The phosphate clamp: a small and independent motif for nucleic acid backbone recognition

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ABSTRACT

The 1.7 Å X-ray crystal structure of the B-DNA dodecamer, [d(CGGAATTCCGG)]₂ (DDD)-bound non-covalently to a platinum(II) complex, [Pt(NH₃)₂μ-trans-Pt(NH₃)₂(NH₂CH₂CH₂NH₃⁺)]NO₃₈ (1, TriplatinNC-A), shows the trinuclear cation extended along the phosphate backbone and bridging the minor groove. The square planar tetra-ammine Pt(II) units form bidentate N-O-N bridges the minor groove. The geometry is conserved and the interaction prefers O2P over O1P atoms (frequency of interaction is O2P > O1P, base and sugar oxygens > N). The binding mode is very similar to that reported for the DDD and [(trans-Pt(NH₃)₂(NH₂CH₂CH₂NH₃⁺)]NO₃₈ (2, TriplatinNC), which exhibits in vivo anti-tumour activity. In the present case, only three sets of Phosphate Clamps were found because one of the three Pt(II) coordination spheres was not clearly observed and was characterized as a bare Pt²⁺ ion. Based on the electron density, the relative occupancy of DDD and the sum of three Pt(II) atoms in the DDD-1 complex was 1:1.69, whereas the ratio for DDD-2 was 1:2.85, almost the mixing ratio in the crystallographic drop. The high repetition and geometric regularity of the motif suggests that it can be developed as a modular nucleic acid binding device with general utility.

INTRODUCTION

Since the discovery of its’ anti-tumour activity the mononuclear platinum drug cis-platin |cis-[PtCl₂(NH₃)₂|, cis-diaminedichloroplatinum(II)), (1,2), and subsequently the related carboplatin |[Pt(NH₃)₂(CBDCA)]|, cis-diammine [1,1-cyclobutanedicarboxylato] platinum(II))| and oxaliplatin {[Pt(R,R-dach)(ox)]}, 1,2-R, R-diaminocyclohexane(oxalato)platinum(II))| have contributed significantly to the anti-cancer drug armamentarium. The drugs all form predominantly short-range 1,2-intrastrand crosslinks on DNA which cause severe conformational distortion, the specific nature of which is thought to be a critical determinant of the biological consequences of platinum drug treatment (3–6). Polynuclear Pt(II) agents are structurally and biochemically distinct from mononuclear Pt(II) agents, and form long-range (Pt,Pt) inter- and intra-strand crosslinks (7–14). The DNA conformational distortions induced by polynuclear agents are thus structurally distinct from those of cis-[PtX₂(amine)₂], and are not necessarily susceptible to the same cellular processing as cisplatin and other cis-[PtX₂(amine)₂] chemotypes (14). The development of cellular resistance to cisplatin (15,16) and also to carboplatin and oxaliplatin (17), through enhanced DNA repair, might thus be circumvented in polynuclear agents. The advancement to Phase II human clinical trials of the prototypical trinuclear drug BBR3464 (for all structures, see Figure 1) validates the search for structure diversity in new platinum analogues (13,18).

Replacement of the substitution-labile chlorides of BBR3464 by ammonia (amine) or amines gives the non-reactive polynuclear agents TriplatinNC-A (1, Figure 1A) and TriplatinNC-B (2, Figure 1B). Crystallographic characterization of a DNA complex with 2 (19) shows that the two mutually cis-oriented platinum-ammine groups of the PtN₄ units form discrete NH≡OP≡HN (amine≡phosphate≡amine)-binding motifs. These ‘Phosphate Clamps’ represent a discrete new ligand–DNA-binding mode, extending the repertoire of binding possibilities by
small ligands beyond intercalation and groove binding. Non-covalent polynuclear platinum compounds and TriplatinNC in particular, have interesting biological properties including *in vitro* cytotoxicity, anti-tumour activity and high cellular accumulation (20–23).

