DNA Hydrolysis by stable metal complexes

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Stable cobalt(III) complexes promote hydrolysis of the DNA phosphodiester backbone.

The development of reagents that promote efficient hydrolysis of phosphate esters is a challenge currently receiving attention in many laboratories as such processes have potential application for the development of novel nucleases for use as therapeutic agents and tools for molecular biology.[1] While many nucleic acid cleavage processes are radical-based[2] and deliver products lacking 3′- or 5′-phosphate groups,[3] hydrolytic cleavage of phosphate esters[4,5] RNA[6] and DNA using lanthanide-based systems are being reported with increasing frequency.[7] To our knowledge no effective non-enzymic hydrolytic cleavage of DNA using stable and well characterised metal complexes has yet been reported. Here we report our finding that certain tetraamine aqua hydroxo cobalt(III) complexes act as artificial nucleases by promoting DNA hydrolysis at physiological pH and temperature. The lesions produced by at least one of these complexes bear functionality suitable for immediate enzymatic manipulation.

The compounds we chose to investigate were $[(en)_2Co(OH)(OH_2)]^{2+}$ **1**, $[(cyclen)Co(OH)(OH_2)]^{2+}$ **2** and $[(tamen)Co(OH)(OH_2)]^{2+}$ **3**‡ as all three of these compounds had previously been studied in the context of their ability to promote hydrolysis of phosphate esters and polyphosphates.[8,9] Plasmid pUC9 DNA was treated with complex **1**, **2** or **3**, after which the DNA was liberated from the cobalt complexes using a previously developed KCN–$Co(ClO_4)_2$ quench method.[9] Fig. 1 shows an agarose electrophoretic gel displaying the course of cleavage reactions§ and reveals that complexes **2** and **3** are indeed capable of promoting cleavage of supercoiled DNA (Form I) into relaxed DNA (Form II). After 6 h, the degree of cleavage promoted by complex **1** was negligible.

Although cobalt(III) complexes **1**, **2** and **3** are known to be oxidatively stable, we were keen to discount the possibility that DNA cleavage was occurring *via* a free radical-based depurination pathway. In a control experiment, incubation of the plasmid DNA with Co^{II} perchlorate under identical conditions led to no observable DNA cleavage, discounting the possibility that Co^{II} contaminants were responsble for the observed cleavage. Furthermore, if a free radical cleavage mechanism were involved, we expected that addition of a competing agent to the reaction mixtures would reduce the observed efficiency of DNA cleavage. This was found not to be the case: addition of up to a 50-fold excess of dATP (relative to the cobalt complex) in these reaction mixtures had no effect on the efficiency of DNA cleavage. Another gratifying aspect of these experiments was that the order of efficiency for DNA cleavage by **1**, **2** and **3**, mirrored that found for the efficiency of hydrolysis of simple phosphate esters,[8b,9b] further supporting our contention that the cleavage processes were hydrolytic.

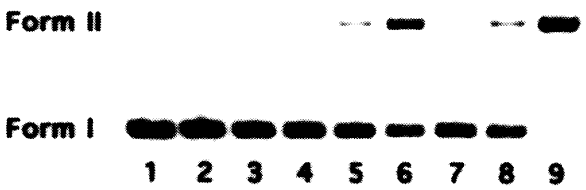
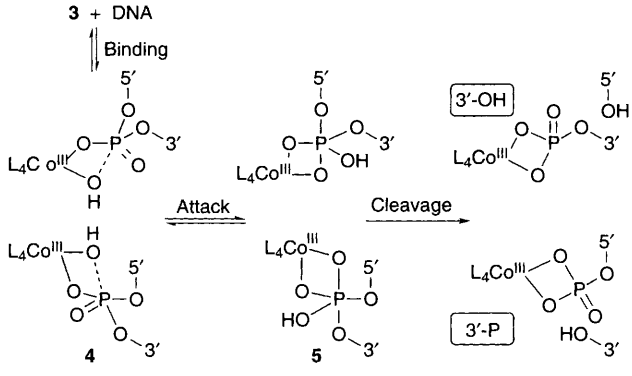


Fig. 1 Hydrolysis of supercoiled pUC9 by complexes **1**, **2** and **3** (1 mmol dm^{-3}) at 37 °C. Coordinated cobalt was liberated from the DNA by treating each reaction with excess KCN and a trace of $Co(ClO_4)_2$ prior to loading on the gel.[9] Lanes 1–3, **1** after 0 min, 100 min and 6 h, respectively; lanes 4–6, **2** after 0 min, 100 min and 6 h, respectively; lanes 7–9, **3** after 0 min, 100 min and 6 h, respectively.

