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DNA Binding Studies of $PdCl_2(LL)$ (LL = Chelating Diamine Ligand: N,N-Dimethyltrimethylenediamine) Complex

S. Kashanian[1]*, N. Shahabadi[2], H. Roshanfekr[2], K. Shalmashi[2], and K. Omidfar[3]

[1]*Department of Chemistry and Nanoscience and Nanotechnology Research Center (NNRC), Faculty of Science, Razi University, Kermanshah, Iran; E-mail: kashanian_s@yahoo.com*
[2]*Department of Chemistry, Faculty of Science, Razi University, Kermanshah, Iran*
[3]*Endocrinology and Metabolism Research Center, Medical Sciences/Tehran University, Tehran, Iran*

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Abstract—The interaction of native calf thymus DNA with the Pd(II) complex, $PdCl_2(LL)$ (LL = chelating diamine ligand: N,N-dimethyltrimethylenediamine), in 10 mM Hepes aqueous solutions at neutral pH has been monitored as a function of metal complex/DNA molar ratio by UV absorption spectrophotometry, circular dichroism (CD), viscosimetry, and fluorescence spectroscopy. The results support two modes of interaction. In particular, this complex showed absorption hypochromism and then hyperchromism, increase in melting temperature, and some structural changes in specific viscosity when bound to calf thymus DNA. The binding constant determined using absorption measurement is $2.69 \cdot 10^3$ M^{-1}. As evidenced by the increasing fluorescence of methylene blue–DNA solutions in the presence of increasing amounts of metal complex, $PdCl_2(LL)$ is able to displace the methylene blue intercalated into DNA, but not so completely, as indicated by partial intercalation. CD spectral changes in two steps and viscosity decrease confirm our conclusions.

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Key words: Pd(II) complex, CT-DNA, partially intercalating, outside binding

Over recent decades, there has been substantial interest in the deoxyribonucleic acid (DNA) binding properties toward a number of metal complexes, with the aim of developing novel reagents that can control genetic information and/or prevent the growth and replication of cancerous cells through the inhibition of transcription [1-5]. The prerequisites for such complexes are that they should be stable, inert in the biological environment, and water-soluble.

Despite a considerable literature on metal complexes and DNA interactions, knowledge of the nature of binding of these complexes to DNA and their binding geometries has remained a subject of debate.

Discovery of the antitumor activity of *cis*-$[PtCl_2(NH_3)_2]$ [6] (cisplatin, *cis*-DDP) and the prevailing view that platinum binds to DNA, the most important intracellular target for antitumor Pt drugs [7-9], have stimulated considerable research on metal ion interactions with nucleic acid constituents.

Reactions of platinum complexes with components present in the media used to dissolve them can produce, and undoubtedly have produced, misleading results in fundamental mechanistic work and in screening studies. A particularly noteworthy example is dimethylsulfoxide (DMSO), which has been used to dissolve platinum compounds, presumably owing to their greater solubility in DMSO than in water. Thus, some interpretations of results from experiments designed to study the mechanism of action of platinum antitumor drugs in which DMSO (or other solvents) has been used could be erroneous [10].

In this work, the interactions have followed in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffer.

The choice of palladium as a center metal is based on three factors: i) it has high solubility in water. Since *in vitro* media must be similar to physiological conditions, these interactions have been followed in aqueous media; thus, the solubility of these complexes in water is important; ii) palladium is less expensive than platinum, and

Abbreviations: CT-DNA) calf thymus DNA; DMSO) dimethylsulfoxide; MB) methylene blue; $PdCl_2(LL)$) Pd(II) complex, where LL = chelating diamine ligand: N,N-dimethyltrimethylenediamine.

* To whom correspondence should be addressed.

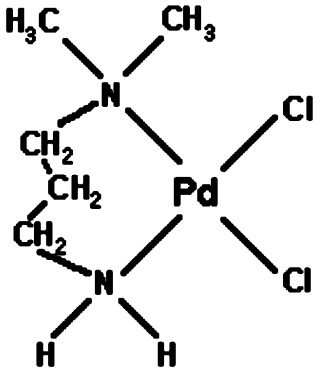

Fig. 1. Schematic structure of $PdCl_2(LL)$ (LL = chelating diamine ligand: N,N-dimethyltrimethylenediamine).