The quasi-independent and modular nature of the PtN4-based phosphate clamp as observed in the TriplatinNC–DNA complex suggests that the interaction can be used as a general DNA-binding element in combination with other nucleic acid binding motifs. The mode of association (B-form backbone tracking, A-form backbone tracking, groove spanning, intra-molecular association, etc.) can be dictated by alteration of linker chain length and rigidity, charge distribution and the number of PtN4 units. To examine the utility and generality of the phosphate clamp we have extended the study of non-covalent platinum complexes to TriplatinNC-A (Figure 1A).

This compound is differentiated from TriplatinNC by the absence of dangling amines on the terminal Pt(II) atoms, resulting in a more compact molecule and an overall charge of 6+ instead of 8+. Here we describe the 3D X-ray crystal structure of 1 bound to the Dickerson–Drew Dodecamer (DDD, Figure 1D) (24), the self-complementary double-stranded DNA [d(CGCGAATTTCGCG)]2. The binding modes and consequent conformational distortions of both platinum complexes are compared and the biological relevance discussed.

**EXPERIMENTAL PROCEDURES**

**Synthesis**

TriplatinNC-A, a gift of Alex Hegmans, was prepared by published procedures (20).

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**Figure 1.** The chemical structures of the trinuclear platinum(II) compounds discussed in this article: (A) TriplatinNC-A, ([{Pt(NH3)3}2-μ-[trans-Pt(NH3)2(NH2(CH2)6NH2)](NO3)6], 1, 0,0,0/t,t,t). (B) Triplatin-NC, ([{trans-Pt(NH3)2(NH2(CH2)6NH3)}2-μ-[trans-Pt(NH3)2(NH2(CH2)6NH2)](NO3)6], 2). (C) BBR3464 containing substitution-labile chlorides ([{trans-PtCl(NH3)2}2-μ-[trans-Pt(NH3)2(NH2(CH2)6NH2)](NO3)4], 3). (D) The double-stranded self-complementary dodecamer duplex, [d(CGCGAATTTCGCG)]2 (DDD), used for this crystallographic study.
Crystallization

The ammonium salt of reverse-phase HPLC-purified d(CGCGAATTCCGCG) (DDD, Integrated DNA Technologies, Coralville, IA, USA) was combined with TriplatinNC-A. The crystallization conditions were the same as for DDD-TriplatinNC complex (19). Crystals were grown in hanging drops by vapor diffusion from a solution initially containing 0.19 mM of the duplex, [d(CGCGAATTCCGCG)]₂, 0.19 mM TriplatinNC-A, 31 mM sodium cacodylate, pH 6.5, 9.6 mM magnesium chloride and 6.9% 2-methyl-2,4-pentanediol (MPD). The crystallization solution was equilibrated against a reservoir of 30% MPD at 20°C in a constant-temperature incubator. Orthorhombic (P2₁₂₁₂₁) crystals appeared within a month. The crystal chosen for data collection was 0.1 x 0.1 x 0.2 mm³.

Data collection

Diffraction data were collected on beamline X26C at the National Synchrotron Light Source, Brookhaven National Laboratory. A total of 120 frames of intensity data (oscillation angle = 1.0°) were collected at 175 K at a wavelength of 1.05 Å and recorded on an ADSC-Quantum 4 CCD detector. Data were merged and reduced with HKL2000 (25).

Structure solution and refinement

The starting DNA model consisted of coordinates of [d(CGCGAATTCCGCG)]₂ (24), [NDB entry bd084 (25)] excluding solvent and ions. After a rotation/translation search and rigid body refinement with CNS version 1.1 (26), the R/R-free factor were 52/55. Strong sum and difference peaks indicated positions of three Pt²⁺ atoms, which were added to the model, giving R/R-free factor of 38/39. The R/R-free factor dropped to 20/26 upon addition of 83 water molecules along with additional atoms of TriplatinNC-A. A partially occupied/disordered TriplatinNC-A was modeled as a bare Pt²⁺ ion. The initial model of TriplatinNC-A was generated with Chem3D Pro version 4.0 (CambridgeSoft).

