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Effects of supercoiling on the sequence-specific photo-modification of DNA by a vanadium(V)-peroxo complex

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Abstract

Sequence-specific photo-modification of DNA has been demonstrated, for the first time, in a vanadium(V)-peroxo complex, $NH_4[VO(O_2)_2(5,6\text{-}Me_2phen)]$ (where 5,6-Me_2phen = 5,6-dimethyl-1,10-phenanthroline). Using molecular cloning technique, a consensus sequence motif of 5′-G(A/G)TA(T/C)C was identified associated with the two specific photo-modification sites, 5′-ATC and 5′-TACC found on a plasmid DNA, pBluescript, by a modified Sanger sequencing technique. DNA supercoiling was shown to be a critical prerequisite for this observed sequence-specific photo-modification activity.

Keywords: Vanadium; Peroxo ligand; DNA damage; Sequence specific; Supercoiling

1. Introduction

With the sequencing of the human genome officially completed, many human diseases with a genetic origin or component can now be understood at the molecular (nucleotide) level [1]. Taking advantage of the considerable progress made in identifying the crucial molecular determinants for site- and sequence-specific interactions with DNA [2–8], the treatment of diseases based on modulation of specific genes is becoming a realizable goal in molecular medicine [9–11]. Following the successful development of cisplatin as an antitumor drug [12], many metal-based complexes capable of DNA and RNA scissions have been designed as specific structural probes for both the primary (sequence) and secondary (conformation) structures of nucleic acids [13–17]. Recently, we have demonstrated substantial DNA cleavage activity in a variety of vanadium(V)-peroxo complexes when photo-irradiated at 365 nm (UV-A) at neutral pH [18]. Singlet oxygen, $^1O_2$, produced from the photolysis of these complexes [18,19], was implicated as the species responsible for the observed DNA scission in these complexes. This deduction was based on the following observations: (1) reduced cleavage activity with increasing concentrations of $^1O_2$ scavengers (e.g., NaN_3) present, (2) enhanced cleavage activity in D_2O where the lifetime of $^1O_2$ is ca. 10-fold longer relative to that in H_2O, (3) enhanced cleavage activity, by ca. two-fold, when alkali-labile site (ALS) treatment was performed on the treated DNA [20]. But interestingly, no good correlation was found between the $^1O_2$ quantum yields measured for 12 of these complexes and their relative DNA photocleavage activities, suggesting that binding interactions between DNA and *some* of the complexes studied might be involved in the observed photocleavage [19].

In this paper, we report additional evidence in support of this notion. Using DNA sequencing technique, two specific photo-modification sites, 5′-ATC and 5′-TACC, were found produced by some vanadium(V)-peroxo complexes on the plasmid DNA studied. Using

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molecular cloning technique, a consensus sequence motif of 5′-G(A/G)TA(T/C)C was identified on this plasmid DNA photo-modified by one of these vanadium(V)-peroxo complexes, namely, $[VO(O_2)_2(5,6\text{-}Me_2phen)]^-$, chosen for a more detailed study. But most significantly, this sequence-specific DNA modification was observed only with supercoiled DNA.

2. Experimental

2.1. Materials

The vanadium(V)-peroxo complexes used in this study were synthesized from NH_4VO_3 (reagent grade, Acros), H_2O_2 (30%, BDH), and their corresponding ancillary ligands using a slightly modified procedure from the literature [21]. These ligands, which included 2,2′-bipyridine, 4-methyl-1,10-phenanthroline, 5-methyl-1,10-phenanthroline, 5,6-dimethyl-1,10-phenanthroline, 4,7-dimethyl-1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 5-nitro-1,10-phenanthroline, were from Aldrich and 5-amino-1,10-phenanthroline was from Polyscience. They were all used as received. The complexes synthesized were characterized by optical absorption, IR and ^{51}V NMR spectroscopies, Ce(IV) potentiometric titration (for their peroxide contents) and elemental analysis [20,22]. The purities of all complexes were determined to be >97% spectrophotometrically using a vanadium(V)-specific chelating agent, *N*-benzoyl-*N*-phenylhydroxylamine [23], from Aldrich, and by ^{51}V NMR spectroscopy.

