Observation of two-mode binding to DNA by bipyridyl-(ethylenediamine)platinum(II): Isothermal titrational calorimetry and midinfrared absorption studies

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The binding of bipyridyl-(ethylenediamine)platinum(II) to calf-thymus DNA has been studied in solution via isothermal titration calorimetry and in unoriented films via midinfrared spectroscopy. The calorimetric data reveal that the ligand binds to DNA at two different sites with the first binding site being filled by about one ligand for every five base pairs. The binding is entropically driven: +25 cal/mol K for the first site and +22 cal/mol K for the second site. Midinfrared absorption data (400–1800 cm^{-1}) from unoriented films show no dependence on the ligand content at relative humidities of 23% and 80%. [S1063-651X(98)09912-7]

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INTRODUCTION

The interactions of DNA with its ligands are of profound importance for both biological and pharmacological reasons. Ligands (such as DNA Polymerases, RNA Polymerases, and others) attach to the DNA molecule during, and are integral components of, the biological processes of replication and transcription. DNA-ligand interactions are also critical for chromosomes, the cellular structures which contain the nuclear DNA in eukaryotic cells and which are composed of DNA wrapped around histone cores. The interactions between the DNA and the histone cores are critical for the stability of the chromosomes themselves. Many anticancer drugs attach directly to the DNA itself, increasing the stability of the double helix. This enhanced stability inhibits the process of replication, slowing the progression of the disease. These different examples of the DNA-ligand interactions show the importance of developing a more complete understanding of the interactions of DNA with various ligands.

DNA is a flexible molecule, adopting four different right-handed conformations (A, B, C, and D) and a left-handed conformation (Z) [1], and this flexibility is believed to be important for the different biological functions of DNA. In replication and transcription, the double helix must be locally unwound so that DNA may serve as the template for a new chain. The mechanism of unwinding is a topological problem, first identified by Watson and Crick [2]. The mechanism for this unwinding is still not understood, but is believed to be mediated by the ligands which attach to DNA during replication and transcription [3]. Significant evidence exists which shows that extended regions of unwound but double-stranded (i.e., a ladder-type conformation) DNA are formed during replication, transcription, and recombination [4].

The conformation of the DNA molecule can be affected by its ligands. Planar molecules which insert themselves between neighboring base pairs, such as ethidium and daunomycin, distort the sugar-phosphate backbone at the intercalation sites [1]. Perhaps the most extreme distortion of DNA is that reported by Arnott *et al.* [5] for oriented fibers of calf-thymus DNA intercalated with bipyridyl-(ethylenediamine)platinum(II) (abbreviated $[(bipy)Pt(en)]^{2+}$) at a relative humidity of 75%. These x-ray diffraction experiments showed that oriented DNA samples with one $[(bipy)Pt(en)]^{2+}$ for every two base pairs adopt a ladder-type conformation (called L-DNA) in which the double-stranded DNA molecule is flat rather than helical.

The DNA$\cdot[(bipy)Pt(en)]^{2+}$ complex is of interest to study since it is the only known example of ladder-DNA which can be routinely prepared in the laboratory. It is, therefore, a model system for the ladder-DNA formed during replication, transcription, and recombination. Experiments which might shed light on the microscopic mechanism by which $[(bipy)Pt(en)]^{2+}$ makes DNA adopt the ladder conformation might provide insight into the interactions between DNA and its ligands when ladder-DNA is formed inside the living cell. Though L-DNA is an ideal model complex for use in the study of the properties of ladder-type DNA, relatively few studies have been reported on this interesting complex to date [1,4,6]. In this paper we report our isothermal titration calorimetric (ITC) [7,8] and midinfrared (MIR) spectroscopic studies of the binding of $[(bipy)Pt(en)]^{2+}$ to DNA. It should be noted that, given the paucity of information about the ladder DNA which is believed to exist in living cells, it is possible that the L-DNA induced by $[(bipy)Pt(en)]^{2+}$ might not be the perfect model. Further experimental work on ladder-DNA will be necessary to resolve this issue.

MATERIALS AND METHODS

ITC experiments were performed with an Omega Isothermal Titration Calorimeter. Solutions of NaDNA (0.86 mM) and $[(bipy)Pt(en)]I_2$ (2.5 mM) were prepared in a 10 mM $NaH_2PO_4\cdot$ 0.01 mM EDTA buffer solution (pH=6.5). During an ITC experiment, 50 injections of 5 μL of the $[(bipy)Pt(en)]^{2+}$ solution were made into 1.35 mL of the DNA solution. Injections were made every 3 min and the experiments were performed at 25 °C.