The three Pt²⁺ positions were confirmed by anomalous maps, which were computed using phases of only a single Pt²⁺ atom, with the program CNS. During the refinement a peak of electron density surrounded by four water molecules, forming a partially disordered octahedron, was observed. The occupancies of TriplatinNC-A were estimated with Chem3D Pro version 4.0 (CambridgeSoft).

RESULTS

Circular dichroism spectroscopy

Circular dichroism (CD) spectra were recorded at room temperature using a Jasco 600 CD spectrophotometer and a 10 mm quartz submicro cuvette. All samples were prepared in 10 mM phosphate buffer solution, pH 7.4 containing 50 mM sodium chloride. The dodecamer (100 μM in nucleotides) was incubated with the corresponding platinum compounds at 37°C for 1 h at variable r values [r_i = (initial number of molecules of platinum complex in solution)/(number of nucleotides of DNA in solution)]. To obtain the presented spectra six scans in the range from 200 to 350 nm were averaged. The background was subtracted electronically.

Phosphate clamps

The Pt-am(mj)ine groups of 1 (TriplatinNC-A) form a total of 12 hydrogen bonds with DNA (Figure 3). The compound, like 2 (TriplatinNC), shows remarkable selectivity for (i) DNA oxygen atoms over nitrogen atoms, (ii) phosphate oxygen atoms over other oxygen atoms and (iii) for O2P over O1P atoms. As a result, 11 of the hydrogen bonding interactions involve oxygen atoms, 10 of which are with phosphate oxygens. The exception is a hydrogen bond to a guanine O6. Only a single hydrogen bond links an NH₃ group of a PtN₄ unit to a nitrogen atom (N7 of G).

The hydrogen-bonding interaction between the ligand (Pt complex) and DNA is best structurally characterized by the formation of phosphate clamps (Figure 3). In the phosphate clamp, a single OP atom accepts two hydrogen bonds, one from each of two adjacent a(m)jino groups of
a PtN₄ unit. Three phosphate clamps involving two PtN₄ coordination spheres of TriplatinNC-A are evident in the electron density. In the case of 2, seven phosphate clamps involving four Pt²⁺ centers were observed (19). Similar to the previous structure, 1 forms an extensive hydrogen bonding and electrostatic network within the DDD crystal, bridging a total of five different symmetry-related duplexes (Figure 3).

In the structure discussed here and the previous one (19), the geometries of Phosphate Clamps are highly conserved. The motif again appears to require cis-orientation of the am(m)ine ligands—mutually trans ligands do not participate in phosphate clamps. Furthermore, the ligands are one Pt-NH₃ (ammine) group and one Pt-NH₂R group. Only two PtN₄ units are observable, which collectively engage in three phosphate clamps. In this structure, one Pt atom, designated Pt(a), was assigned as a bare cation since the electron density around Pt(a) is not sufficient to determine the geometry of the coordination sphere.

**Backbone tracking**

The observable portion of TriplatinNC-A is extended along the backbone of one strand of the DDD (Sym 0 in Figure 3). One Phosphate Clamp [Pt(b)] engages the

**Table 1. Data collection and refinement statistics**

| Parameter                                      | Value                          |
|-----------------------------------------------|--------------------------------|
| Unit cell dimensions                          | a = β = γ = 90°                |
|                                              | a = 23.440 Å, b = 39.007 Å     |
|                                              | c = 65.922 Å                   |
| DNA (asymmetric unit)                         |                               |
| Space group                                    |                               |
| Temperature of data collection (K)            |                               |
| Number of all reflections                     |                               |
| Number of reflections in resolution range (Å) | 6843                           |
| Number of reflections used in refinement (F > 3σF) | 6292                           |
| RMSD of bonds from ideality (Å)               | 0.026                          |
| RMSD of angles from ideality (°)              | 1.995                          |
| Max. resolution of observed reflections (Å)   | 1.26                           |
| Max. resolution of refinement (Å)             | 1.70                           |
| Number of DNA atoms                           | 486                            |
| Number of TriplatinNC-A atoms                 | 15                             |
| Number of water molecules, excluding sodium first shell | 83                             |
| Number of sodium ions plus coordinating water molecules | 5                             |
| R-factor (% , F > 3σF)                        | 19.64                          |
| R-free (% , all)                              | 25.68                          |

