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Specific cleavage of DNA at CG sites by Co(III) and Ni(II) desferal complexes*

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It is demonstrated that Co(III) and Ni(II) complexes of desferal, a siderophore chelating drug, show specificity for cleavage of DNA at CG sites, while the corresponding Cu(II) complex cleaves DNA sequences at both CG and AT sites.

Desferal; DNA cleavage; Sequence specificity

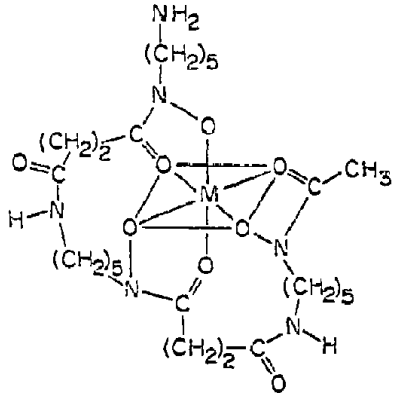
1. INTRODUCTION

In recent years, there has been growing interest in interaction of metal complexes with nucleic acids and in particular for their ability to cleave DNA [1–5]. Some important nucleolytes are Cu(II)-OP [2], Fe(II)-BLM [3], Fe(II)-EDTA [4], metalloporphyrins [5], Cu and Co complexes of substituted phenanthrolines [6] and Ni(II) complexes of azamacrocycles [7] and a tripeptide Gly-Gly-His [18]. These reagents cleave DNA either by oxidative degradation of deoxyribose moiety or by base modification, leading ultimately to strand scission. We have recently shown [9] that Cu(II) **1**, Co(III) **2** and Ni(II) **3** complexes of a siderophore chelating drug desferal cleave plasmid DNA, in contrast to the corresponding Fe(II) complex which is inert in DNA scission reaction. In this paper, by employing synthetic oligonucleotides, we demonstrate that while the Cu(II) complex of desferal has only marginal sequence preference for cleavage reactions (CG > AT), Co and Ni complexes cleave DNA only at CG sites and are ineffective at AT sites.

2. MATERIALS AND METHODS

The oligonucleotides $d(CG)_5$, $d(AT)_n$, $d(A_3T_3)$ and $d[(CG)_nA_nT_n(CG)_n]$ were synthesised on a Pharmacia Gene Assembler Plus (0.2 μmol) using β-cyanoethyl phosphoramidite monomers. They were then purified by FPLC (Pharmacia) on a Mono-Q 5/10 column (anion exchange) using the solvent system: *Buffer A* (10 mM aq. NaOH + 0.1 M NaCl); *Buffer B* (10 mM aq. NaOH + 0.9 M NaCl) and desalted on NAP-10 (Pharmacia) column. The synthetic oligonucleotides were 5'-end labelled [10] using T4 polynucleotide kinase (10 units/200 pmol) and [γ-^{32}P]ATP (spec. act. 3000 μCi/mmol). The labeled DNA was precipitated by ethanol with the help of carrier calf thymus DNA. The Cu, Co and Ni complexes of desferal (**1–3**) were prepared and purified as before [9]. The cleavage reactions were carried out using oligonucleotides (8000 cpm) in tris buffer (10 mM) and sodium acetate (1.2 mM) and additional reactants depended on metal complex, with the following effective concentrations: (a) Cu-DFO (300 μM), ME (0.5 mM), H_2O_2 (4 mM); (b) Co-DFO (150 μM), H_2O_2 (4 mM); (c) Ni-DFO (150 μM), H_2O_2 (4 mM). The reactions were carried out by incubating the above mixture at 37°C for 30 min followed by freezing at −20°C and dried on a Savant SpeedVac concentrator. This was then loaded in formamide/Bromophenol blue (5 μl), either directly or after piperidine treatment (100 μl, 1 M, 90°C, 30 min), into different wells on 20% polyacrylamide gel for analysis by electrophoresis at 15 mA, 400 V until the dye reached about 75% of the gel length. Subsequently the gels were visualised after autoradiography.

HPLC analysis of cleavage reactions were done on C18 (reverse phase) column using the solvent system: *Buffer A* (10% acetonitrile in



1. M = Cu^{II}; 2. M = Co^{III}; 3. M = Ni^{II};

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Abbreviations: DFO, desferrioxamine; ME, mercaptoethanol; Pip, piperidine; DTT, dithiothreitol; OP, phenanthroline; BLM, bleomycin; EDTA, ethylenediamine tetraacetic acid; PAGE, polyacrylamide gel electrophoresis. Cu(II), Ni(II) and Co(III) desferal complexes are denoted as Cu-DFO, Ni-DFO and Co-DFO, respectively.

