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Unique Cooperative Binding Interaction Observed between a Minor Groove Binding Pt Antitumor Agent and Hoeschst Dye 33258

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The trinuclear compound $[\{Pt(NH_3)_3\}_2\mu\text{-}\{trans\text{-}Pt(NH_3)_2(H_2N(CH_2)_6\text{-}NH_2)\}_2]^{6+}$ (0,0,0/t,t,t) binds to DNA only through noncovalent hydrogen bonding and electrostatic interactions. The presence of this 6+ cation allows discrimination of binding modes for common DNA ligands: binding of minor-groove agents such as Hoechst 33258 is cooperative, and dye–DNA interaction is enhanced whereas intercalation as exemplified by ethidium bromide is competitively inhibited.

DNA, as a template, affects the rate of substitution reactions of coordination complexes occurring within its domain.[1,2] Metal complexes can be designed to target particular sequences or structural features of the DNA helix, and upon binding will then influence DNA structure.[3]

The introduction of positive charge by cations and small molecules interacting with DNA can also impart significant steric and electronic effects on the polynucleotide and alter the template structure and reactivity.[4] Preassociation of transition metal cations to DNA by electrostatic and hydrogen-bonding interactions prior to possible covalent bond formation may also significantly affect local structure and sequence specificity of the final adducts.[5,6] In this Communication, we show how localization of charge on the polynucleotide by polynuclear platinum complexes results in cooperative effects with other DNA binding ligands. Additionally, we demonstrate that this cooperativity affects the sequence specificity of the ligand and differentiates between DNA minor groove and intercalator binding modes. Polynuclear platinum complexes present a particularly interesting set of compounds to study for these effects, because the charge and its distribution may be systematically altered.

The trinuclear platinum clinical agent 1,0,1/t,t,t (BBR 3464) binds to DNA giving interstrand adducts that are structurally distinct from those formed by cisplatin.[7] Examination of the kinetics of cross-link formation in the sequences $(5'\text{-ATATGTACATAT-}3')_2$ and $(5'\text{-TATGTATACATA-}3')_2$ produced evidence for preassociation in the minor groove.[8] Minor groove contacts are also observed in the structure of the isolated 1,4 cross-link formed from BBR 3464 and the 8 mer $(5'\text{-ATGTACAT-}3')_2$.[6] To further examine the role of preassociation in platinum complex binding to DNA, the "noncovalent" analogues (0,0/t,t and 0,0,0/t,t,t) were prepared, where the chloride leaving groups are replaced by the inert ammonia ligand to prevent covalent binding to DNA (Figure 1).[9]

The dinuclear and trinuclear "noncovalent" compounds carry a positive charge of 4+ and 6+, respectively. Competition binding experiments between these compounds and both intercalator and minor groove ligands were studied. The 10 mer, $(5'\text{-GGTAATTACC-}3')_2$, is a high affinity sequence for Hoechst dye 33258.[10,11] The fluorescence of the dye is increased upon binding in the minor groove of DNA due to the exclusion of water and fluorescence intensity is an indicator of the strength of dye binding.[12] In the presence of the trinuclear platinum complex, the fluorescence attributed to the dye−DNA interaction actually increased in the presence of the cation, Figure 2A. Thus, the binding is enhanced in the presence of II; inhibition of binding would lower fluorescence compared to control. Other cations such as the dinuclear 0,0/t,t (IV) or the well studied $[Co(NH_3)_6]^{3+}$ also demonstrate this effect but to a much lesser extent than the 6+ agent. Interestingly, this effect could also be produced

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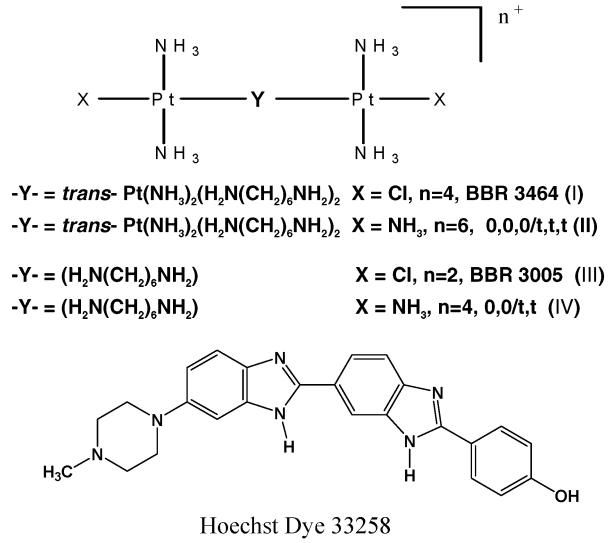

Figure 1. Structures of polynuclear platinum anticancer agents, their "noncovalent" analogues, and Hoechst dye 33258.