**Figure 2.** Electron density maps surrounding the DDD/1 (TriplatinNC-A) complex. (A) Sum (2Fₒ-Fₑ) electron density surrounding the DNA, contoured at 1.3σ. (B) Sum electron density around 1 contoured at 1.0σ.
O2P oxygen atom of C15 while the other [Pt(c)] engages the O2P atom of the adjacent G16 (Figure 4). In contrast, in the DDD–TriplatinNC complex the PtN4 units forms phosphate clamps with A17 and G16, but not with C15 (19). This difference in binding interactions may be due to the increased charge caused by the presence of the dangling hexanediamine, which forms hydrogen bonds with the O2P of G14 (19). This extra interaction appears to induce the trinuclear unit to bend, pushing the one PtN4 coordination sphere away from the phosphate of C15, forming a total of three phosphate clamps.

Groove spanning

TriplatinNC-A participates in Groove Spanning by bridging between two DNA strands across the minor groove of the DNA duplex (Duplex Sym 1 in Figure 3) through clamps at the phosphate of T7 and one incomplete clamp, a PtN4-O2P hydrogen bond at C21 (Figures 3 and 5). In contrast, in the TriplatinNC–DDD complex the Groove Spanning is achieved by two intact phosphate clamps. Overall, the two compounds bind by remarkably similar motifs—the Backbone Tracking and Groove Spanning are facilitated through the Phosphate Clamp, although the specific positions and interacting parts of the DDD are not exactly the same.

Sodium ions

In many previously-described high-resolution DDD crystal structures, a fully hydrated magnesium ion $[\text{Mg(H}_2\text{O)}_6]^{2+}$ is located in the major-groove near one end of the duplex (25,29,30). The first shell water molecules of the Mg$^{2+}$ ion form hydrogen bonds to the G(2)–C(23) and C(3)–G(22) base pairs. In the DDD-1 complex, a hydrated sodium ion $[\text{Na(H}_2\text{O)}_4]^{+}$ is observed on the other end of the duplex in the major groove adjacent to G(10)–C(15) and C(11)–G(14). The Na$^+$ does not form first-shell contacts with the DNA. Instead, the four first-shell water molecules that coordinate the Na$^+$ form hydrogen bonds to the guanine O6 and N7 positions of G(14) and to the O6 position of G(10), Figure 3. These hydrogen bonds are similar to those of $[\text{Mg(H}_2\text{O)}_6]^{2+}$ at the other end of the DNA fragment. This Na$^+$ ion is nearby to where 1 tracks the DNA backbone. In the DDD-2 complex a hexa-coordinate sodium ion $[\text{Na(H}_2\text{O)}_6(O6)]^{+}$ is also observed, with the sixth ligand being the O6 from residue G(22). The first shell water ligands interact with G(2), C(21) and G(22).
and the complex is located at the opposite end of DDD from where 2 forms the Backbone Tracking (19). Accordingly, the location and hydrogen bonding properties of hydrated sodium cations, although observed in both cases, were different between the two structures.

DNA conformation

To examine the DNA conformational changes induced through binding by this class of ligand, we compare the DDD–TriplatinNC-A (1) to DDD–TriplatinNC (2) and to other high resolution DDD structures, in particular structure bdl084, which is the highest resolution, non-modified DDD structure determined thus far (25). Phosphodiester backbone torsion angles, glycosyl angles and sugar puckers are given in Table 2. The major-groove and minor-groove widths of DDD–TriplatinNC-A are intermediate between those of DDD–TriplatinNC and the control DDD, Figure 6. From these parameters, the superimposition of both polynuclear complexes onto the control DDD is shown in Figure 7. Base-base and inter-base parameters are shown in Supplementary Figures S1 and S2.

