Nucleic Acids Condensation

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Substitution-Inert Trinuclear Platinum Complexes Efficiently Condense/Aggregate Nucleic Acids and Inhibit Enzymatic Activity**

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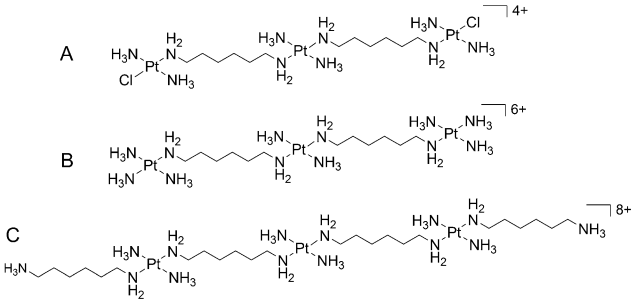
Abstract: *The trinuclear platinum complexes (TriplatinNC-A $[\{Pt(NH_3)_3\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{6+}$, and TriplatinNC $[\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_3^+)\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{8+}$) are biologically active agents that bind to DNA through noncovalent (hydrogen bonding, electrostatic) interactions. Herein, we show that TriplatinNC condenses DNA with a much higher potency than conventional DNA condensing agents. Both complexes induce aggregation of small transfer RNA molecules, and TriplatinNC in particular completely inhibits DNA transcription at lower concentrations than naturally occurring spermine. Topoisomerase I-mediated relaxation of supercoiled DNA was inhibited by TriplatinNC-A and TriplatinNC at concentrations which were 60 times and 250 times lower than that of spermine. The mechanisms for the biological activity of TriplatinNC-A and TriplatinNC may be associated with their ability to condense/aggregate nucleic acids with consequent inhibitory effects on crucial enzymatic activities.*



Scheme 1. The chemical structures of trinuclear linear Pt compounds A) cytotoxic drug BBR3464 $[\{trans\text{-}PtCl\text{-}(NH_3)_2\}\text{-}\mu\text{-}trans\text{-}Pt(NH_3)_2\{NH_2\text{-}(CH_2)_6NH_2\}_2]^{4+}$; B) TriplatinNC-A $[\{Pt(NH_3)_3\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(NH_2\text{-}(CH_2)_6NH_2)_2\}]^{6+}$; and C) TriplatinNC $[\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6\text{-}NH_3^+)\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{8+}$.

Polynuclear platinum complexes represent a discrete class of anticancer drugs which are structurally distinct from the mononuclear compounds. The trinuclear TriplatinNC-A $([\{Pt(NH_3)_3\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{6+}$ and TriplatinNC $[\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_3^+)\}_2\text{-}\mu\text{-}\{trans\text{-}Pt\text{-}(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{8+}$,) (Scheme 1 B, C) bind to DNA through noncovalent interactions. The basis of these interactions is the phosphate clamp, a third mode of ligand binding to DNA, discrete from canonical intercalation and minor-groove binding.[1,2]

Polynuclear platinum compounds, and TriplatinNC in particular, have interesting biological properties including in vitro toxicity in several ovarian carcinoma cell lines,[3,4] high cellular accumulation, and transformed cell selectivity.[5] The modular nature of the polynuclear Pt complex binding results in a high DNA binding affinity with subsequent stabilization and induction of B-DNA to Z-DNA and B-DNA to A-DNA conformational transitions in susceptible sequences at concentrations lower than those required by cobalt hexammine[6] and displacement of ethidium bromide from DNA.

The molecular details of phosphate-clamp binding to DNA has analogies with the arginine fork mode of recognition on nucleic acids.[1,2] The structure, especially of the noncovalent compounds, also bears analogy to the naturally occurring polyamines, such as spermidine and spermine. We previously reported on the DNA binding affinity, condensation properties, and sequence selectivity of the dinuclear polyamine(spermidine/spermine)-linked complexes and the trinuclear complex TriplatinNC-A.[7] All polynuclear platinum compounds induce DNA condensation at concentrations which are more than one order of magnitude lower than conventional condensing agents, such as the naturally occurring polyamine spermine^{4+} itself $(H_2N(CH_2)_3NH(CH_2)_4NH\text{-}(CH_2)_3NH_2)$. TriplatinNC-A binds to DNA in a sequence-dependent manner protecting DNA from enzymatic cleavage by endonuclease deoxyribonuclease I (DNase I).[7] This result is consistent with the structurally demonstrated spanning of the minor groove by TriplatinNC-A,[2] its protection of the minor groove toward alkylation,[8] and the increase of the DNA-binding affinity of Hoechst dye.[9]