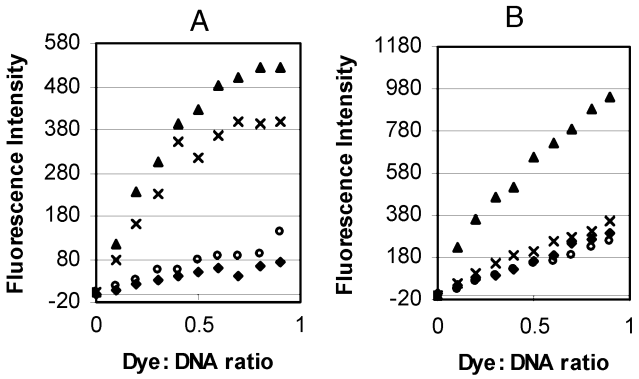

Figure 2. Fluorescence of Hoechst 33258 interacting with DNA in the presence of transition metal cations: ◆ DNA plus Hoechst dye, ▲ II, × IV, ○ Co(hex). (A) 1 μM (5′-GGTAATTACC-3′)$_2$, (B) 1 μM (5′-ATATGTACATAT-3′)$_2$. While a decrease or maintenance of the control fluorescence was expected, a large increase was observed. Note the different scales for panels A and B. No enhancement is seen for dye + compound in absence of template DNA.

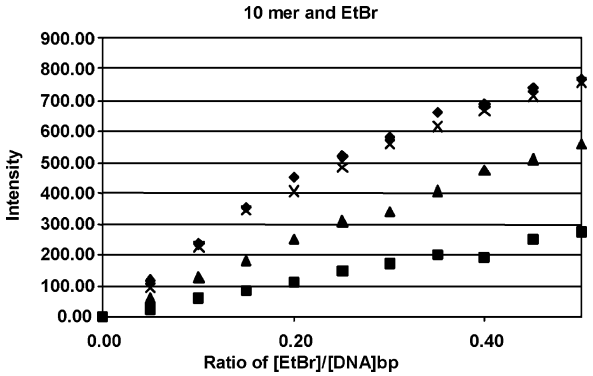

Figure 3. Fluorescence of ethidium bromide in the presence of (5′-GGTAATTACC-3′)$_2$ (1 μM) and transition metal cations: ◆ DNA plus EtBr, ▲ II, × IV, ■ EtBr with no DNA.

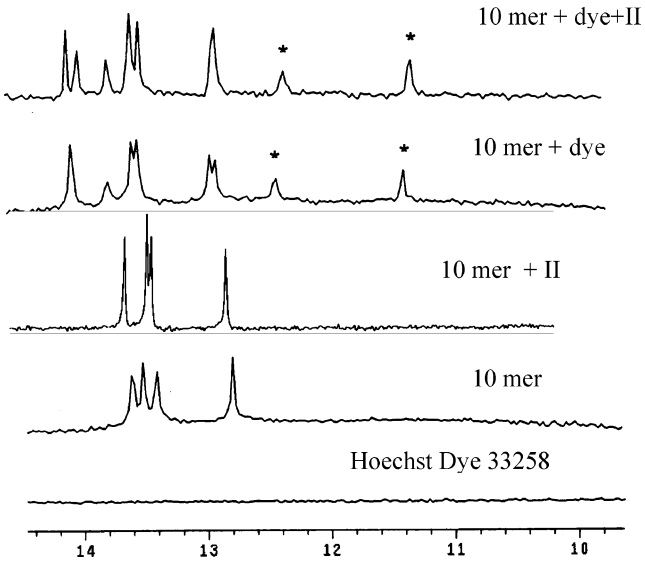

Figure 4. 1D ^{1}H NMR spectra of Hoechst dye in the presence of 10-mer (5′-GGTAATTACC-3′)$_2$ (0.5 mM) and II (1:1 II/dye ratio) shows the appearance of the imino protons of the dye at 11.2 and 12.3 ppm as well as the thymine imino proton at 14 ppm. These signals are enhanced upon the addition of II. No enhancement of these NMR signals is seen for dye + compound in absence of template DNA.

by moderate levels of NaCl (50 mM). Scatchard plot analysis was nonlinear, preventing the calculation of a meaningful binding constant at this time. Nevertheless, the effect is clear.

