative cleavage of these structures. This complex also has the advantage that it exhibits only a low affinity towards double-stranded DNA. Moreover, it does not show the cleavage reactivity toward single-stranded DNA.



The happ ligand was prepared by a modification of the procedure[8] for the condensation of two molecules of 2,9-dichloro-1,10-phenanthroline at 200 °C under a flowing ammonia gas.[9] Its Co^{II} complex was prepared by treating the happ ligand with cobalt(II) acetate in TFA/MeOH. The X-ray crystal structure of the $[Co^{II}(tfa)_2(happ)]$ complex (Figure 1) reveals that the complex contains two fused 1,10-phenanthroline moieties, with all four pyridinium nitrogen atoms sitting on the same coordination plane, and two labile axial tfa ligands.[10] The average Co–N bond length is approximately 1.86 Å. The EPR spectrum of the Co^{II} complex gave a g_{av} value of 2.005 – 2.331 in methanol, which is indicative of an octahedral Co^{II} complex. The addition of one equivalent of pyridine rapidly displaces one of the axial tfa ligands under ambient conditions, as monitored by EPR spectroscopy, which suggests that the tfa ligands are labile enough to be substituted by nucleophiles.

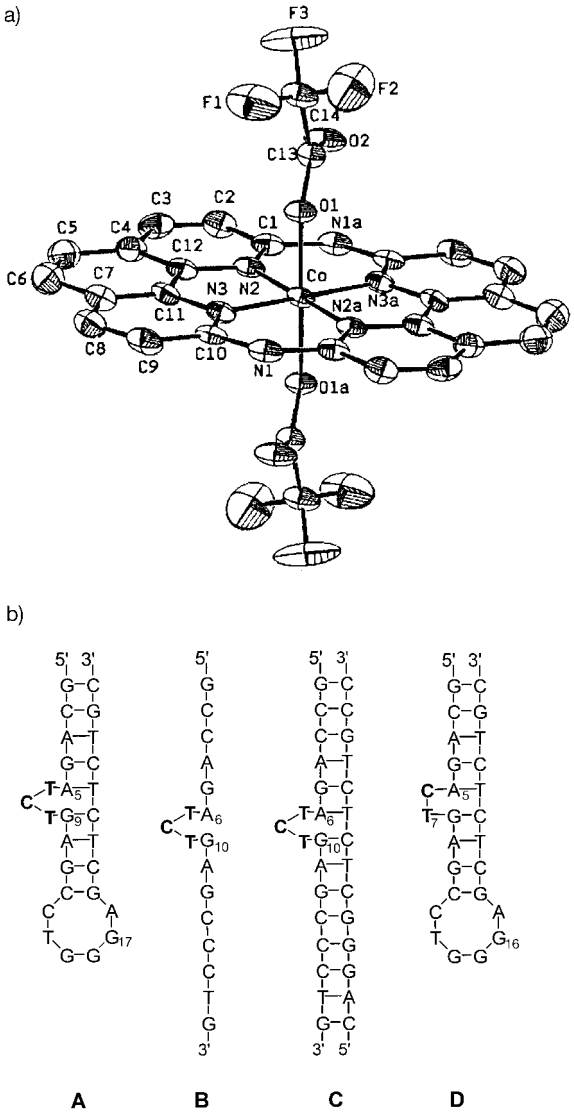


Figure 1. a) ORTEP representation of $[Co^{II}(tfa)_2(happ)]$ in the crystal showing the numbering of the atoms. b) The various DNA substrates used in this study.

Since the 1,10-phenanthroline ligand in the tetrahedral $[Cu^{I}(phen)_2]$ complex is known to intercalate into DNA, it might be expected that $[Co^{II}(tfa)_2(happ)]$, which also contains this flat macrocyclic ligand, might also act as a DNA intercalator. However, in the absence of H_2O_2 and under noncleavage conditions (see below), neither the topoisomerase I assay[11] showed any sign of DNA unwinding associated with DNA intercalation, nor did the native gel mobility shift

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assay reveal the presence of high-molecular-weight bands attributable to the $[Co^{II}(happ)]^{2+}$–DNA adducts in polyacrylamide gel electrophoresis (PAGE). In addition, the melting temperature of calf thymus DNA (60 μM per nucleotide) incubated with $[Co^{II}(happ)]^{2+}$ (8 μM) displayed only a modest change of 0.5–1.0 °C. The incubation of DNA with ethidium bromide under similar conditions resulted in a DNA adduct with a melting temperature T_m that differed by 12–13 °C from the control. The DNA-binding constant of $[Co^{II}(happ)]^{2+}$ with calf thymus DNA was determined by spectral titration at 399 nm as approximately $1 \times 10^4\,M^{-1}$ in 10 mM phosphate buffer at pH 7. In contrast, the corresponding four-coordinated copper complex, $[Cu^{II}(happ)]^{2+}$, is a stronger intercalator in DNA with a binding constant of $10^5\,M^{-1}$, as determined by the ethidium displacement assay.[9] It is evident that the axial ligands of the octahedral $[Co^{II}(happ)(X)_2]$ (X = tfa or H_2O) complex provides a significant steric hindrance to limit the insertion of the Co^{II} complex between the adjacent bases of the double-stranded DNA.