Fourier transform MIR experiments were performed using a Nicolet SX-60 FTIR spectrometer. NaDNA gels were made by dissolving solid NaDNA in the same buffer solution

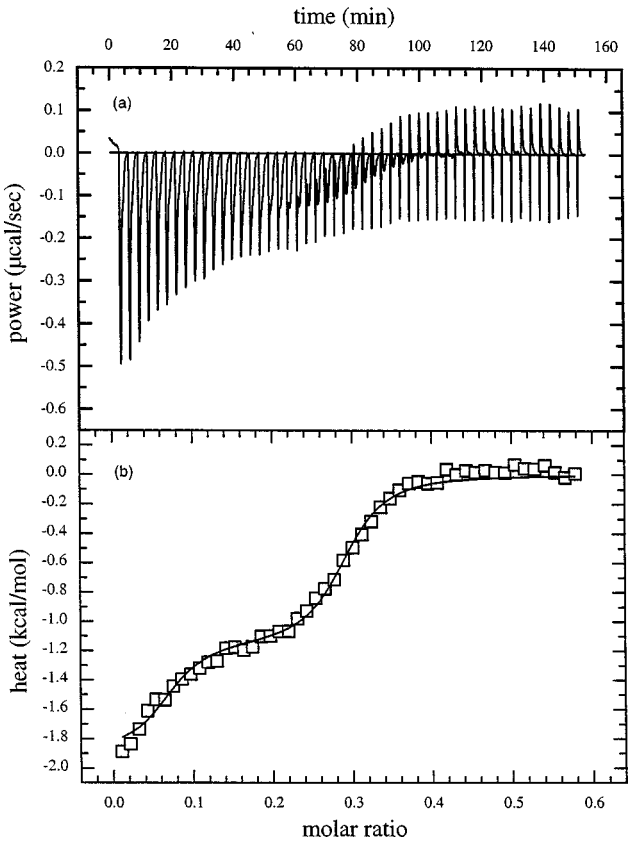

FIG. 1. (a) Raw binding data for the titration of calf-thymus NaDNA with $[(bipy)Pt(en)]I_2$ at 25 °C and (b) the integrated titration curve (open squares) with a fit (solid line) with a two-independent-site model.

employed in the ITC experiments. The appropriate amount of dissolved $[(bipy)Pt(en)]I_2$ was added yielding a gel with a final concentration of about 10 mM. The gel was thoroughly mixed using a mechanical mixer for about 15 min. The gels were left standing for 12 h to allow air bubbles to escape, as judged by visual inspection. A small amount (roughly 1 mL) of the gel was spread onto a ZnSe window. The window was placed in a dessicator at a relative humidity of 0% for 24 h to dry the film. The window was then mounted on a sealed IR cell with the DNA· $[(bipy)Pt(en)]^{2+}$ film on the inside. The relative humidity (RH) inside the cell was controlled by adding small amounts of the appropriate saturated salt solution to a well at the bottom of the cell [9]. IR measurements were performed on a pure DNA film as well as six DNA· $[(bipy)Pt(en)]^{2+}$ films with molar ratios of $[(bipy)Pt(en)]^{2+}$ to DNA base pairs of 0.083, 0.17, 0.23, 0.29, 0.47, and 0.58.

The $[(bipy)Pt(en)]^{2+}$ molecular ion was synthesized as the iodide salt following the method described by Watt and Upchurch [10]. Calf-thymus NaDNA was purchased from Sigma Chemical Co.

III. RESULTS AND DISCUSSION

Figure 1(a) shows the power output required to maintain a constant temperature of 25 °C for each injection of the $[(bipy)Pt(en)]^{2+}$ solution into the DNA solution. Only the heat of dilution is observed after about 30 injections (about 100 min), showing that the binding sites of the DNA are filled. The open squares of Fig. 1(b) show the integrated titration curve, yielding the total heat liberated with each injection. Note the plateau near a molar ratio of about 0.20 $[(bipy)Pt(en)]^{2+}$ to DNA base pairs, indicating that the binding of $[(bipy)Pt(en)]^{2+}$ to DNA is a two-mode process (i.e., binding to two different sites).

