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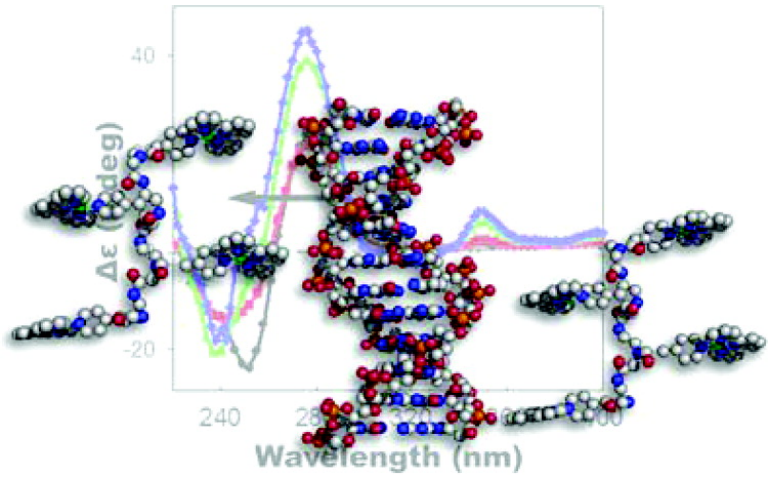

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Tetraplatinated Artificial Oligopeptides Afford High Affinity Intercalation into dsDNA

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Molecular probes that bind selectively and with high affinity to biological molecules have uses in gene therapy, antiviral therapeutics, and as tools for biotechnology and molecular biology. Polyvalent species are particularly intriguing because of their potential for very high affinity binding. While there are several examples of polyvalent organic compounds,[1] inorganic complexes offer an advantage for studying the role that molecular design and geometry play in binding with DNA because of their relative ease in preparation and modification.[2] In the search for new chemotherapeutic agents, inorganic complexes that function by intercalation of their aromatic ligands into the base pairs of DNA are of intense interest.[3] Recent reports have shown that binding affinity is enhanced either by anchoring a metal intercalator to an oligopeptide which hydrogen bonds to DNA[4] or by tethering two metal complexes to a hydrocarbon chain.[5] We have recently reported the synthesis of artificial oligopeptides bearing ligands for the stoichiometric complexation of a variety of inorganic ions and complexes.[6] The peptides chosen for these multimetallic structures are based on the structure of the peptidic analogue of DNA, peptide nucleic acid (PNA).[7] As such, the spacing between metal complexes tethered to the peptide mimics the distance between nucleic acids on the DNA sugar phosphate backbone. We reasoned that having multiple metal complexes on a single oligopeptide strand should dramatically increase the binding affinity with DNA as a result of polyvalent interactions.

We have initially chosen to test this hypothesis by examination of the interaction of 12 base pair (12 bp) double strand (ds) DNA with a tetrapeptide scaffold to which are tethered four $[Pt(tpy)(py)]^{2+}$ complexes (Scheme 1). Complexation of $[Pt(tpy)]^{2+}$ to the pyridine tetrapeptide scaffold leaves four aromatic tpy ligands projecting into solution.[6b] The sequence of the oligomeric DNA shown in Scheme 1 is 5′-CGT GAC CAG CTG-3′ bound to its complement and contains 75% GC content both to improve hybridization efficiency and to specifically avoid hairpin formation and alternative registers. The size of the metalated artificial tetrapeptide is ca. half that of the dsDNA.

Isothermal titration microcalorimetry (ITC) was used to measure the heat of reaction upon titration of a solution containing the 12 bp dsDNA with $[Pt(tpy)(py)]_4^{8+}$ tetrapeptide. The titration curve (Supporting Information) was fit to simultaneously determine the binding stoichiometry (n), binding constant (K_B), and thermodynamic parameters (enthalpy and entropy changes). These data reveal that *two* $[Pt(tpy)(py)]_4^{8+}$ tetrapeptide molecules (i.e., 8 total Pt complexes, $n = 0.67$ Pt/bp) bind to each 12 bp dsDNA, with a K_B of 1.7×10^6 M^{-1} (Table 1). In separate ITC experiments with nonmetalated oligopeptide and 12 bp dsDNA, no binding was observed (Supporting Information). In comparison, the measured binding stoichiometry of the monometallic complex $[Pt(tpy)(pic)]^{2+}$ was determined by ITC to be two Pt complexes with one 12 bp DNA (i.e., $n = 0.16$ Pt/bp), with a K_B 2 orders of magnitude smaller than that of the tetrametallic peptide (Table 1). The small molecule binding data are in excellent agreement with literature values for binding of $[Pt(tpy)(pic)]^{2+}$ with calf thymus (ct) DNA,[8] indicating that these results are not dependent on the length of the dsDNA sequence used.