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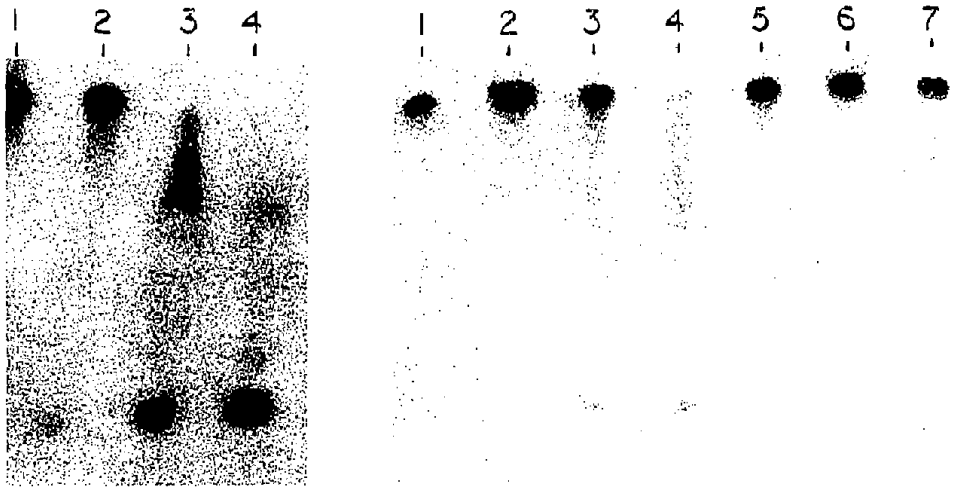

Fig. 1. 20% PAGE autoradiogram of cleavage reactions $d(CG)_9$ and $d(AT)_9$ with metallodesferals: (a) Lane 1, $d(CG)_9$; lane 2, $d(CG)_9$ + Pip (1 M); lane 3, $d(CG)_9$ + Cu-DFO (300 μM) + ME (0.5 mM) + H_2O_2 (4 mM); lane 4, 3 + Pip (1 M), (b) Lane 1, $d(AT)_9$; lane 2, $d(AT)_9$ + Pip (1 M); lane 3, $d(AT)_9$ + Cu-DFO (300 μM) + ME (0.5 mM) + H_2O_2 (4 mM); lane 4, 3 + Pip (1 M); lane 5, $d(AT)_9$ + Co-DFO (150 μM) + H_2O_2 (4 mM); lane 6, 5 + Pip (1 M); lane 7, $d(AT)_9$ + Ni-DFO (150 μM) + H_2O_2 (4 mM) + Pip (1 M). For experimental details see Materials and Methods.

0.1 M TEAA) and *Buffer B* (15% acetonitrile in 0.1 M TEAA); *Gradient* (A to B in 20 min, at B for 10 min).

3. RESULTS AND DISCUSSION

3.1. *Cleavage reactions with $d(CG)_n$ and $d(AT)_n$ oligonucleotides*

Fig. 1 shows the electrophoretic gel results of DNA cleavage reactions of $d(CG)_9$ and $d(AT)_9$ with Cu(II), Co(III), and Ni(II) complexes of desferal (**1–3**). Treatment of $d(CG)_9$ (Fig. 1a) with Cu-DFO leads to initial cleavage fragments (lane 3), which on treatment with piperidine results in complete degradation (lane 4). Comparison of controls, lanes 1 and 2 (Fig. 1a and b) indicate that synthetic DNA is stable to piperidine under these conditions and the degradation seen (lane 4, Fig. 1a) is due to the fact that the initial products of the reaction are chemically modified and hence susceptible to piperidine action. Both Co-DFO and Ni-DFO behaved similarly in reactions with $d(CG)_9$, leading to full degradation of DNA after piperidine treatment (not shown). The optimum concentrations required for cleavage varies with the metal and was determined by changing the concentration of the metal complexes over a wide range of values (200 μM to 2 mM). It was also observed that the dinucleotide d(CG) and tetranucleotide $d(CG)_2$ failed to undergo cleavage with metallodesferals while $d(CG)_3$ exhibited cleavage reactions.