The $[Co^{II}(tfa)_2(happ)]$ complex cleaves DNA catalytically in the presence of H_2O_2. Reactions of $[Co^{II}(happ)]^{2+}$ (0.6 μM) with various 5′-end ^{32}P-labeled oligonucleotides[12] (4–5 μM) were performed in the presence of H_2O_2 (0.005–0.05 %) for 5 min at ambient temperature. The DNA fragments produced were analyzed by high resolution PAGE. These experiments were carried out on the 27-mer DNA substrate 5′-GCA-GATCTGA-GCCTGGGAGC-TCTCTGC-3′, whose secondary structure is comprised of a three-base bulge, a six-base single-stranded loop, as well as double-stranded domains (**A** in Figure 1). This DNA sequence was based on the RNA hairpin from the *trans*-activation response element (TAR-RNA).[5, 13–14] After piperidine treatment the strand scission was found to occur predominantly at the DNA-bulge site (T6, C7, and T8) and only very weakly at the DNA hairpin loop (C13–A18), despite the fact that both sites contain the same DNA 5′-CTG-3′ sequence, as shown in Figure 2.

Minor cleavage was also found at the sites near the flanking junctions of these nucleobases. Interestingly, no significant oxidative cleavage was observed at the 5′-GGG-3′ region in the DNA hairpin loop. The guanine residues in this DNA loop have been reported to be the sites most susceptible to oxidative cleavage as a result of its accessibility[13] and low reduction potential.[15] The equivalent DNA cleavage in the absence of H_2O_2 required a higher Co^{II} complex concentration (> 50 μM) as well as a longer reaction time (> 40 min). Moreover, competitive inhibition by the DNA intercalator $[Pt(terpy)(het)]^+$, which targets the DNA bulge site, was found to reduce the extent of cleavage at the bulge site remarkably. These observations support the bulge-specific DNA cleavage by the $[Co^{II}(happ)]^{2+}$ complex in the presence of H_2O_2.

The complex $[Co^{II}(tfa)_2(happ)]$ shows a low reactivity toward single-stranded DNA. When $[Co^{II}(happ)]^{2+}$ was allowed to react with a single-stranded 16-mer DNA containing the sequence 5′-GCCAGA**TCT**G-AGCCTG-3′ (**B** in Figure 1) in the presence of H_2O_2 under identical conditions, no specific cleavage was observed at the 5′-TCT-3′ site, even when a 20-fold excess of the Co^{II} complex was used. Upon annealing this single-stranded DNA with its complementary