(iii) the remarkable analogy between Pt(II) and Pd(II) coordination stereochemistry has promoted studies of Pd(II) compounds as anticancer drugs [11-15].

Recent advances in this area have focused on Pd(II) complexes with one N,N-diamino- [16-19] or N,S-aminothioether [20-22] chelating ligand that impels *cis*-coordination with more labile ligands (containing two chlorine or nitrate moieties or one dicarboxylate moiety). In such cases, the chelating ligand prevents *cis-trans* isomerization around Pd(II) in spite of its greater lability (about 10^5-fold) relative to its Pt(II) [12, 16, 23] analogs. This significant kinetic difference makes Pd(II) compounds useful as models for Pt(II) compounds in mechanistic and kinetic [12], potentiometric [16], and spectrophotometric studies [24, 25].

On the other hand, the low antitumor activity of Pd complexes has been attributed to rapid hydrolysis leading to easy dissociation of leaving groups in solution and to the formation of very reactive species unable to reach their pharmacological targets [26]. One approach to solving this problem is identifying novel substances used as binding blocks for Pd antitumor drugs. Chelating ligands such as diamines or thioether amino acids are needed to impose the *cis*-coordination to salient labile ligands as a suitable way to synthesize antitumor complexes [27].

In this paper, the palladium complex of the $[PdCl_2(LL)]$ type (LL = chelating diamine ligand: N,N-dimethyltrimethylenediamine) (Fig. 1) was synthesized and used as a model for platinum and other palladium complex derivatives with five- and six-membered chelate rings to study their interaction with calf thymus DNA (CT-DNA).

MATERIALS AND METHODS

Chemicals and materials. Commercial pure chemicals such as $PdCl_2$, N,N-dimethyltrimethylenediamine, and Hepes (Sigma, Spain) were used as purchased. Experiments were carried out in Hepes buffer (10 mM, pH 7.0). Solutions were prepared with distilled water. High polymer CT-DNA was purchased from Sigma.

The stock solution of DNA was prepared by dissolving DNA in 10 mM Hepes buffer, pH 7.0. The solution of CT-DNA had a ratio of UV absorbance at 260 and 280 nm of more than 1.8, indicating that the DNA was sufficiently free from protein [28].

The DNA concentration (monomeric units) of the stock solution was determined by UV spectrophotometry, in properly diluted samples, using the molar absorption coefficient 6600 $M^{-1}\cdot cm^{-1}$ at 260 nm [29]. The stock solution was stored at 4°C.

To study the interaction of palladium complex with DNA, stock solutions were prepared by dissolving the complex in Hepes buffer to a final concentration of 1 mg/ml. Stock solutions were freshly prepared before use (less than 1 h).

Instrumentation. The complex was characterized by elemental analysis, UV-VIS, and NMR (^1H and ^{13}C) spectroscopy. Elemental analysis was performed using the Heraeus CHN elemental analysis system. Absorbance spectra were recorded using an HP spectrophotometer (Agilent 8453) equipped with a thermostatted bath (Huber polystat cc1). The ^1H- and ^{13}C-NMR spectra were recorded using a Bruker Avance DPX 200 MHz (4.7 Tesla) spectrometer using d_6-DMSO as solvent and sodium 3-(trimethylsilyl)tetradeuteriopropionate as internal standard. Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter. For viscosity measurements, a viscosimeter (SCHOT AVS 450) thermostatted at 25°C by a constant temperature bath was used. Fluorescence measurements were carried out with a JASCO spectrofluorometer FP6200.

General procedure in preparation of Pd complex. The $[PdCl_2(LL)]$ complex was prepared according to general procedures reported in the literature for complexes with ethylenediamine, with certain modifications to avoid the presence of impurities [30, 31].