Scheme 1. Structure of the Tetrametallic $[Pt(tpy)(py)]_4^{8+}$ Tetrapeptide, and Comparison of Its Space-Filled Molecular Model with the dsDNA

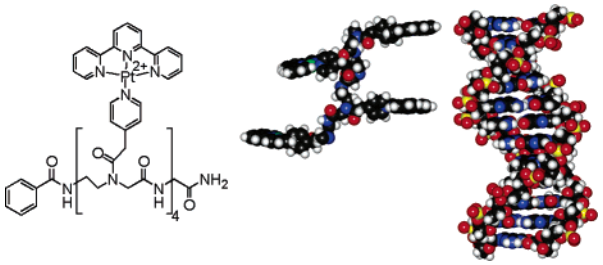

Table 1. Thermodynamic Data from ITC at 30 °C

complex and dsDNA sequence	$n^{a,b}$	K_B 10^6 $(M^{-1})^b$	ΔH (kcal/mol)	ΔS (cal/mol K)	ΔG (kcal/mol)
$[Pt(tpy)(py)]_4^{8+}$ + 12 bp DNA[c]	0.67	1.7 ± 0.5	-14.1 ± 0.6	-18 ± 3	-8.5 ± 0.8
$[Pt(tpy)(pic)]^{2+}$ + 12 bp DNA[c]	0.17	0.025 ± 0.002	-10.7 ± 0.5	-15 ± 1	-6.2 ± 0.1
$[Pt(tpy)(pic)]^{2+}$ + ct DNA[e]		0.035			−6.2

[a] Pt/bp DNA at equivalence point. [b] From ITC curve fit. [c] DNA sequence as in Scheme 1. [d] pic = 4-methyl pyridine. [e] From ref 8, at 25 °C.

For the tetrametallic oligopeptide, the free energy change for the reaction with 12 bp dsDNA was determined to be −8.5 kcal/mol; in comparison with its small molecule analogue (Table 1), the difference in free energy change of the reaction, $\Delta\Delta G$, is −2.3 kcal/mol. Because the change in entropy (ΔS) for the reaction is approximately the same for both the $[Pt(tpy)(py)]_4^{8+}$ tetrapeptide and the $[Pt(tpy)(py)]^{2+}$ small molecule, the $\Delta\Delta G$ is attributed to the greater exothermicity associated with binding the multimetallic complex. This larger enthalpy change (ΔH) is attributed to multiple noncovalent interactions, such as polyvalent binding. The magnitude of the enhancement observed for our multimetallic peptides is equal to or greater than previously reported inorganic polyintercalators.[9]

The decrease in free energy implies that binding the $[Pt(tpy)(py)]_4^{8+}$ imparts structural stability to the dsDNA. To further examine this, the effect of oligopeptide binding on the melting temperature (T_m) of the dsDNA was measured by monitoring the temperature-dependent UV absorption. Figure 1A shows melting curves for the 12 bp dsDNA in the presence of varying amounts of tetrametalated peptide. An increase in the T_m is observed as the quantity of metalated tetrapeptide is increased: at the stoichiometric quantity of two oligopeptides to one dsDNA, the T_m increases by 25 °C (from 60 to 85 °C); additional quantities of added metalated tetrapeptide do not further affect the T_m. This dramatic rise in T_m indicates a highly stabilized DNA helix and is large in comparison to the increase observed when two $[Pt(tpy)(pic)]^{2+}$ molecules bind to the 12 bp dsDNA. Binding of this small molecule with the same 12bp dsDNA causes an increase in T_m of only 15 °C, similar to values reported for Pt complex monointercalators.[8] The much

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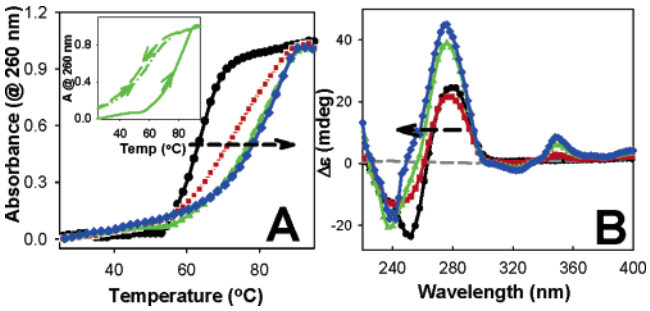

Figure 1. (A) Melting curves obtained during the initial heating cycle of 40 μmol 12 bp dsDNA in phosphate buffer solution (black line) and with (red line) 40 μmol; (green line) 80 μmol; and (blue line) 120 μmol $[Pt(tpy)(py)]_4^{8+}$. Inset: Melting curves of the solution containing 2 mol $[Pt(tpy)(py)]_4^{8+}$: 1 mol dsDNA during sequential heating (——), cooling (- - -), and reheating (—··—). (B) Circular dichroism spectra of the same solutions, compared to a solution containing solely 400 μmol $[Pt(tpy)(py)]_4^{8+}$ (- - -).

greater effect of the metalated tetrapeptides supports a multisite intercalation mechanism.