The mechanism of the biological activities of polynuclear platinum complexes, which bind to DNA through noncovalent interactions, is not fully understood. However, our results suggest that the mechanism may be associated with the unique ability of these compounds to condense DNA along with their sequence-specific DNA binding, and possibly on competition with naturally occurring polyamines for intra-

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cellular binding sites, but with altered function. Herein, we compare their nucleic acid binding effects by biophysical methods and report on the condensation and aggregation of the DNA and tRNA (transfer RNA) induced by TriplatinNC and TriplatinNC-A in cell-free media. Furthermore, because DNA-binding ligands in general may impair crucial enzymatic processes acting directly on DNA or RNA, we have investigated their effects on DNA transcriptional activity and the activity of eukaryotic DNA topoisomerase I (topo I).

Total intensity light scattering[10] was employed to determine the efficacy of TriplatinNC and TriplatinNC-A to condense/aggregate calf thymus (CT) DNA and tRNA. This method is based on the measurement of the intensity of light scattered by the diluted DNA or RNA solution at 90°. The scattered light intensity is low in the absence of, or at low concentrations of, the condensing agent, however a marked increase in the intensity of the scattered light appears at a critical concentration of added agent because of the formation of condensed DNA or RNA particles. The increase in the intensity of the scattered light is concentration dependent up to a certain concentration of the condensing agent and then it levels off. The efficacy of various condensing agents to induce DNA or RNA condensation can be quantified by determining the EC_{50} value, which is the concentration of a condensing agent at the midpoint of the condensation. As can be seen in Table 1, TriplatinNC, having a higher charge of 8+, was more efficient in inducing CT DNA condensation than TriplatinNC-A with a 6+ charge. The EC_{50} value of TriplatinNC (0.15 ± 0.01 μM) is approximately 27 times lower than that of spermine obtained under the same conditions.[7]

Table 1: EC_{50} values [μM] of spermine and Pt compounds.[a]

	CT DNA	tRNA
spermine	4.1 ± 0.5[b]	> 50
TriplatinNC-A	0.20 ± 0.01[b]	0.27 ± 0.01
TriplatinNC	0.15 ± 0.01	0.24 ± 0.01

[a] All measurements were conducted in sodium cacodylate buffer (10 mM) pH 7.2, at 298 K. EC_{50} values were determined by plotting the scattered light intensity against the concentration of condensing agents. Data are mean ± SD (standard deviation) of three separate measurements. [b] Data previously reported.[7]

The molecules of CT DNA which have an average length of several kilobase pairs can be compacted by either monomolecular condensation with distinguishable morphologies[11,12] or multimolecular aggregation with an irregular morphology. In contrast, the tRNA molecules are typically 60–95 nucleotides in length and are too short to be individually condensed. The only way tRNA particles can be formed is by multimolecular aggregation.[13,14]

TriplatinNC and TriplatinNC-A were also shown to be efficient in tRNA aggregation, as summarized in Table 1. Notably, the conventional DNA condensing agent spermine was unable to induce tRNA aggregation at concentrations up to 200 times higher than those of the Pt complexes. Therefore, TriplatinNC-A and TriplatinNC represent unique agents capable of condensing tRNA. The outstanding and unprecedented efficiency to condense/aggregate nucleic acids was confirmed by UV/Vis absorption spectroscopy, gel retardation assays, and by investigating the effect of TriplatinNC on the morphology of DNA or tRNA condensates by atomic force microscopy (Figure 1 and Figures S1–S8 in the Supporting Information).

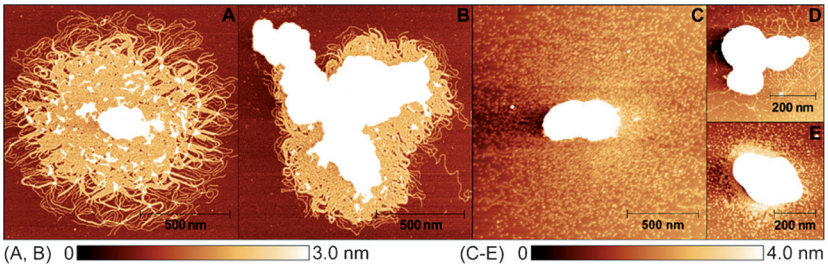

Figure 1. Representative AFM images of linearized plasmid pSP73 DNA (A, B) and tRNA (C–E) in the presence of TriplatinNC (6.25 μM). See Figures S7 and S8 for further details. Scale bars in (A–C) = 500 nm; scale bars in (D, E) = 200 nm.