In this procedure, 0.5 g $PdC1_2$ was dissolved under reflux in a mixture of 10 ml H_2O and 3 ml HCl (conc.). The clear solution was filtered and the pH was adjusted to 2-3 by adding NaOH.

Then the ligand was added drop-wise to the warm solution of $PdC1_4$ in the mole ratio of 1 : 1. The pH of the solution was constantly adjusted to 2-3 by the addition of HCl. In basic medium, no complex formation could be observed and irreversible hydrolysis interfered. After addition of the ligand, a yellow precipitate formed in the light brown solution, which disappeared with pH adjustment with HCl. At this point, the solution turned dark brown. After the yellow precipitate of $[PdCl_2(LL)]$ was observed, it was stirred for 24 h at 20°C. Then the precipitate was filtered off and thoroughly washed with water, ethanol, and diethyl ether. The product appeared as a yel-

low powder with a high yield (92-95%). This complex is more soluble in water than cisplatin. $C_5H_{14}N_2Cl_2Pd$. Anal. Calc.: C, 21.46; H, 5.00; N, 10.02. Found: C, 21.6; H, 5.1; N, 10.

Absorbance measurements were performed in two ways: i) by keeping the concentration of the DNA constant ($8.18 \cdot 10^{-5}$ M) while varying the complex concentrations from $8.18 \cdot 10^{-6}$ to $57.2 \cdot 10^{-6}$ M; ii) by using a fixed complex concentration to which increments of the DNA stock solution were added. Complex solutions employed were 50 μM in concentration, and DNA concentrations were from $2.5 \cdot 10^{-6}$ to 10^{-3} M.

All of the samples were incubated at 37°C for 24 h, and the spectra were recorded in the range of 200-400 nm.

Our experiments for determining the melting temperature were carried out for CT-DNA in the absence and presence of different amounts of Pd(II) complexes. The melting plot of DNA ($8.18 \cdot 10^{-5}$ M) was monitored by plotting the UV maximum absorption of DNA at 258 nm versus temperature.

Preparation of the samples for CD was similar to that for UV, where the concentration of DNA must be kept constant.

For viscosity measurements, a viscosimeter (SCHOT AVS 450) thermostatted at 25°C by a constant temperature bath was used. Flow time was measured with a digital stopwatch; the mean value of three replicated measurements was used to evaluate the viscosity (η) of the samples. The complex concentration was $(0.5\text{-}9) \cdot 10^{-5}$ M and DNA concentration was constant at $5.0 \cdot 10^{-5}$ M. The data is reported as $(\eta/\eta_0)^{1/3}$ versus [$PdCl_2$(LL)]/[DNA] ratio [32], where η_0 is the viscosity of the DNA solution alone.

In fluorescence studies, methylene blue (MB) and DNA concentrations in all samples were constant ($5 \cdot 10^{-6}$ and $5 \cdot 10^{-5}$ M, respectively) and complex concentrations were from 10^{-5} to $6 \cdot 10^{-5}$ M. These solutions were then mixed, diluted to volume with Hepes solution, and incubated for 4-5 h. Pure MB and MB–DNA solutions were also prepared in a similar manner.

RESULTS AND DISCUSSION

Chemistry. $PdCl_2$(LL) shows an intense absorption band at about 221 nm.

Data corresponding to the ^{1}H-NMR and ^{13}C-NMR spectra of the complex recorded in d_6-DMSO are given in Table 1.

The ^{1}H-NMR spectrum of the complex showed a quintet at 1.8 ppm caused by the protons of the inner methylene group, a triplet at 2.52 ppm arising from the four protons of the outer methylene groups, and a singlet signal observed at 2.92 ppm corresponds to $N(CH_3)_2$ protons.

Signals of the ^{13}C-NMR spectrum of $PdCl_2$(LL) observed at 60, 53, and 25 ppm were assigned to inner methylene, methyl, and outer methylene carbons, respectively.