The solid line shown in Fig. 1(b) is the isotherm calculated with a two-independent-site model using a Marquardt minimization routine [11]. This fit yields the enthalpies of binding (ΔH) and equilibrium binding constants (K) for both below and above the transition. The binding free energies are calculated from the equation $\Delta G = -RT \ln K$. These thermodynamic parameters are listed in Table I along with the same information for other ligands of DNA [12–16]. Although the binding of $[(bipy)Pt(en)]^{2+}$ is exothermic, the large favorable binding free energies are derived from the positive entropy contributions. Similar behavior has been observed in the binding of Δ-$[Ru(phen)_2DPPZ]^{2+}$, Λ-$[Ru(phen)_2DPPZ]^{2+}$, Δ-$[Ru(phen)_3]^{2+}$, and Λ-$[Ru(phen)_3]^{2+}$, as shown in Table I. The positive entropy change is likely due to the counterion dispersal caused by the binding of the doubly charged $[(bipy)Pt(en)]^{2+}$.

TABLE I. Comparison of thermodynamic parameters for ligands binding to DNA. Our buffer solution was 10 mM NaH_2PO_4· 0.01 mM EDTA buffer solution (pH=6.5). The value for the enthalpy refers to a racemic mixture of Δ- and Λ-$[Ru(phen)_3]^{2+}$.

Ligand	$K/10^5$ (M^{-1})	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/mol K)
ethidium[a]	12.5	−8.3	−8.8	−1.4
daunomycin[b]	6.9	−9.1	−10.4	−4.7
Δ-$[Ru(phen)_2DPPZ]^{2+}$[c]	32.0	−8.9	+0.2	+30.8
Λ-$[Ru(phen)_2DPPZ]^{2+}$[c]	17.0	−8.5	+2.9	+38.1
Δ-$[Ru(phen)_3]^{2+}$[d]	0.09	−5.4	+2.6*	+26.8
Λ-$[Ru(phen)_3]^{2+}$[d]	0.11	−5.5		
$(bipy)Pt(en)^{2+}$ below the transition	81±31	−9.4±0.23	−1.9±0.11	+25±0.84
$(bipy)Pt(en)^{2+}$ above the transition	3.7±0.88	−7.6±0.14	−1.2±0.048	+22±0.50

[a]These data are from Refs. [12] and [13].
[b]These data are from Ref. [14].
[c]These data are from Ref. [15].
[d]These data are from Ref. [16].

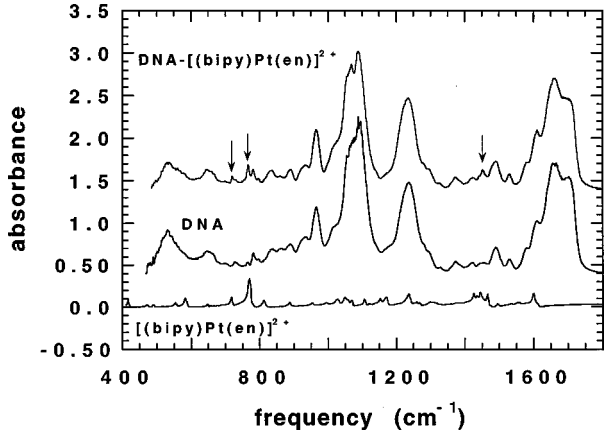

FIG. 2. The IR spectra of the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ complex (with 0.17 molar ratio of $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ to DNA), pure DNA, and pure $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ at a relative humidity of 23%. The spectra of pure DNA and the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ complex has been shifted up by 0.4 and 1.4 absorbance units, respectively. The vibrational modes of the $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ molecular ion appearing at 716, 769, and 1445 cm^{-1} in the spectrum of the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ complex are denoted by arrows.

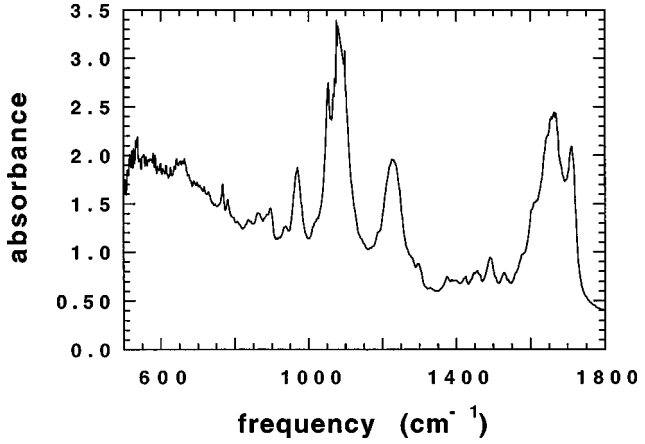

FIG. 3. The IR spectrum of the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ complex (0.17 molar ratio of $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ to DNA) at 80% RH.

