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RECOGNITION AND CLEAVAGE OF DNA BY A DISTAMYCIN-SALEN•COPPER CONJUGATE

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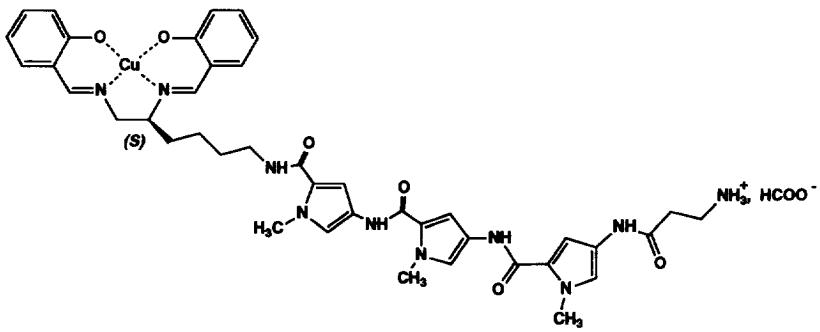
Abstract: Linkage of a salen•Cu complex to a distamycin analogue leads to a hybrid molecule that binds selectively to AT-rich sequences in DNA. The cleavage of DNA in the presence of a reducing agent remains efficient but weakly selective. © 1997 Elsevier Science Ltd.

Bis(salicylidene)ethylenediamine, usually referred to as salen, efficiently cleaves nucleic acids via metal-mediated redox or hydrolytic processes. Complexes of tetradentate salen-type Schiff bases with Cu^{II}, Co^{II} and Mn^{III} have proved useful as synthetic catalysts to investigate nucleic acids conformation.[1-4] Recently we reported that a functionalized salen-Cu^{II} complex is capable of triggering single-stranded and non-specific DNA cleavages after activation with appropriate reducing agents such as 2-mercaptopropionic acid (MPA).[5] Subsequently, we showed that the coupling of the salen moiety to anthraquinone derivatives significantly promotes their affinity for DNA but has no effect on the cleavage reaction.[6] In an effort to increase the specificity and to direct the cleavage within the minor groove of DNA, we have coupled the salen•copper complex to the antibiotic distamycin known to act as a recognition element.[7,8] Here we report on the synthesis of this distamycin-salen•Cu^{II} conjugate and the evaluation of its capacity to bind and to cleave DNA sequences.

Figure 1.



The salen moiety functionalized with a butyl-amino side chain can be synthesized either as a metal-free Schiff base or directly as a copper complex according to a sequential procedure described recently.[5,9] It was condensed with the amino propionamido-trispyrrole-carboxylic acid block[10] mimicking the distamycin antibiotic, via a conventional coupling procedure using dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt). The N-terminal BOC protecting group was removed under mild acidic conditions using formic acid without destroying the complex. Attemps with TFA or HCl were unsuccessful.[11]

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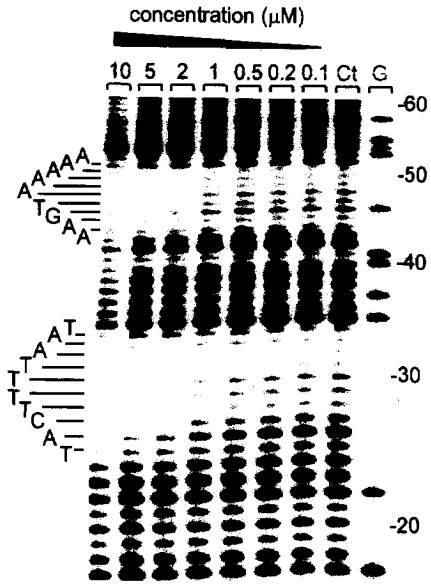

Figure 2. DNAase I footprinting of the distamycin-salen•Cu^{II} conjugate bound to the *tyr T* DNA fragment. The DNA was 3'-end labelled with [α-^{32}P]dATP in the presence of AMV reverse transcriptase. The drug concentration (μM) is shown at the top of the appropriate gel lanes. The track labelled "Ct" contained no drug. The track labelled "G" represents a dimethylsulphate-piperidine marker specific for guanines. Numbers on the side of the gels refer to the numbering scheme of the fragment. DNAase I cleavage products were resuspended in 5 μl 80% formamide containing 10 mM EDTA and 0.1% tracking dyes. Samples were heated to 90 °C for 4 min and then chilled in an ice-bath just before being loaded on a sequencing gel (8% polyacrylamide, 7 M urea). The two sequences protected from cleavage are indicated.