This phenomenon is not restricted to sequences of high affinity for the dye. Upon addition of dye to a solution of II:DNA (3:1) in 50 mM NaCl, where the DNA is the 12 base pair oligonucleotide, (5′-ATATGTACATAT-3′)$_2$, the fluorescence also increased in a concentration dependent manner, Figure 2B. In this case, the effect is unique to the trinuclear compound and no effect is seen for the smaller cations or in the presence of 50 mM NaCl alone.

When similar competition experiments were performed with the intercalator ethidium bromide (EtBr) using the same sequences, the ethidium fluorescence decreased in the presence of the Pt complexes, indicating the ability of the cation to displace or prevent the binding of ethidium to the DNA, in agreement with previous results.[7,13] The 6+ cation II is significantly more efficient than IV or $[Co(NH_3)_6]^{3+}$ (not shown) at preventing intercalation, indicating a very strong interaction (Figure 3).

Thus, the deposition of a high concentration of charge (6+) in a localized region of DNA causes cooperative binding of a minor groove ligand but inhibits intercalation. Further, "low-affinity" minor groove binding sites may be converted to "high-affinity" ones, and again, the 6+ agent is more efficient than either the 4+ or 3+ cation. The molecular details for this differentiation are of some interest. 1-D ^{1}H NMR spectra of the ternary (5′-GGTAATTACC-3′)$_2$/dye/cation solution show the appearance at 11.2 and 12.3 ppm of the dye imino protons upon making hydrogen bonding contacts in the minor groove (and subsequent diminution of solvent exchange).[14] These signals appear somewhat enhanced in the presence of II, and new signals also appear in the exchange region (Figure 4). The thymine imino protons seen at approximately 14 ppm are sharpened by the addtion of II also indicating an effect on the minor groove.

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Preliminary experiments utilizing 2-D NMR techniques show that both II and the Hoechst dye make hydrogen bonding contacts in the minor groove, as well as showing NOE contacts with each other.

Extensive structural analysis of Hoechst 33258−DNA interaction indicates that minor groove ligand recognition involves a combination of induced fit and conformational selection.[8,14] Factors contributing to the degree of induced fit include changes in base step parameters, suppression of DNA bending modes at TA steps, and changes to sugar−phosphate backbone conformation.[14] The latter factor is especially likely to be affected by charge neutralization. Critical to a consideration of electrostatic effects on DNA is the counterion condensation theory of Manning.[16] This has been extensively explored in the context of small cations such as $[Co(NH_3)_6]^{3+}$ or the naturally occurring polyamines spermidine (3+) and spermine (4+) as well as the naturally occurring Na^+ and Mg^{2+}.[16] The bending and flexibility of the double-stranded DNA template is affected by charge neutralization, but the 3+ and 4+ cations are considered to be randomly distributed throughout the helical surface.[16] Prebending by charge neutralization may enhance minor-groove recognition but not intercalation. A single-crystal X-ray diffraction structure determination of a noncovalent multinuclear platinum compound with the dodecamer d(CGCGAATTCGCG)$_2$ does in fact show some bending of the helix axis.[17] It is of interest to note that bending of cisplatin-bound DNA promotes ternary adduct formation with normally unreactive intercalators such as ethidium[18] in contrast to competitive displacement. This reaction has significant steric requirements, double stranded DNA and the *cis* rather than the *trans* platinum isomer,[19] further emphasizing the diversity of metal complex binding modes in modulation of solution dynamic behavior of DNA.

The behavior observed here suggests that charge effects are not simply cumulative as the charge is increased and a "break" may occur with DNA binding ligands such as II which can deposit up to 6+ in a well-defined localized region of the polynucleotide. This allows observation of hitherto unexpected effects involving both cooperativity and sequence selectivity of DNA-binding ligands. These new agents may find utility in extending the study of the effects of electrostatic phenomena to higher charges and further assist in understanding the role of electrostatic deformation in DNA−protein interactions.

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