The identities of the two binding sites are of great interest. The x-ray experiments of Arnott *et al.* [5] were performed on oriented fibers of calf-thymus DNA which had been saturated with $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$. For such conditions, $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ intercalates in a nearest-neighbor exclusion manner, yielding samples in the ladder conformation with one $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ for every two base pairs. One interpretation of the ITC data is that the $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ intercalates the DNA for both sites but that the first site is intercalation of helical B-DNA and the second site is intercalation of the ladder L-DNA.

IR absorption experiments were performed in order to test this hypothesis. A recent theoretical and experimental study of the vibrational modes of a DNA·daunomycin [17] complex has shown significant frequency shifts for those modes localized in the regions of the molecules distorted by the formation of the complex. Daunomycin binds to DNA by intercalating its planar heterocyclic part between base pairs and by groove binding of its amino sugar which lays in the minor groove of the DNA [18]. Intercalation of a planar group (such as daunomycin or $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$) causes the neighboring base pairs to move farther apart, which distorts the backbone connecting the base pairs. Theoretical work by several groups [19–24] has shown that the observed modes in the 800–1000 cm^{-1} range are localized in the DNA backbone. Frequency shifts of these modes were observed for the DNA· daunomycin complex [17]. The changes in the backbone geometry reported in the formation of L-DNA by the intercalation of $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ [5] are larger than caused by daunomycin, suggesting that MIR experiments should reveal the presence of even larger frequency shifts due to the intercalation by $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$.

Unoriented films of DNA hydrated to either 23% or 80% RH were used instead of solutions because of the very intense and broad absorption of water which obscures the backbone region (800–1000 cm^{-1}). Experiments were performed at 23% RH since the water content is very low at this RH, minimizing the water signal. Experiments were not performed at 0% RH since such extreme dehydration frequently caused the films to crack. Figure 2 shows the MIR spectra of pure DNA, pure $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ and the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ complex at 23% RH. These spectra display virtually no absorption features due to water. It should also be noted that almost all of the modes observed from the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ complex are DNA modes: the only $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ modes observable in the DNA· $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ spectra are the modes at 716, 769, and 1445 cm^{-1}. The bases of double-helical samples of pure DNA are destacked at 23% RH [25], making the evaluation of these data somewhat problematic. The bases are properly stacked at 80% RH. However, the broad absorption feature below about 900 cm^{-1} observed in MIR spectra from samples at 80% RH, such as shown in Fig. 3, is due to absorption by water. This feature lowers the signal-to-noise ratio of the backbone modes. This RH was also chosen since it closely matches the hydration conditions of the x-ray experiments of Arnott *et al.* [5].

Table II lists all the observed vibrational frequencies for samples at 23% and 80% RH. An examination of this table shows that none of the modes localized in either DNA or $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ experience a significant frequency shift ($\geqslant 10$ cm^{-1}) due to the formation of the complex. These MIR data argue that the geometry of neither molecule is significantly affected by the formation of the complex. Intercalation of $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ between the base pairs (as observed for L-DNA) would not be expected to cause a significant perturbation of the geometry of $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$. However, the geometry of the DNA phosphodiester backbone is radically altered by the formation of L-DNA. The distance between neighboring base pairs would double when a $[(\text{bipy})\text{Pt}(\text{en})]^{2+}$ intercalates between them. This change in geometry causes a significant change in the geometry of the backbone connecting these neighboring base pairs. This change in backbone geometry would be expected to shift the frequency of the vibrations localized in the backbone. In contrast, the data of Table II show virtually no difference for any modes.

The torsional modes of DNA are expected to be very sensitive to the changes in the molecular geometry induced by the formation of L-DNA. Given the relatively weak re-

TABLE II. Vibrational frequencies observed from pure DNA, pure $[(bipy)Pt(en)]^{2+}$, and DNA· $[(bipy)Pt(en)]^{2+}$ (0.47 molar ratio of $[(bipy)Pt(en)]^{2+}$ to DNA). The asterisks denote a $[(bipy)Pt(en)]^{2+}$ mode observable in the DNA· $[(bipy)Pt(en)]^{2+}$ spectra.