Fig. 1b shows reactions of $d(AT)_9$ with Cu, Co and Ni-DFO complexes under conditions similar to that with $d(CG)_9$. While Cu-DFO treatment (lanes 3 and 4) led to efficient cleavage, it is seen that Co and Ni complexes (lanes 5–7) do not cleave $d(AT)_9$ sequences even after piperidine treatment of the initial reaction products. Thus Co and Ni complexes are inert to AT sequences and show specificity towards cleavage of CG

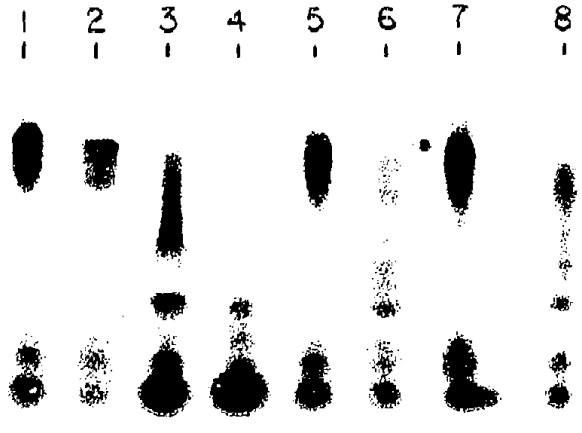

Fig. 2. 20% PAGE autoradiogram of cleavage of $d[(CG)_2A_2T_2(CG)_2]$ with metallodesferals: Lane 1, control DNA; lane 2, 1 + Pip (1 M); lane 3, 1 + Cu-DFO (300 μM) + ME (0.5 mM) + H_2O_2 (4 mM); lane 4, 3 + Pip (1 M); lane 5, 1 + Co-DFO (150 μM) + H_2O_2 (4 mM); lane 6, 5 + Pip (1 M); lane 7, 1 + Ni-DFO (150 μM) + H_2O_2 (4 mM); lane 8, 1 + Cu(II)-OP (170 μM) + ME (0.5 mM) + H_2O_2 (4 mM) + Pip (1 M).

sequences while Cu complex cleaves both CG and AT containing sequences. It may also be mentioned that while Cu-DFO reactions necessarily require a reducing agent (e.g. ME, DTT) to promote cleavage, Co and Ni complexes bring about scission under oxidative conditions. These facts may have important bearing on the possible mechanistic differences of cleavage reactions using these complexes.

3.2. *Cleavage reactions with d[(CG)$_n$A$_n$T$_n$(CG)$_n$]*

In view of the characteristic behaviour of Co and Ni complexes in promoting cleavage at CG sites and remaining passive towards AT sites, cleavage reactions were performed on oligonucleotides containing mixed sequences. Fig. 2 shows electrophoretic analysis of cleavage reactions of d[(CG)$_2$A$_2$T$_2$(CG)$_2$] with Cu, Co and Ni-DFO complexes. As expected all three complexes were active in cleavage reactions (lanes 3–7). Although the cleavage patterns were similar, differences were noticed in relative intensities of product bands. In each case, it is seen that piperidine treatment following the initial reaction with metallodesferal leads to further degradation in a way similar to that observed in d(CG)$_n$ and d(AT)$_n$ sequences. The reaction was most efficient in case of Cu-DFO which led mostly to faster moving products on the gel (lane 4). For comparison, the pattern of products obtained from a similar cleavage reaction with Cu-phenanthroline are shown (lane 8).

We also examined the cleavage reactions using HPLC. Fig. 3 shows HPLC profile of products obtained during cleavage reaction of d[CG)$_3$A$_3$T$_3$(CG)$_3$]. After

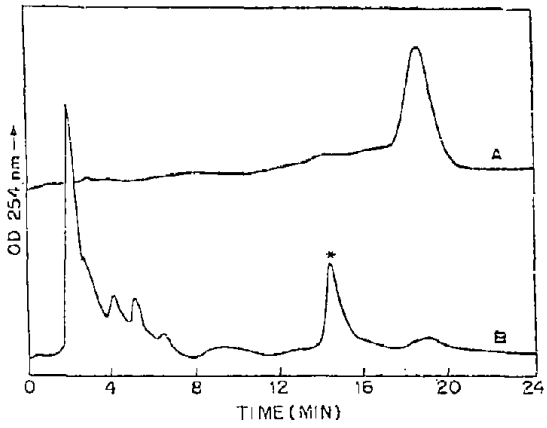

Fig. 3. HPLC analysis of cleavage reactions of d[(CG)$_3$A$_3$T$_3$(CG)$_3$] with metallodesferals: (A), DNA only; (B), A + Cu-DFO (300 μM) + ME (0.5 mM) + H_2O_2 (4 mM).

the cleavage reaction with Cu-DFO, treatment with piperidine gave a number of low molecular weight products (< 8 min), accompanied by a distinct peak at 14.2 min (Fig. 3B). This peak was collected and subjected to a DNase I treatment upon which only two peaks were seen. This pattern was similar to DNase I hydrolytic product of d(AAATTT), suggesting that the peak at 14.2 min is a direct cleavage product containing only A and T bases. These experiments clearly indicated a CG-associated preference in cleavage reactions promoted by DFO complexes.