Footprinting studies were performed to investigate the capacity of the conjugate to recognise specific sequences in DNA. Figure 2 shows the results of a typical DNAase I footprinting experiment using the *tyr*T fragment that has been previously used to map distamycin binding sites.[12,13] The gel shows clearly that the linkage of the salen moiety does not hinder the capacity of the distamycin portion of the hybrid to bind selectively to AT-rich sequences. Two well-resolved footprints encompassing the sequences 5'-AAAAATGAA and 5'-TAATTTCAT can be detected using micromolar drug concentrations. Regions protected from DNAase I cleavage by the hybrid ligand are confined to the same AT-rich sequences as those detected with distamycin.[11,12] Complementary DNAase I footprinting experiments were carried out with two other restriction fragments from plasmid pBS and in each case, the footprints detected with the distamycin-salen conjugate were located exclusively within AT-rich sequences (not shown). Experiments using DNAase II and micrococcale nuclease as a DNA cleaving agent led to the same conclusion indicating that the minor groove binding element imposes its preference for AT-rich sequences.

The footprinting data together with the positive circular dichroism signals obtained with the hybrid-DNA complex at 320 nm (not shown) attest that, as expected, the distamycin portion of the hybrid locates in the minor groove of DNA. A topoisomerization assay was used to investigate intercalative binding of the salen moiety as it has been suggested for a modified salen•Cu complex.[4] This test provides direct means to probe drug-induced unwinding of closed circular duplex DNA which is a good criterion for DNA intercalation. Neither the salen•Cu moiety alone nor distamycin affect the relaxation of supercoiled DNA by topoisomerase I whereas a noticeable effect can be seen with the conjugate molecule (Figure 3A). The topoisomerization reaction was not totally inhibited as is the case with classical intercalating agents (e.g. ethidium) but however, the electrophoretic mobility of the relaxed DNA molecules is markedly reduced. This suggests that the conjugate is tightly bound to DNA and may partially intercalate its salen moiety into DNA.

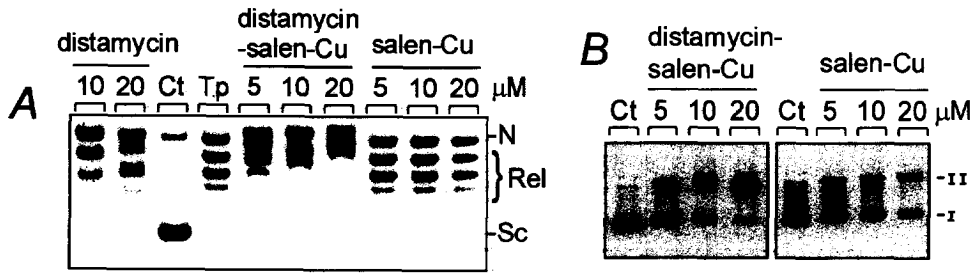

Figure 3. (A) DNA unwinding experiments. Supercoiled DNA (2 μg) was incubated with 5 units DNA topoisomerase I at 37°C for 1 h in 50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA in the absence (Tp) and presence of the drug. (B) Cleavage of closed circular DNA. Supercoiled DNA (1 μg) was incubated at 37°C for 30 min. with the drug at 5, 10 or 20 μM and 0.5 mM MPA. Control lanes (Ct) refer to the plasmid DNA incubated without drug in the presence of MPA. Forms I and II refer to the supercoiled and nicked DNA forms, respectively. In both cases, electrophoresis was performed in a 1% agarose gel for about 2 hours at 80V in TBE buffer (89 mM tris-borate pH 8.3, 1 mM EDTA). Gels were stained with ethidium bromide (1μg/ml) then destained for 20 min in water prior to being photographed under UV light.

DNA cleavage was first analysed by monitoring the conversion of supercoiled plasmid DNA (form I) to the nicked circular molecules (form II) and linear DNA (form III). The tests were performed under aerobic conditions in the presence of 2-mercaptopropionic acid (MPA) as a reducing agent. As shown in Figure 3B, the distamycin analogue equipped with a salen•Cu^{II} functionality is able to catalyze reductive cleavage of DNA. Incubation of the plasmid at 37°C for 30 min. with 10 μM drug causes the almost complete conversion of the form I to the nicked form II and at a higher concentration, linearized DNA molecules (form III) become apparent. We therefore conclude that the activation of the copper complex leads to efficient single strand cleavage of duplex DNA.