	23% RH		80% RH	
pure DNA (cm^{-1})	pure $[(bipy)Pt(en)]^{2+}$ (cm^{-1})	DNA· $[(bipy)Pt(en)]^{2+}$ (cm^{-1})	pure DNA (cm^{-1})	DNA· $[(bipy)Pt(en)]^{2+}$ (cm^{-1})
	416			
	471			
	489			
532		534		
	552			
	582			
	646			
649		649		
698		698		
	716	719*		716*
728		728	728	726
764	769	766*	764	765*
781		780	780	780
796		796		
	811	805	811	
837		832	838	837
861		859	863	863
			885	883
888	887	889		
			896	896
930		929	937	937
	952			
965		964	968	968
	994			
1021		1021	1020	1020
	1025			
	1048			
1057	1056	1055	1055	
1073	1068	1068		1073
1091		1088	1087	
	1106			
	1121			
	1152			
	1169			
			1189	1189
1232	1235	1230	1223	1228
	1259			
	1267			
1278		1279	1277	
1293		1294	1298	1294
	1298			
	1304			
1328		1325	1329	1332
1373		1373	1375	1374
			1399	1399
1421	1425	1423	1423	1424
	1435			
1446		1442		1449
1455	1445	1452*	1453	
	1465			1464
1489	1495	1486	1491	1490
				1506
1529	1533	1529	1529	1528
	1556		1560	
1578		1580	1583	1578
	1600			
1609		1610	1612	1609
1660		1660	1659	1653
1700		1696	1711	1706

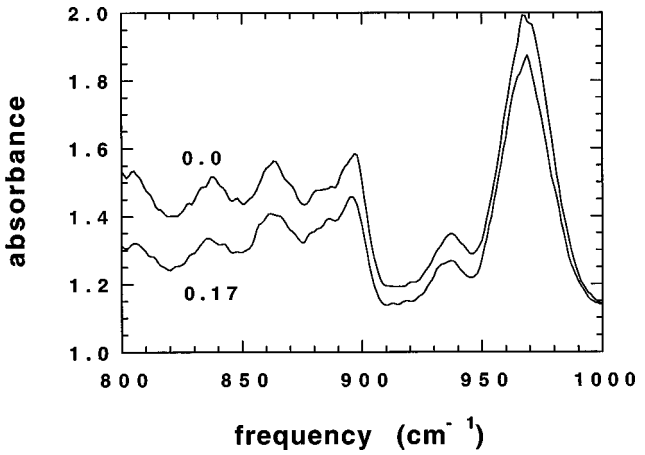

FIG. 4. Backbone region of IR spectra of pure DNA and the DNA· $[(bipy)Pt(en)]^{2+}$ complex (0.17 molar ratio of $[(bipy)Pt(en)]^{2+}$ to DNA) at 80% RH.

storing forces associated with torsional motions, these modes are expected to be very low in frequency, in the far-IR (FIR) range. However, these modes have never been observed from helical DNA, to the best of our knowledge.

Several studies of the conformationally sensitive vibrational modes of DNA have been performed [20–24,26–28]. In the backbone region of MIR spectra, A-DNA is characterized by the presence of vibrational modes at 807, 864, 877, and 899 cm^{-1} and B-DNA is characterized by modes at about 835 and 894 cm^{-1}. The differences in geometry of the right-handed A and B conformations of DNA are relatively small when compared to the differences in geometry between the B and L conformations of DNA. The fact that the backbone vibrations shift by measurable amounts between A- and B-DNA suggests that these modes are very sensitive to minor shifts in geometry. The fact that our MIR experiments show no significant shifts for the DNA backbone modes when $[(bipy)Pt(en)]^{2+}$ attaches to DNA is strong evidence that the DNA backbone has not undergone any substantial modifications in our experiments.

Figure 4 shows the backbone region of the MIR spectra for pure DNA and a DNA· $[(bipy)Pt(en)]^{2+}$ sample with a molar ratio of 0.17 at 80% RH. Note the presence of the 807, 864, and 877 cm^{-1} A-DNA marker bands as well as the B-form marker band at about 835 cm^{-1}. This shows that our samples are a mixture of A- and B-DNA. Oriented fibers and films of NaDNA have been found to be in the A conformation between about 50% and 92% RH [29]. Several studies [29–31] have shown that the strong intermolecular bonds of the crystalline state are important for stabilizing the A conformation. Unoriented samples in this range of hydration are frequently not pure A-DNA, as observed in our experiments at 80% RH.