The observed distribution of supercoiled and relaxed DNA in the agarose gels provides a measure of the extent of hydrolysis of the first phosphodiester bond in each plasmid DNA and we used these data to perform simple kinetic analyses.¶ Measurement of both the rate of appearance of Form II DNA and the rate of disappearance of Form I DNA for each complex indicated that the approximate pseudo first-order rate constants are $\ll 10^{-6}$ s^{-1} for hydrolysis promoted by **1**, 1×10^{-5} s^{-1} for **2** and 5×10^{-5} s^{-1} for **3**. These rate constants represent an approximate rate enhancement of 10^9 relative to that of unpromoted phosphate diester hydrolysis.[10]

The mechanism by which complexes such as **2** and **3** hydrolyse phosphate esters has been studied extensively[4,9] and in this case implicitly involves loss of one cobalt water ligand and replacement by a phosphoryl oxygen to form the activated phosphodiester **4**. The adjacent coordinated hydroxide then acts as an intramolecular nucleophile and attacks the phosphorus atom leading to a five-coordinate oxy-phosphorane **5**. Collapse of this intermediate results in phosphodiester hydrolysis (Scheme 1).[11] Based on this mechanism, two types of cleavage product are possible, 3′-OH and 3′-P where the cleavage products would respectively bear a coordinated phosphate at either the 5′- or 3′-side of the lesion. These would arise from the orientation of the Co–OH nucleophile at two of the faces of the tetrahedral P-centre.‖

In order to determine whether the cleavage reaction gave a preponderance of either 3′-OH or 3′-P products (Scheme 1) or displayed any form of sequence selectivity, we repeated the cleavage with complex **3** and a 35-mer oligonucleotide as substrate.** In the event, oligodeoxynucleotide cleavage reactions were performed under identical conditions described for plasmid cleavages on a double-stranded 35-mer (**A**) and its two single-stranded constituents (**a** and **a′**) where the double-



Scheme 1 '3'' and '5'' refer to the 3′ and 5′ ends of the DNA backbone

stranded oligomer had been designed to incorporate restriction sites for endonucleases *Asp*I and *Bst*EII. Given the relatively slow cleavage of DNA by **3** in order to minimize radiolytic DNA damage, the DNA cleavage products were 5′-end labelled directly, subsequent to cleavage by **3**.†† In experiments where radiolabelling was performed prior to cleavage, autoradiography revealed distinct decomposition of gel-purified control oligonucleotides. Consequently we adopted the above 'post-labelling' protocol. Electrophoretic comparison of the cobalt-derived cleavage products with products formed by either *Asp*I or *Bst*EII digestion (Fig. 2) shows that between the 18-mer and 12-mer *BstEII* digestion products (lane 1), the products (lanes 4, 6 and 8) display six characteristic pairs of bands. Likewise, between the 27-mer product of *Asp*I digestion (lane 2) and the 18-mer *Bst*EII digestion product, there are nine pairs of bands. We attribute these pairs of products to the two populations of products 3′-OH and 3′-P with the more mobile band of each pair being the doubly phosphorylated oligonucleotides. For single-stranded DNA, the intensity ratio 3′-OH : 3′-P was approximately 1 : 4 and for double-stranded DNA 3′-OH : 3′-P was 1 : 2.‡‡

Our experiments so far indicate that DNA hydrolysis occurs non-specifically with respect to nucleotide sequence. However, the cleavage products formed in these reactions are substrates for DNA-modifying enzymes. Treatment of isolated Form II DNA with T4 DNA ligase leads to ligation with 35% efficiency indicating that about 35% of the product DNA bears a 5′-phosphoryl moiety: an outcome consistent with the results of our oligonucleotide cleavage experiments for double-stranded DNA (data not shown).