It has been previously demonstrated[15] that DNA transcriptional activity occurs as a function of spermine concentration. When the concentration of spermine was kept below approximately 1 mM, the DNA transcriptional activity is enhanced, whereas increasing the concentrations of spermine above approximately 1 mM results in an inhibition of the DNA transcription. The procedure of Luckel et al.[15] was employed to examine the transcriptional activity of the circular form of the plasmid pBR322 DNA in the presence of TriplatinNC-A and TriplatinNC. The DNA transcription was monitored by measuring the fluorescence intensity resulting from the separation of the AmNS (aminonaphthalenesulfonate) group from the terminal phosphate group of UTP (uridine triphosphate) in the fluorescent molecular probe UTP-gamma-AmNS during RNA polymerization. The actual amounts of transcription products in the presence of various concentrations of spermine, TriplatinNC-A, and TriplatinNC as a function of time are shown in Figure 2A. It can be seen that the DNA transcriptional activity was inhibited in the presence of 0.4 μM and 2 μM of TriplatinNC and TriplatinNC-A, respectively. In contrast, a concentration of spermine as high as 800 μM still had a slightly enhancing effect on the transcriptional activity. The plots of relative transcriptional activity (Figure 2B), defined as the ratio of RNA products at given TriplatinNC and TriplatinNC-A concentrations to the amount of RNA products in the absence of any complex, show that the addition of approximately 4 μM of TriplatinNC and 20 μM TriplatinNC-A completely inhibited transcription of the DNA. These concentrations are approximately 350 times and 70 times lower than the concentration of spermine necessary to achieve the same effect (> 1400 μM).[15]

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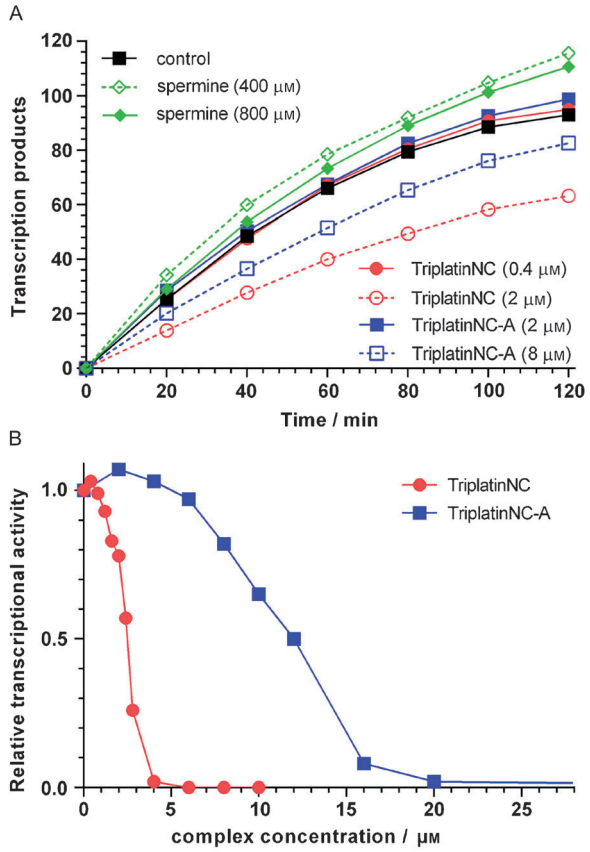

Figure 2. A) Transcription kinetics of circular pBR322 plasmid DNA in the absence and in the presence of various concentrations of spermine, TriplatinNC-A, and TriplatinNC. B) Relative transcriptional activity of pBR322 plasmid DNA as a function of TriplatinNC (red circles) and TriplatinNC-A (blue squares) concentration.

The results show that TriplatinNC and TriplatinNC-A are approximately 27-fold and 20-fold more potent inducers of mammalian CT DNA condensation than spermine, and that unlike spermine, both complexes cause aggregation of relatively small tRNA molecules (typically 60–95 nucleotides in length). The high resistance of DNA and tRNA condensates/aggregates formed by TriplatinNC-A and TriplatinNC in particular against treatment by high concentrations of NaCl (Figures S2 B, S3, and S6) indicates the strong interaction of both complexes with nucleic acids. Therefore, we suggest that the high affinity of TriplatinNC-A and TriplatinNC for nucleic acids and the high efficiency of these polynuclear platinum complexes in condensing DNA preclude reading DNA sequences by RNA polymerases when they produce a complementary, antiparallel RNA strand (i.e. a primary transcript). Notably, DNA compaction is expected to prevent the binding/sliding of RNA polymerase to DNA.[16–18] RNA polymerases function like a molecular motor that can convert chemical energy into the work of translocation along the DNA during transcription.[19] Thus, it is also possible that this DNA translocation mechanism is insufficiently powerful to displace TriplatinNC and TriplatinNC-A which is tightly bound to condensed template DNA. As TriplatinNC and TriplatinNC-A are markedly more effective in condensing DNA and inhibiting the transcriptional activity of DNA compared to conventional spermine, our findings suggest an enhanced regulatory role of TriplatinNC and TriplatinNC-A at the level of genome transcription in comparison with spermine. The activity of platinum drugs against cancer is mediated by a combination of processes, including cell entry, drug activation, DNA-binding, and transcription inhibition.[20] Our results reveal a correlation between the high affinity of TriplatinNC and TriplatinNC-A for nucleic acids and the high efficiency of these polynuclear platinum complexes to condense/aggregate DNA on one hand, and the ability of these complexes to inhibit DNA transcription on the other.