Previously we suggested that an observed increase in the minor groove width of DDD–TriplatinNC in comparison to the ‘free’ DDD is related to the groove spanning by the complex. The cationic centers of 1 interact with the same phosphates as TriplatinNC. In both structures, two Phosphate Clamps are juxtaposed across the minor groove. However, in the present case, one of the two Phosphate Clamps of the Groove Spanning mode is only partially formed (see above). The weaker Groove Spanning interactions might result in slightly lesser deformation of the duplex. For example, the major-groove width of DDD-1 at A(6)–T(19), in which the major-groove width of DDD–TriplatinNC and the control DDD show the largest deviation, was found to be the median value of the other two structures, Figure 6.

The backbone: BI versus BII. The DNA complexed with TriplatinNC-A is wholly in the BI conformation, with $\varepsilon-\xi$ is in the range of $-160$ to $+20$. B-DNA conformation is characterized by BI, the most frequent, or BII, depending on the torsion angles $\varepsilon$ and $\xi$ (31,32). These torsion angles influence the positions of phosphate groups and modulate the width of the minor groove. The difference between $\varepsilon$ and $\xi$ is a useful indicator of BI/BII. An $\varepsilon-\xi$ of roughly $-90^\circ$ indicates BI whereas a value of roughly $+90^\circ$ indicates BII.

The backbone: sugar puckers. Of the 24 sugar puckers of DDD-1, 11 differ from those of the corresponding residues in the control DDD. A total of 14 differ from those of DDD–TriplatinNC. Large differences in sugar pucker are found in G(12) ($-103$) and (C23) (+99) compared to the control DDD. Differences are also found in G(2) (+168), C(3) (+93) C23 (+99) relative to DDD–TriplatinNC.

Global conformation. The axial bending and axial shortening of the DDD-1 complex are very similar to those of DDD–TriplatinNC and significantly greater than those of the DDD. In the program Curves 5.3 (28), UU is a measure of axial bending, giving the angle formed by the axis segments of the terminal sections of the DNA duplex. PP is the angle between the vectors formed between the two axis reference points at either end of the fragment. UU and PP for the DDD-1 complex were found to be $28^\circ$ and $38^\circ$, both of which are very close to the
corresponding value of the DDD–TriplatinNC complex and significantly larger than those of the control DDD. This trend is also the same in axial bend and the axial path length shortening ratio (DDD–1: 28°/2.0%; DDD–TriplatinNC: 27°/2.4%; DDD: 12.9°/0.66%).

Conformation in solution

The similarity in binding mode of the two polynuclear complexes is also confirmed in solution. The CD spectra of the DDD titrated with both 1 and 2 are very similar and further confirm the essential B-nature of the conformational changes upon complex binding (Figure 8). The spectra are characterized by a negative shift (decrease) in ellipticity for the peak centered at 280 nm and a concomitant positive shift (increase) for the peak centered at 206 nm. Thus the difference in peak height is decreased.

DISCUSSION

The results reported here confirm the generality and utility of the PtN₄ unit for formation of Phosphate Clamps with DNA, involving two modes of binding, Backbone Tracking and Groove Spanning. Phosphate clamps form six-membered rings, with two hydrogen bonds from cis-oriented Pt-a(m)amines to a common phosphate oxygen. It is a discrete and modular DNA-binding device with high potential as a drug-design scaffold. In solution, conformational changes are equivalent for the two complexes whose structures have been determined, further emphasizing the similarity in binding mode.