Then we investigated the cleavage reaction using sequencing gels and the *Tyr*T fragment was employed as a substrate The distamycin-salen•Cu complex alone does not cleave DNA but addition of MPA initiated cleavage of DNA. Surprisingly, the cleavage proved to occur at random. At low drug concentration (< 5 μM) the regions between the binding sites inferred from the footprinting experiments (e.g. around nucleotide positions 35 and 55) are slightly more reactive than the others but at higher concentrations the cutting occurs all along the DNA fragment (Figure 4). We tested various experimental conditions but in each case non-specific cleavage was observed.

The above results indicate that the introduction of the DNA-cleaving group does not abolish the capacity of the drugs to recognise selectively AT-rich sequences in DNA. However, it does seem clear that there is no simple correlation between the binding data and the cutting profile of the conjugate. The cleavage of DNA proved to be non-specific whereas the hybrid molecule binds selectively to AT-rich tracts as judged from the various footprinting experiments. Linkage to sequence-selective minor groove binders such as netropsin and distamycin, as the means of targeting of cutting agents to DNA has been explored by others in recent years. Among metal complexes, EDTA•Fe,[14] peptide•Cu[15] and bleomycin models•Fe complexes (e.g. PYML[16] and AMPHIS[17]) have been linked to distamycin derivatives with the aim of producing artificial nucleases. In agreement with the results reported here, it has been observed generally that the AT selectivity conferred by the oligopyrrole moiety can be retained but the metal complex-induced DNA cleavage efficiency is not necessarily enhanced. Further hybrid molecules containing other types of metal complexes are now being synthesized with the aim of finding conjugates capable of inducing double-strand DNA cleavage.

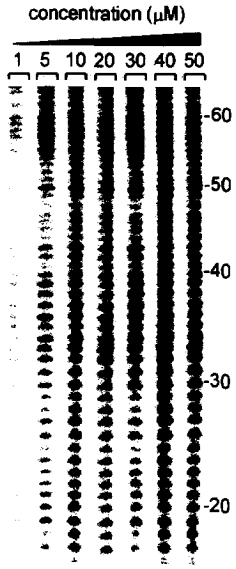

Figure 4. Cleavage of the *tyr T* DNA fragment in the presence of increasing concentrations of the distamycin-salen•Cu^{II} conjugate. The DNA ^{32}P-labelled at its 3'-end labelled DNA was treated with the drug in the presence of 1 mM MPA. After 1 hour incubation at 37°C, samples were precipitated with cold ethanol and the DNA was resuspended in 5 μl 80% formamide containing 10 mM EDTA and 0.1% of tracking dyes. Samples were heated to 90 °C for 4 min and then chilled in an ice-bath just before being loaded on a sequencing gel (8% polyacrylamide, 7 M urea).

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11. Analytical data for (i) the BOC-protected conjugate: 41 %, mp 172-174 °C; IR (KBr): ν 3300, 2910, 1690, 1650, 1620 cm^{-1}; MS (MALDI$^+$): 938.5 $(M+1)^+$, 960.5 $(M+Na)^+$, 976.5 $(M+K)^+$; Rf (MeOH) 0.23; ESR: $A_{//}$ 191 G, $g_{//}$ 2.23. Anal. calcd for $C_{46}H_{54}N_{10}O_8Cu$: C, 58.89; H, 5.81; N, 14.94. Found: C, 59.01; H 5.72; N, 14.85.; (ii) the conjugate: 79 %, mp 193-195 °C; IR (KBr): ν 3500, 2930, 1720, 1690, 1635 cm^{-1}; MS (MALDI$^+$): 838.5 $(M-HCOO^-)^+$, 861.5 $(M+Na-HCOO^-)^{2+}$; Rf (MeOH) 0.0; ESR: $A_{//}$ 189 G, $g_{//}$ 2.22. Anal. calcd for $C_{42}H_{48}N_{10}O_8Cu$: C, 57.06; H, 5.48; N, 15.85. Found: C, 57.12; H, 5.35; N, 15.89.
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