Table 1. ^{1}H-NMR and ^{13}C-NMR spectral data for the $PdCl_2$(LL) complex

	N–C*H_2–C	C–C*H_2–C	–N(C*H_3)$_2$
δ (^{13}C) ppm	25	60	53
	N–CH*$_2$–C	C–CH*$_2$–C	–N(CH*$_3$)$_2$
δ (^{1}H) ppm	2.13	1.8	2.52

Electronic spectra. Electronic absorption spectra are initially employed to study the binding of complexes to DNA. The binding of intercalative drugs to DNA helix has been characterized classically through absorption spectral titrations, by following the changes in absorbance (hypochromism) and shift in wavelength (red shift) [33].

In the present study, the interaction of Pd(II) complex with CT-DNA was monitored in aqueous solution. Aliquots of the DNA solution at a constant concentration ($8.18 \cdot 10^{-5}$ M, ~27 μg/ml) were incubated with Pd complex at [complex]/[DNA] ratio (r) values of 0.1-0.7 in 10 mM Hepes (pH 7.0) at 37°C.

The UV band of DNA at about 258 nm was monitored in the absence and presence of different amounts of Pd(II) complex. Bathochromic shifts from 258 to 265 nm accompanied by hyperchromicity are generally observed (Fig. 2a), which concurs with the data recently reported for Pd analogs like [$PdCl_2$(en)] and [PdL(en)] complexes (en = ethylenediamine and L = 1,1-cyclobutanedicarboxylate) [25] and also [$PdCl_2$(1,2-pn)] and [$PdCl_2$(1,3-pn)] complexes (pn = diaminopropane) [34]. Also, bathochromic shift upon reaction with Pd complex indicates association of Pd with DNA bases [10].

This complex has a six-membered chelate ring that is conformationally similar to the corresponding cyclohexane derivative [35]. By introducing steric hindrance on ethylenediamine-type ligands, the lability of the metal center can be tuned to control its reactivity in possible catalytic and biological applications [12]. These results indicate a pronounced interaction of the Pd complex with DNA.

When complex concentration is constant, two peaks are seen. $PdCl_2$(LL) shows an intensive absorption band at about 221 nm, which was significantly perturbed by the addition of increasing amounts of DNA, and free DNA in solution shows a peak at 260 nm. In detail, the spectral variations occur in two steps as a function of [DNA]/[complex] ratio (R) (Fig. 2b).

At low biopolymer concentrations ($R < 1$), hypochromism of about 40% is observed with a very small bathochromic shift (2 nm). When R increases, the maximal intensity increases, until hyperchromicity is seen