Forks and clamps

The interactions of PtN₄ coordination units with DNA have some analogy to the interactions of the guanidino group of arginine with polynucleotides. The limiting 'canonical' structures for both arginine forks and phosphate clamps are shown schematically below for comparison. It was previously suggested that arginine shows an OP clamping ability in which two adjacent NH groups of a guanidino group interact with two oxygen atoms of a common phosphate (33–35). This motif would result in an eight-membered ring (including hydrogen atoms) stabilized by two hydrogen bonds. In the ‘Arginine Fork’, one guanidino group may be engaged in two of these interactions (33). The apparent mimicry of arginine by the platinum–tetra(m)mine unit led us re-evaluate the frequency and geometry of Arginine Forks. We therefore examined ribosomal structures such as 1JJ2 (35) and 2J00/2J01 (34). The large subunit of the Haloarcula Marismortui ribosome (1JJ2), for example, contains 336 arginines and nearly 3000 phosphate groups. From analysis of such structures we conclude that the guanidino group of arginine, like PtN₄, binds to nucleic acids with high selectivity for OP atoms, i.e. for non-bridging phosphate oxygens: PtN₄ > ARG. The guanidino group does form clamp-like structures, consisting of eight-membered rings, at some measurable frequency but again, PtN₄ >> ARG. However PtN₄ forms forks while the guanidino group of arginine does not; the frequency at which one guanidino group engages in two phosphate clamps is essentially zero. In sum, PtN₄ appears to be unique in its combined selectivity for OP atoms, forking propensity, and highly conserved geometry of binding. Thus the PtN₄ unit has

Figure 5. Groove spanning. Hydrogen bonds between 1 and DNA (Sym 1, Figure 3) are indicated by dashed lines. There are two Phosphate Clamps, with which TriplatinNC-A bridges two strands across the minor groove of a DNA duplex, at the lip of the minor groove, defined in Sines et al. (29). (A) View perpendicular to the helical axis. (B) View along the helical axis. Atoms are colored and coded as in Figure 4.
potential as a general DNA/RNA-binding element and drug design scaffold.

Binding modes compared to other DNA-binding ligands

Because there is some analogy between the polynuclear platinum structure (especially non-covalent compounds such as 1 and 2) and polyamines such as spermine and spermidine, it is of interest to note that polyamines generally bind preferentially in the major groove rather than to phosphates (36). Key features in differences in recognition patterns between the two classes are the distances between amino groups and the fact that the cis-\[Pt(NH3)(NH2R)\] unit in fact is sterically rigid because of the strict adherence to square-planar geometry (\(\sim 90^\circ\) bond N-Pt-N bond angle) for Pt complexes. Polyamines are conformationally flexible and optimal distances to achieve phosphate clamp binding—provided by the square planar geometry and also to a lesser extent the sterically rigid guanidine group—are not as readily available.

Non-covalent ligand–DNA complexes are stabilized by a composite of hydrogen-bonding, van der Waals and electrostatic interactions. These interactions most generally occur within the minor groove or between base pairs (37,38). More recently Arya and coworkers have developed major groove binders (39). Appropriate
combinations of modular binding elements gives high affinity and specificity (37) but, comparing all cases, the discrete nature of the Phosphate Clamp is clear.

DNA distortion

We previously suggested that a change in DNA conformation may arise upon substitution of a hydrated Mg\textsuperscript{2+} in the native DDD for a partially dehydrated Na\textsuperscript{+} ion in the DDD–TriplatinNC complex (19). The Na\textsuperscript{+} ion is located directly on the floor of the major groove (interacting with O6 of a G). It was difficult to isolate the ligand, a hydrated cation or TriplatinNC, both directly interacting with DNA, as the primary contributor to DNA deformation. In the DDD-1 crystal structure, no hydrated cation is observed in the position of the hydrated Mg\textsuperscript{2+} or Na\textsuperscript{+} in the control DDD or DDD–TriplatinNC. Accordingly, all the hydrated cations found in the control DDD, DDD–TriplatinNC, and DDD-1 are different in atom species, hydrogen bonding properties, and interacting areas but deformation directions of TriplatinNC and 1 from the control DDD appear to be the same. Therefore, the ligands controlling the DNA deformation seem to be the trinuclear Pt(II) complexes, and hydrated cations are selected and fit to a preformed pocket of the DNA.