Heating the dsDNA−oligopeptide conjugates causes denaturation of the duplex DNA, and upon cooling these samples (95 to 0 °C), the T_m is observed to return to a value approximately the same as pure dsDNA (i.e., an absence of metalated tetrapeptide, Figure 1A inset). The T_m remains constant during subsequent temperature cycling; no re-binding of the $[Pt(tpy)(py)]_4^{8+}$ is observed, even after hours (Supporting Information). Similar hysteresis in the melting curves is observed for the monometallic $[Pt(tpy)(pic)]^{2+}$ analogue (Supporting Information). In both cases, the observed hysteresis suggests that the oligopeptide−dsDNA interaction breaks upon the initial denaturation step; cooling the sample permits rehybridization of the DNA while excluding the inorganic complexes. When a fresh aliquot of the metalated oligopeptide is added to the cooled dsDNA (following a heating cycle), binding (and an increase in T_m) is again observed. Thus, the inability to re-bind the tetrapeptide after an initial heating cycle results from an irreversible chemical transformation in the $[Pt(tpy)(py)]_4^{8+}$ tetrapeptide, and not a structural changes of the DNA. In separate experiments, the temperature-dependent UV−visible spectra were measured for the metalated peptide in buffer (in the absence of DNA) and in pure water, and nonreversible spectral changes at elevated temperatures are observed that likely result from coordination with water and acetate anions (Supporting Information). In the presence of dsDNA, these spectral changes are not observed until the duplex denatures. Therefore, while the metalated peptide stabilizes the DNA duplex (and raises T_m), conversely the dsDNA *stabilizes the tetrapeptide structure* and prohibits structural changes as a function of elevated temperature until denaturation causes disassociation.

To further understand the nature of the interaction of the tetrapeptide with dsDNA, and to directly measure any concomitant structural changes, circular dichroism spectra of the 12 bp dsDNA sequence were also acquired in the presence of varying amounts of the $[Pt(tpy)(py)]_4^{8+}$ tetramer. Figure 1B shows the change in the CD spectra as a function of increasing quantities of metalated tetrapeptide; the observed shifts remain constant at a 2:1 binding stoichiometry, consistent with the ITC and melting experiments. A new peak appears at 355 nm that is associated with a $\pi-\pi^*$ transition[6b,8b] in the $[Pt(tpy)(py)]^{8+}$ tetrapeptide. Since a solution containing only the metalated tetramer does not display a CD signal (dashed line in Figure 1B), appearance of the peak at 355 nm in the presence of dsDNA arises as a result of its intercalation into the helical DNA duplex. In the CD spectra, the changes in intensity and shifts of the peaks at wavelengths less than 330 nm are consistent with the 12 bp dsDNA unwinding from its original B conformation.[10] Temperature-dependent CD spectra (Supporting Information) indicate that dehybridization of the dsDNA at elevated temperatures is accompanied with a disappearance of the peak at 355 nm, consistent with exclusion of the Pt species upon denaturation.

Taken together, the ITC, melting and CD experiments conclusively demonstrate high affinity binding of two $[Pt(tpy)(py)]_4^{8+}$ tetrapeptide molecules with a single 12 bp dsDNA via intercalation of the pendant aromatic ligands. It is widely understood that intercalation compounds will (at most) insert between every other nucleic acid, causing the helix to unwind.[11] On the basis of the spacing of the $[Pt(tpy)(py)]^{2+}$ complexes tethered to the peptide (Scheme 1), we hypothesize that only two of the four pendant $[Pt(tpy)]^{2+}$ groups per peptide chain insert into DNA and appear to do so while strengthening the helix without disassociating the strands. These results are a step toward understanding the relationship between the number and spacing of the tethered metal complexes on the binding affinity and stoichiometry with duplex DNA. Furthermore, they represent an important advance in the development of inorganic, multivalent intercalators for the application and design of more potent anticancer agents. Our continuing efforts aim toward assessing binding selectivity with a DNA library and further understanding the role of metalated oligopeptide structure on affinity.

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Supporting Information Available: Complete experimental details and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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