The supercoiled plasmid DNA used in this study was prepared from a single colony of JM109/pBluescript. The detailed extraction and purification of the plasmid DNA is given in Section 2.2. The 33-base oligodeoxyribonucleotide (33-mer), 5′-AATTCGAT**ATC**AAGCTTATCGA**TACC**GTCGACC, and its complementary strand used in the Maxam-Gilbert sequencing experiment were obtained from Perkin–Elmer Biosystem, Inc. Further purification of these oligodeoxyribonucleotides was performed by 20% polyacryamide gel electrophoresis (recovery > 90%) before use. Methylene blue was from Aldrich and NaN_3 was from Acros. Dimethyl sulfate and mercaptoethanol, used in preparing the G-lane marker, were from Aldrich and Sigma, respectively, and were used as received. The phosphate buffer (pH 7) was prepared from disodium hydrogen orthophosphate (Sigma) and sodium dihydrogen orthophosphate (Sigma). The water used in these experiments was of Milli-Q grade. The primers used in the Sanger dideoxy sequencing experiments, i.e., the M13 universal (forward) and reverse primers, and the enzyme used in the cycle-sequencing, i.e., *Taq* DNA polymerase, were from Amersham Pharmacia Biotech. For the radioactive labeling of DNA substrates, the enzyme used, i.e., T4 polynucleotide kinase (PNK), was from New England Biolabs, Inc. and the [γ-^{32}P]dATP and [α-^{32}P]dATP were from Amersham Pharmacia Biotech.

2.2. Extraction and purification of plasmid DNA (pBluescript)

The extraction and purification of the plasmid DNA (pBluescript) were conducted according to the literature procedure [24,25] which comprised three main steps: (i) the culturing of the bacteria, (ii) the harvesting and lysis of the bacteria, and (iii) the purification of the plasmid DNA using reagents and enzymes from the Qiagen Master Max kit. In the bacterial culturing process, a single colony of *Escherichia coli* JM109/Bluescript was incubated in an autoclaved L. Brath (LB) medium (500 mL) in the presence of ampicillin (concentration 50 μg/mL). The LB medium was then placed in a shaker and kept at 37 °C for overnight. After the culturing process, the bacteria were harvested and lysed according to the procedure given in the Qiagen Master Max kit: for a 500 mL bacterial culture solution, the bacteria were first recovered by centrifugation at 12,000 rpm at 4 °C for 10 min in a centrifuge bottle. Then the supernatant was decanted and the pellet was resuspended in 10 mL P1 buffer (100 μg/mL RNase A, 50 mM Tris–HCl and 10 mM EDTA, pH 8). After that, 10 mL P2 buffer (0.2 M NaOH in 1% SDS) was added to this mixture which was then mixed gently and incubated at room temperature for 5 min. After incubation, 10 mL P3 buffer (2.55 M potassium acetate, pH 4.8) was added to the incubation mixture and mixed immediately but gently. Centrifugation was then carried out at 15,000 rpm at 4 °C for 20 min in order to get a clear supernatant. The clear supernatant was loaded on a QIAGEN-tip 500 column which had previously been equilibrated with 10 mL QBT buffer (750 mM NaCl, 50 mM Mops, 15% ethanol, and 0.15% Triton X-100, pH 7.0). The supernatant was allowed to enter the resin in the column by gravity flow. The resin was then washed with 30 mL QC buffer (1.0 M NaCl, 50 mM Mops, 15% ethanol, pH 7) and the DNA was eluted by 15 mL QF buffer (1.25 M NaCl, 50 mM Mops, 15% ethanol, pH 8.2). The eluent was mixed with three volumes of 3 M sodium acetate/ethanol (1:25, v/v) and kept at −20 °C overnight. The DNA pellet was obtained after centrifugation. The pellet was then redissolved in a minimum amount of Tris–EDTA (TE) buffer (89 mM Tris base and 2 mM EDTA, pH 8) and stored at −20 °C.

2.3. Plasmid DNA-relaxation assay

DNA-cleavage activity was measured by a plasmid DNA-relaxation assay. The protocol of this assay is given as follows. Plasmid DNA (pBluescript), enriched with the supercoiled conformer, and the one-phor-all buffer (which contained 10 mM Tris–acetate, 10 mM