The work here expands the description of platinum–DNA-binding modes, and indeed metal complex–DNA interactions in general. The NDB database shows only a very limited number of crystal structures of duplex DNA bound with cis-platin [intrastrand GG and the cisplatin–DNA duplex associated with the HMG protein (40), interstrand (GC)\textsuperscript{2} (41); oxaliplatin (intrastrand GG) (42) and cis-[Pt(NH\textsubscript{3})(cyclohexylamine)Cl\textsubscript{2}] (intrastrand GG) (43) and a Z-DNA-forming sequence d(CGTA\textsubscript{NH2}ACG)\textsubscript{2} stabilized by [Pt(NH\textsubscript{3})\textsubscript{2}]\textsuperscript{+} (44). With the exception of the latter, all of the DNA–platinum structures belong to the cis-[PtX\textsubscript{2}(amine)\textsubscript{2}] family and the work has delineated the 3D structural distortions of bifunctional binding and the factors affecting protein (HMG) recognition of the 1,2-GG intrastrand adduct. Indeed few crystal structures of non-covalently binding transition metal complexes are available—most recently the structures of a three-way DNA junction (3WJ) stabilized by metal-based supramolecular helicates have been described (45,46). The other high-resolution crystal structure entries appear limited to a sequence-specific rhodium intercalator (47,48) and the use of [Co(NH\textsubscript{3})\textsubscript{6}]\textsuperscript{3+} and [Ru(NH\textsubscript{3})\textsubscript{6}]\textsuperscript{3+} in stabilizing Z-DNA sequences (49,50).

Biological relevance of non-covalent Pt–DNA interactions

Both trinuclear compounds 1 and 2 bind to DNA exclusively by non-covalent interactions. Biophysical studies showed that the complexes stabilized duplex DNA and
the higher the electrical charge of the complexes the greater the stabilization observed. The complexes induced both B→A and B→Z conformational changes in canonical DNA sequences (20). The binding affinity is sufficiently high such that they are not readily displaced from the helix by intercalators such as ethidium bromide, resulting in an essential irreversibility of the conformational change. The interaction with DNA of the minor-groove binding dye Hoechst 33258 is cooperatively enhanced in the presence of the charged compound (22). Given the overall similarity of the binding modes, it is not surprising that the affinity of both molecules for DNA is also very similar—as would be expected since the overall binding differs only in charge and extra hydrogen bonding capability of the ‘dangling’ amines. Yet, the complexes differ in their biological consequences. Especially TriplatinNC (2, Figure 1) is cytotoxic at micromolar concentrations and exhibits in vivo anti-tumour activity. In contrast, 1 is ~5–10 times less potent than TriplatinNC. The enhanced cytotoxicity has been attributed to greater cellular accumulation of the 8+ species. The differences in cytotoxicity between 1 and 2 may reflect differences in cellular accumulation—the higher positive charge produces greater accumulation which may provide a stronger driving force for access to DNA, resulting in a higher frequency of phosphate clamps. Note that TriplatinNC is also significantly more potent than other non-covalent platinum compounds reported (20–23,51,52).

CONCLUSIONS

The Phosphate Clamp is a general feature of DNA complexes of PtN₄ containing non-reactive polynuclear platinum agents. The backbone selective binding is distinct from ‘classic’ minor-groove binding and intercalation or the more recently described major groove binding. The molecules described here contain numerous PtN₄ groups acting as DNA-binding ligands and connected by flexible linkers—polymorphism in solution may arise from the number of PtN₄ per binding ligand and flexible connections between them. Both minor-groove binders and intercalators have undergone extensive study for their cytotoxic and anti-tumor properties. The modular nature of the polynuclear platinum binding allows for significant further complex design (aliphatic/aromatic linkers, shorter/longer chain length, or cis/trans geometry) to enhance biological activity. Platinum-DNA binding triggers downstream cell cycle signals eventually leading to apoptosis and cell killing. Given that so little of
cisplatin actually gets to DNA, the Phosphate Clamp motif may be a much more effective method to trigger a cellular response. The absence of covalent binding to biomolecules in general may also result in less potential side effects than currently used agents. These considerations suggest that polynuclear platinum agents capable of non-covalent interactions represent a further class of platinum-based anti-tumour agents, discrete from covalently-binding mononuclear (cis-platin) and polynuclear (BBR3464) agents. Their overall profile relative to DNA-modifying agents deserves further investigation.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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