The frequency of two intense modes in the MIR spectra shows significant dependence on the molecular conformation. The antisymmetric phosphate stretching mode has a frequency of 1240 cm^{-1} for A-DNA, 1225 cm^{-1} for B-DNA, and 1215 cm^{-1} for Z-DNA. An in-plane base double-bond stretching mode has a frequency of 1705 cm^{-1} for A-DNA, 1715 cm^{-1} for B-DNA, and 1695 cm^{-1} for Z-DNA. Figure 5 shows the frequency of these modes (plus the 895 cm^{-1} mode) as a function of the $[(bipy)Pt(en)]^{2+}$ content. The letters A, B, and Z are included in the figures to show the exact frequency of these modes for A-, B-, and Z-DNA, respectively. The observed frequencies show no dependence on $[(bipy)Pt(en)]^{2+}$ content. This strongly suggests that the attachment of $[(bipy)Pt(en)]^{2+}$ to the DNA does not cause any significant change in the geometry of the DNA double helix. It should be noted that the frequencies of these modes for the pure DNA samples show a mixture of A- and B-DNA.

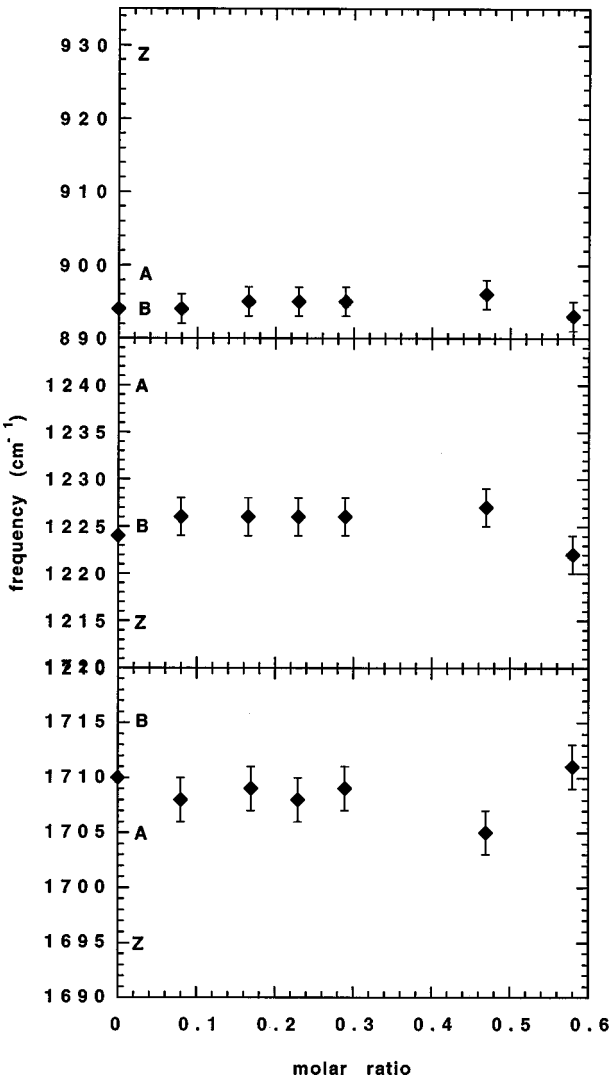

FIG. 5. Frequency of three conformationally sensitive modes of the DNA· $[(bipy)Pt(en)]^{2+}$ at 80% RH as a function of $[(bipy)Pt(en)]^{2+}$ content. The letters A, B, and Z denote the frequencies of these modes for A-, B-, and Z-DNA, respectively.