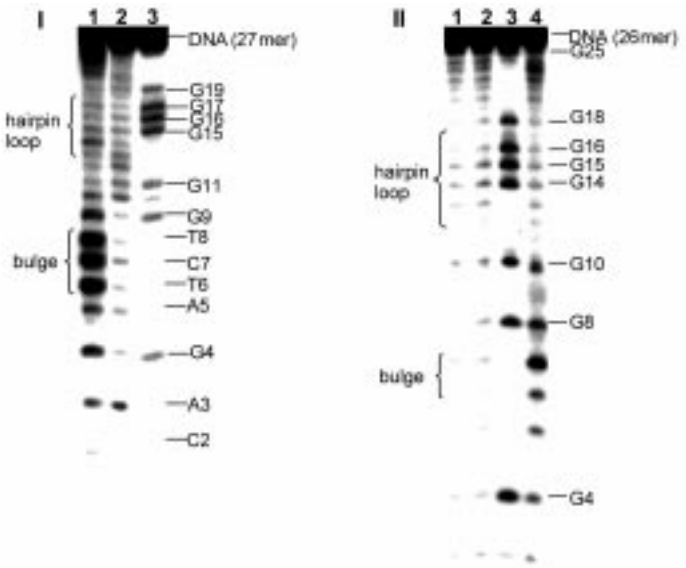

Figure 2. The autoradiogram of a denaturing polyacrylamide gel (20 %; 7 M urea) showing the results of oxidation of a 5′-end-labeled DNA (4 μM) with $[Co^{II}(tfa)_2(happ)]$ activated by H_2O_2 (0.005 %) in 10 mM sodium phosphate buffer (pH 6.9) at 25 °C for 5 min. All reactions were heated with 0.7 M piperidine at 90 °C for 30 min. I) DNA substrate **A** (see Figure 1). Lane 1: DNA with Co^{II} (0.6 μM) and H_2O_2; lane 2: DNA with H_2O_2; lane 3: G-reaction of the Maxam–Gilbert sequence. II) DNA substrate **D** (see Figure 1). Lane 1: DNA only; lane 2: DNA with H_2O_2, lane 3: G-reaction of the Maxam–Gilbert sequence; lane 4: DNA with Co^{II} (0.6 μM) and H_2O_2.

DNA strand, a three-base bulge DNA duplex (**C** in Figure 1) was formed, as monitored by native PAGE. An enhanced DNA cleavage was observed at the 5′-TCT-3′ site in the DNA bulge region upon subjecting this duplex to the same DNA cleavage reaction with the $[Co^{II}(happ)]^{2+}$ complex. These results further support the conclusion that $[Co^{II}(happ)]^{2+}$ serves as a DNA bulge-specific cleavage reagent without the reactivity toward the corresponding domain in the single-stranded DNA.

When magnesium monoperoxyphthalic acid (MMPP) or oxone ($KHSO_5$) were used instead of H_2O_2, no significant DNA cleavage was observed with supercoiled plasmid DNA. Since the addition of superoxide dismutase and D_2O into the reaction medium did not reduce the concentration of circular DNA (Type II) formed in the DNA cleavage products mediated by this Co^{II} complex, it is unlikely that superoxide and singlet oxygen species are involved in this process. However, when mannitol, a hydroxyl radical scavenger, was added into the DNA-cleavage assay medium, the amount of circular DNA (type II) was found to be reduced by half. These results suggest that the hydroxyl radical, induced by the reaction of $[Co^{II}(happ)]^{2+}$ with H_2O_2, is likely to be one of the reactive species responsible for the DNA cleavage. In general, the hydroxyl radical is a diffusable species and lacks specificity in the oxidative DNA cleavage. Nevertheless, the DNA-strand scission induced by $[Co^{II}(happ)]^{2+}$ was found to be directed specifically at the bulge site. Consequently, this specific DNA cleavage must be derived from a specific interaction between the Co^{II} complex, most probably through their ligands, and the bulge region in the DNA. Recently, the solution structure of a two-base DNA bulge has been

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determined by NMR spectroscopy. This structure has been reported to produce a triangular prism pocket to bind with the enediyne analogues.[16] Indeed, when the two-base bulge of a 26-mer DNA containing one base (T6) less than the three-base bulge of a 27-mer DNA (**D** in Figure 1) was used as the DNA substrate, enhanced cleavage activity and specificity was observed towards the T7 residue of the 26-mer DNA bulge shown in Figure 2. Thus, the shape and size of the binding pocket of the DNA bulge are probably important controlling factors in the specific recognition by the $[Co^{II}(tfa)_2$-(happ)] complex. The addition of the intercalator $[Pt(terpy)(het)]^+$ to DNA substrate D[12] was, as for DNA substrate A, found to significantly inhibit the cleavage at the bulge site induced by $[Co^{II}(happ)]^{2+}$ in the presence of H_2O_2.

This study provides the first attempt in utilizing a novel Co^{II} complex to show specific targeting and cleavage of a DNA bulge site. No significant reactivity was observed toward the corresponding sequence in a single-stranded DNA region. We assume that the intercalation of the 1,10-phenanthroline ligand of $[Co^{II}(tfa)_2(happ)]$ toward double-stranded DNA is probably inhibited by the two axial ligands in this octahedral complex. On the other hand, it is possible that $[Co^{II}(tfa)_2$-(happ)] recognizes a binding pocket in the DNA bulge of a specific shape and size, and this recognition may be responsible for the observed bulge-specific DNA cleavage by the diffusable hydroxyl radical produced from the reaction between the cobalt(II) complex and H_2O_2.

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Functional Monolayers with Coordinatively Embedded Metalloporphyrins**

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In memory of Jacqueline Libman

The expression of porphyrin functions in supramolecular systems depends on their immediate environment and mutual orientation.[1] Incorporation of porphyrins into artificial bilayers[2] or their assembly onto solid supports[3] neither resulted in uniform orientation of the chromophores, nor

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