The results of cleavage experiments using well defined

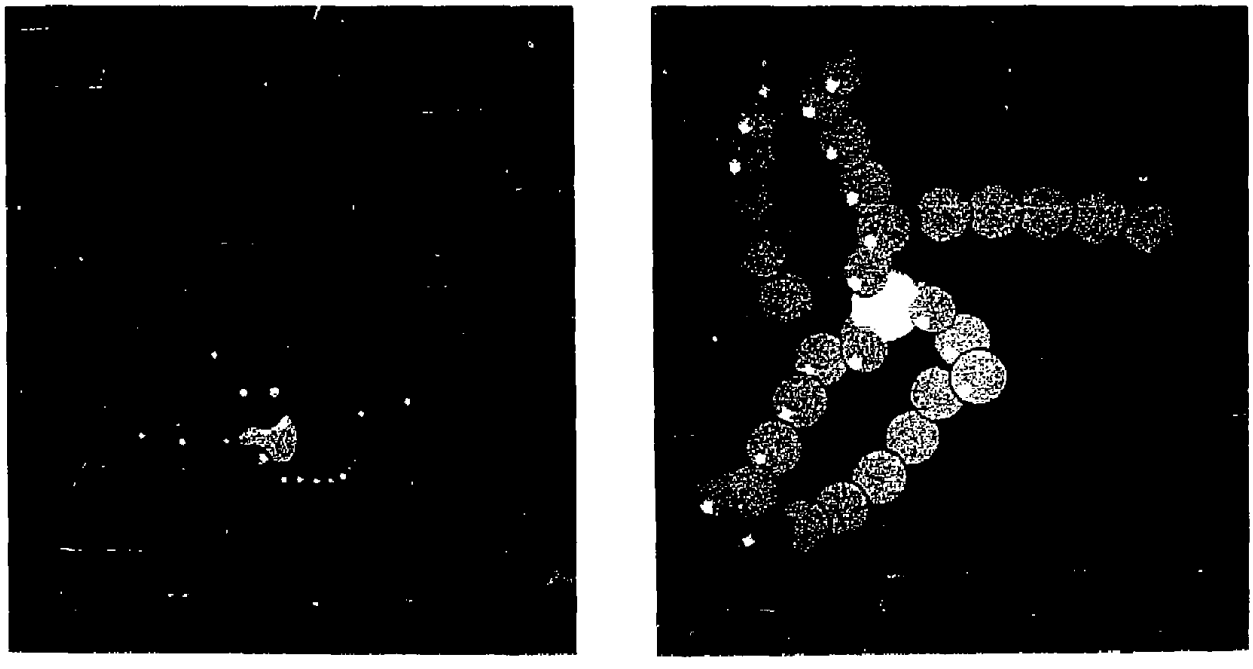

Fig. 4. Energy minimised (DTMM software) structures of C-backbone of Ferrioxamine B. The co-ordinates for the structure are from [11].

synthetic oligonucleotides reported in this communication demonstrate that metallodesferal complexes show an inherent preference for cleavage at CG sites. To understand the possible origin of specificity, we attempted molecular modelling of metallodesferal using DTMM software. The structure was generated from the co-ordinates of crystal structure of Ferrioxamine E [11], in which part of the structure was excised to transform it into Ferrioxamine B. The energy minimised structure (Fig. 4) shows interesting features. The two peptide groups are oriented trans, with their carbonyls along with the co-ordination carbonyls of hydroxamine residues located on the same side of the molecule. The peptide hydrogens point away from the carbonyls on the opposite site. The interpeptide distance (~13 Å) is roughly 4 times the average helix rise per residue in B-DNA, suggesting a possible geometrical fit based on hydrogen bonding between the peptide groups and nucleobases. The guanine N7 can also directly co-ordinate with the metal centre. Further studies are aimed towards understanding the molecular mechanism of cleavage using oligonucleotide-metallodesferal covalent conjugates.

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