Topoisomerases are ubiquitous and vital enzymes because of their role in the control of the topological state of DNA. Thus, topoisomerases participate in nearly all events related to DNA metabolism including replication, transcription, and recombination. Topoisomerases are now viewed as important therapeutic targets and in particular, topoisomerase inhibitors are considered promising anticancer agents.[21–23]

We utilized a DNA relaxation assay to examine whether the activity of eukaryotic topoisomerase I (topo I) is affected in the presence of TriplatinNC and TriplatinNC-A. This assay is based on monitoring the conversion of naturally negatively supercoiled pSP73 KB plasmid DNA preincubated with increasing concentrations of TriplatinNC, TriplatinNC-A, and spermine into relaxed covalently closed circular DNA. As can be seen in Figure 3, TriplatinNC and TriplatinNC-A completely inhibited topo I-catalyzed DNA relaxation at concentrations as low as approximately 16 μM and 64 μM, respectively. These values are approximately 250- and 60-fold lower than the concentration of natural polyamine spermine (4 mM) which gives rise to the same effect (Figure S9).

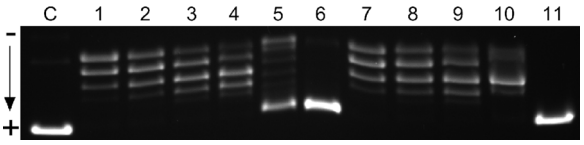

Figure 3. Inhibition of relaxation of negatively supercoiled pSP73 KB plasmid DNA by topo I in the presence of increasing concentrations of TriplatinNC-A and TriplatinNC analyzed by agarose gel electrophoresis. Lane C: nonmodified plasmid DNA in the absence of topo I; lane 1: nonmodified plasmid DNA in the presence of topo I; lanes 2–6: plasmid DNA in the presence of topo I and TriplatinNC-A (at concentrations 4, 8, 16, 32, and 64 μM, respectively); lanes 7–11: plasmid DNA in the presence of topo I and TriplatinNC (at concentrations 1, 2, 4, 8, and 16 μM, respectively). DNA concentration in the samples: 200 μM (0.5 μg).

The inhibitory effect of TriplatinNC-A on topo I-mediated plasmid DNA relaxation is comparable to that of some synthetic hexamines examined previously,[24] whereas TriplatinNC is approximately four times more potent. Interestingly, both trinuclear platinum complexes induce DNA topo I inhibition at concentrations that are similar or even lower than those of several conventional topo I inhibitors, such as camptothecin derivatives, which are used in the treatment of cancer and induce a similar inhibitory effect.[25]

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It has been reported[24] that the types of structures of the DNA aggregates formed by different polyamines might play a key role in topo I catalysis. For instance, DNA aggregation induced by the natural polyamine spermine yields a highly fluid DNA aggregate. In contrast, the polyamines with the longest central chains produce very different non-fluid aggregates with a high packing density of DNA molecules. Polyamines yielding non-fluid aggregates were markedly more efficient inhibitors of DNA relaxation by topo I than considerably shorter natural polyamines (spermine) yielding the fluid aggregates.[7,24]