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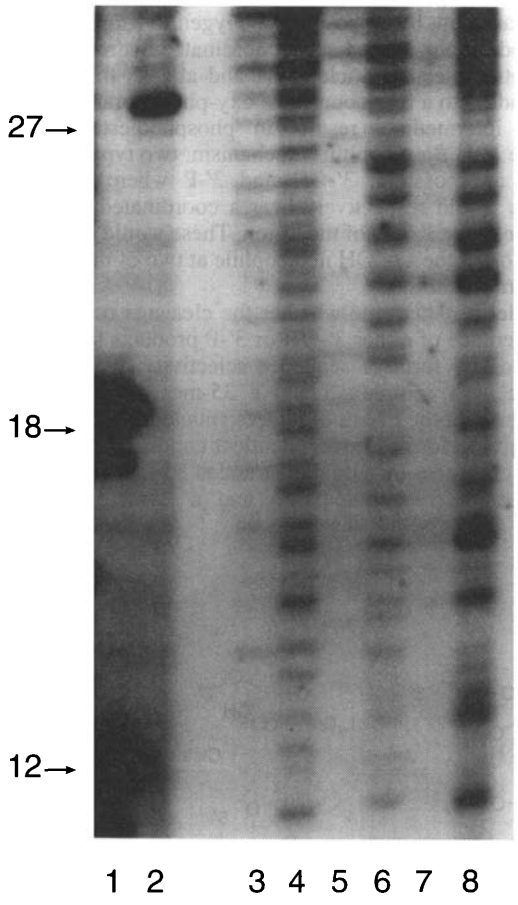

Fig. 2

Footnotes

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‡ en = ethanediamine, cyclen = 1,4,7,10-tetraazacyclododecane, tamen = 6-(4-amino-2-azabutyl)-6-methyl-1,4-diazacycloheptane.

§ In a typical cleavage reaction, pUC9 DNA (23 μmol dm^{-3}) in Tris buffer (20 mmol dm^{-3}, pH 7.6) was treated with a freshly prepared solution of **1**, **2** or **3** (1 in 20 mmol dm^{-3} Tris, pH 7.6) at 37 °C in the dark. Agarose electrophoresis was performed using 1% agarose and Tris-borate-EDTA buffer containing 0.5 μg cm^{-3} ethidium bromide, followed by visualisation with a UV transilluminator.

¶ To perform kinetic analyses, Forms I and II DNA were visualised with the aid of ethidium bromide on a UV transilluminator at 254 nm and photographed with Polaroid film. The photographic negatives were scanned on a UVR scanner set on transmission mode. Band densities for the various time points were measured using the public domain software 'NIH Image'.

‖ An examination of DNA models implies that addition at the face *trans* to the 5′-site would be preferred, leading to a preference for the 3′-P product.

** In these experiments we anticipated that ^{32}P end-labelling of the oligonucleotide cleavage products and subsequent electrophoretic separation would reveal two populations: those bearing only the 5′-phosphate derived from the end-labelling reaction (corresponding to 3′-OH, Scheme 1) and those bearing a phosphate group at both the 3′- and 5′-termini (corresponding to 3′-P, Scheme 1). Following electrophoresis, quantification of the respective band intensities would then reveal the ratio of the two cleavage products.

†† Only one of the oligonucleotide cleavage products from cleavage in the 3′-OH mode can be end-labelled using the polynucleotide kinase. Consequently, if 3′-OH and 3′-P cleavage modes occurred with equal efficiency, the ratio of band intensities for 3′-OH : 3′-P would appear as 1 : 2 if one cleavage per oligonucleotide occurred. However, if multiple cleavages occur, the ratio would be significantly less (*ca.* 1). To avoid the well-known 3′-phosphatase activity of polynucleotide kinase, we used a mutant polynucleotide kinase free of 3′-phosphatase activity (Boehringer Mannheim) for 5′ end-labelling.

‡‡ Band intensities were measured by phosphorimager scanning. This selectivity is consistent with the expectations arising from examination of DNA models. Treatment of cyclic nucleotide phosphate esters with related cobalt complexes has also been shown to deliver a distribution of products.[9]

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