Additional information about the molecular conformation of our samples can be obtained by analyzing the strength of the absorbance of the modes at 835 and 860 cm^{-1}. As noted earlier, the 835 cm^{-1} mode is the B-form marker band [26] which indicates the presence of B-DNA with its C-2′-*endo* sugar pucker. The 860 cm^{-1} mode is observed in A-DNA with its C-3′-*endo* sugar pucker [27]. These modes can be used to probe the relative amounts of sugars with C-2′-*endo* pucker and C-3′-*endo* pucker. In an elegant set of experiments, Taillandier *et al.* [28] used the integrated absorbances of these two modes to monitor the B to A conformational transition while dehydrating the sample. The ratio

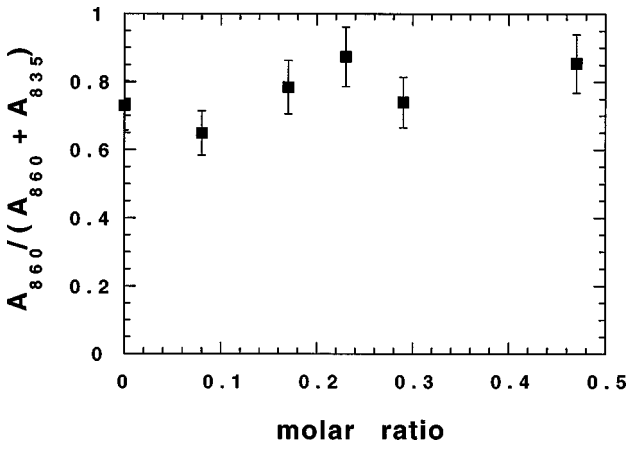

FIG. 6. Ratio $A_{860}/(A_{860}+A_{835})$ as a function of $[(\text{bipy})\text{Pt(en)}]^{2+}$ content at 80% RH where A_{860} and A_{835} are the integrated absorbances of the 860 and 835 cm^{-1} modes, respectively.

$A_{860}/(A_{860}+A_{835})$ is zero when the sample is entirely B-DNA and one when the sample is entirely A-DNA, where A_{860} and A_{835} are the integrated absorbances of the 860 and 835 cm^{-1} modes, respectively. Measuring this ratio as a function of $[(\text{bipy})\text{Pt(en)}]^{2+}$ content would show whether the formation of the complex changes the relative amounts of C-2′-*endo* and C-3′-*endo* sugars in the sample.

Figure 4 shows the backbone region of the MIR spectra for a pure DNA sample and a DNA$\cdot[(\text{bipy})\text{Pt(en)}]^{2+}$ sample with 0.17 molar ratio of $[(\text{bipy})\text{Pt(en)}]^{2+}$ at 80% RH. Four broad (and overlapping) modes are observed between 800 and 900 cm^{-1}. The relative absorbances of each mode have been evaluated by performing a nonlinear least-squares fit to these modes for each of the six different $[(\text{bipy})\text{Pt(en)}]^{2+}$ contents. Figure 6 shows the $A_{860}/(A_{860}+A_{835})$ ratio as a function of $[(\text{bipy})\text{Pt(en)}]^{2+}$ content at 80% RH. This ratio is near 0.70 for pure DNA and 0.85 for a molar ratio of 0.47, suggesting that the addition of $[(\text{bipy})\text{Pt(en)}]^{2+}$ has caused more of the DNA to adopt the A conformation. This is contrary to the expectation based upon the x-ray study [5] of L-DNA. That work found that for each base-pair, the sugar moiety on one strand is in the C-3′-*endo* pucker and the sugar moiety on the other strand is in the C-2′-*endo* pucker. It should be noted that this measurement of the $A_{860}/(A_{860}+A_{835})$ ratio is complicated by the fact that the DNA molecules of our samples are not all in the A conformation for the pure DNA samples. The relative amounts of A- and B-DNA in the pure samples cannot be evaluated. It is possible, though not likely, that the relative amounts of C-2′-*endo* and C-3′-*endo* sugars would not change due to the addition of $[(\text{bipy})\text{Pt(en)}]^{2+}$ to the DNA.

Our MIR results do not support the structural model of this complex proposed by Arnott *et al.* [5]. It should be noted the samples of the two studies are different in one crucial aspect. Our samples are in solution and unoriented films while Arnott *et al.* used oriented fibers. Far fewer strong intermolecular bonds are expected to be present in our unoriented samples than were present in the ordered samples of Arnott *et al.* As mentioned earlier, significant work [29–31] has shown that such bonds play an important role in A-DNA. Perhaps strong intermolecular bonds are also important for the formation of L-DNA. Our unoriented samples lack such strong intermolecular bonds which might preclude the formation of L-DNA.

SUMMARY

In summary, bipyridyl-(ethylenediamine)platinum(II) has been shown to bind to two different sites on the DNA molecule. The binding is driven by a large entropy increase, presumably due to the dispersion of the Na^+ counterions. MIR experiments suggest that the conformation of the DNA molecule is unaffected by the binding process.

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