Thus, trinuclear platinum complexes tested herein, and in particular TriplatinNC, can be grouped with longer polyamines yielding the non-fluid aggregates (Figure S7) responsible for inhibition of DNA relaxation by topo I.[24] The observation that TriplatinNC-A is a less efficient inhibitor of DNA topo I activity than TriplatinNC (Figure 3) is consistent with the fact that the DNA condensates induced by TriplatinNC-A are less compact than those induced by TriplatinNC. Similarly, the enhanced inhibition efficiency of TriplatinNC corresponds to our observation that at higher concentrations of TriplatinNC, DNA particles are predominantly formed instead of the flat, single-layered compact DNA patterns typically found for TriplatinNC-A (see Ref. [7]). Moreover, it has been proposed[24] that, in contrast to spermine, the longer polyamines, such as hexamines, could form more crosslinks (crosslinks through electrostatic interactions and hydrogen-bonding interactions which bridge the groove of the helices or helix[26–28]) between different DNA molecules or different parts of the same DNA molecule leading to the formation of the highly condensed non-fluid aggregates that are responsible for the inhibition of the topo I activity. Thus, it is reasonable to assume that the ability of the longer trinuclear Pt complexes tested herein to form crosslinks similar to those formed by hexamines might contribute to the formation of highly condensed non-fluid aggregates and consequently to the inhibition of the topo I activity by these agents. The details of the mechanism of the inhibition of the topo I activity by the condensed non-fluid aggregates formed by long polyamines, such as TriplatinNC-A, TriplatinNC, or hexamines, are unclear. A plausible suggestion, which needs experimental confirmation, might be that formation of the highly condensed non-fluid aggregates does not allow topo I to access its specific recognition sites, or if the enzyme binds to DNA it is unable to cleave and transfer the densely packed DNA strand.[24]

Both trinuclear platinum complexes TriplatinNC and TriplatinNC-A demonstrate high efficiency in inducing the condensation and aggregation of nucleic acids. The higher overall charge (8+) and greater length of TriplatinNC are probably responsible for its greater ability to induce DNA condensation and tRNA aggregation in comparison with the (6+) TriplatinNC-A. The presence of dangling amine substituents on the terminal Pt^{II} atoms of TriplatinNC also extends the length of the molecule, and, combined with the increased charge, might enable the formation of crosslinks by condensing counterions between the different DNA or RNA molecules or between the different domains of the same DNA or RNA molecule. The process of condensation or aggregation induced by TriplatinNC bridging the groove of the helices occur through electrostatic interactions and hydrogen bonding.[26–28] AFM studies of DNA condensation in the presence of TriplatinNC have revealed (Figure S7, S8) that the morphologies of DNA condensates become more compact with increasing concentration of the platinum complex. The higher concentrations of TriplatinNC lead to the formation of DNA particles instead of rather flat and single-layered compact DNA patterns typically found for TriplatinNC-A. The results from AFM experiments (Figure 1, Figures S7 and S8) correlate well with the results of other experiments and confirm that TriplatinNC and TriplatinNC-A condense DNA with much higher potency than spermine. In contrast to spermine, both platinum complexes induce aggregation of short tRNA molecules. These properties also distinguish the phosphate clamp DNA-binding mode of polynuclear platinum complexes (with their high positive charge) from typical minor-groove binders, despite the affinity for A–T-rich sequences.[7] Thus, this class of polynuclear Pt complex has distinctly different properties to both the polyamine class and the minor-groove binders.

The high affinity and DNA conformational changes induced by the complexes also have consequences for protein–DNA interactions. The enzymatic studies have shown that TriplatinNC and TriplatinNC-A inhibit DNA transcriptional activity and topo I-mediated relaxation of supercoiled DNA at markedly lower concentrations than naturally occurring spermine. These results, combined with the demonstration that TriplatinNC targets the nucleolus,[29] suggest new approaches to the eventual inhibition of cellular proliferation, distinct from the covalent Pt–DNA bond formation approach.[30]

Within the concept of a noncovalently binding polynuclear Pt agent, this paper and previous studies have investigated DNA affinity and conformational changes by systematically modifying the modular nature of the chemotype. These changes have been achieved by employing {Pt(tetraamine)} moieties instead a polyamine central linker and by using dangling amine groups in place of NH_3 as the ligand to add further overall charge. Athough the biological consequences of DNA binding are similar for all compounds independent of specific structure, it is clear that there are some differences. TriplatinNC in particular is cytotoxic at low micromolar concentrations whereas TriplatinNC-A is consistently 5–10 times less potent. Both compounds are significantly more cytotoxic than the dinuclear polyamine-linked analogues.[31,32] For the two compounds studied herein, cytotoxicity also correlates with cellular accumulation mediated by heparan sulfate binding on the cell surface.[33,34] Thus, although the effect of nucleic acid condensation may contribute overall to the general effects of these compounds on cells, differentiation between cytotoxic effects may reflect differences in cellular accumulation.[5,9] This distinction may allow for consideration of these compounds as dual-function agents where traditional DNA-binding effects may be complemented by effects on the cell surface.[30] Additionally, these trinuclear platinum complexes may also have potential for gene delivery because the transfection of DNA in gene therapy largely depends on the

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possibility to achieve its condensation by simple artificial molecules.[35–37]

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