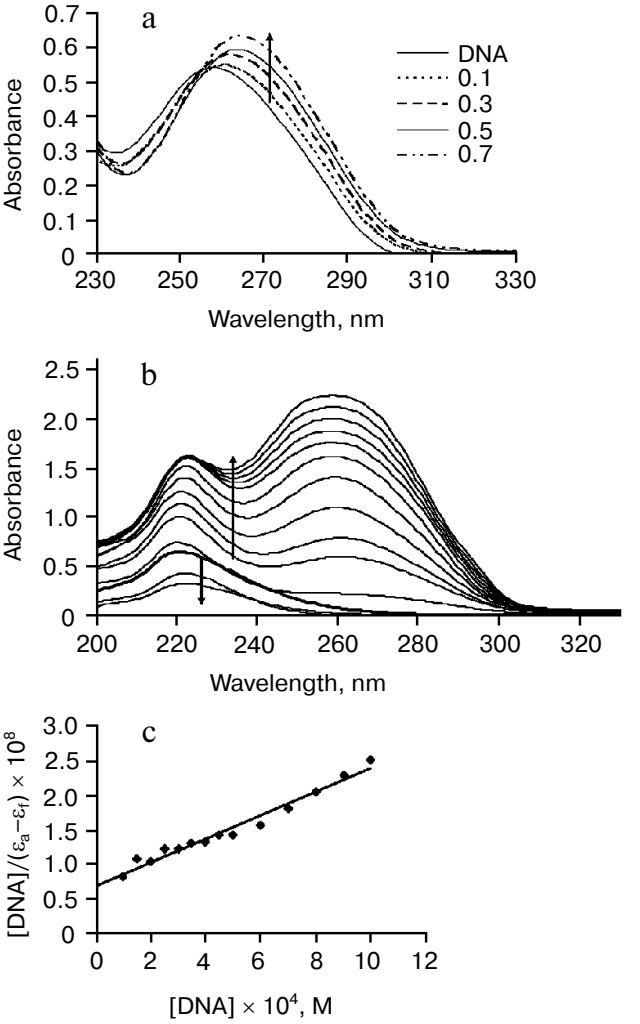

Fig. 2. Absorption spectra: a) DNA ($8.18 \cdot 10^{-5}$ M) in the absence (solid line) and presence of increasing amounts of Pd complex (r = [complex]/[DNA] = 0.0, 0.1, 0.3, 0.5, 0.7); b) Pd complex (50 μM) in the absence (solid line) and presence of increasing amounts of DNA (R = [DNA]/[complex] = 0.0, 0.05, 0.4, 1, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20). c) [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] plot.

through the addition of DNA up to stoichiometric ratio [$DNA_{phosphate}$]/[complex] = 1. The magnitude of hypochromicity is comparable with the intercalation mode of binding, but red shift is lower than those observed for typical classical intercalators. Moreover, the intensity begins to increase immediately, thus intercalation interaction is ruled out. Its intrinsic binding constant, K_b, with CT-DNA was determined from the decay of the absorption spectrum (Fig. 2b) according to the following equation [36]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f),$$

where the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to A_{obs}/[Pd], the extinction coefficient for the free palladium complex, and the extinction coefficient for the palladium in the fully bound form, respectively. In plots of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], K_b is given by the ratio of the slope to the intercept. A representative plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] for [$PdCl_2$(LL)] is shown in Fig. 2c. The binding constant, K_b, was calculated to be $2.69 \cdot 10^3$ M^{-1}. The K_b value is lower than those observed for typical classical intercalators, e.g. K_b for ethidium bromide–DNA complex is equal to $7 \cdot 10^7$ M^{-1} in 40 mM Tris-HCl buffer, pH 7.9 [37], and $1.4 \cdot 10^6$ M^{-1} in 40 mM NaCl/25 mM Tris-HCl [38]. This indicates that DNA–complex binding has an affinity less than that of the classical intercalators.

We propose that the unexpected change in intensity is a result of change in the mode of interaction. Primary hypochromicity is indicative of partial intercalation established in the next sections, and after that hyperchromicity considering K_b value suggested outside binding.

In the experiments where DNA concentration was constant, a considerable red shift and hyperchromicity was observed by addition of complex, which indicates considerable association with bases. However, these methods, by themselves, cannot definitively establish the DNA binding modes of this complex.

Thermal denaturation experiments. The consequences of adduct formation on the stability of the double helix in CT-DNA were assayed by recording the DNA melting profiles. Thermal behavior of DNA in the presence of complexes can give insight into their conformational changes when temperature is raised, and offers information about the interaction strength of complexes with DNA. With the exception of metal ions that bind strongly to nucleobase heteroatoms [39], upon interaction with a cationic species the double helix stability increases [40] and so does the DNA melting temperature. Although the increase in denaturation temperature is not specific to any particular type of noncovalent interaction, ΔT_m values give some indications regarding the binding mode. Large increases in melting temperature are observed only for the strongest type of interaction, i.e. intercalation.

Table 2 reports increase in DNA melting temperature in the presence of the complex under study and of the complexes $[Pt(bpy)(py)_2]^{2+}$ and $[Pt(en)(py)_2]^{2+}$. The latter substance, which cannot intercalate, has been used by Barton et al. [41] as a reference of non-intercalating

Table 2. Increase in melting temperature of CT-DNA upon interaction with complexes

Complex	ΔT_m
$[Pt(bpy)(py)_2]^{2+}$	10.5
$[Pt(en)(py)_2]^{2+}$	2.9
$PdCl_2$(LL)	7

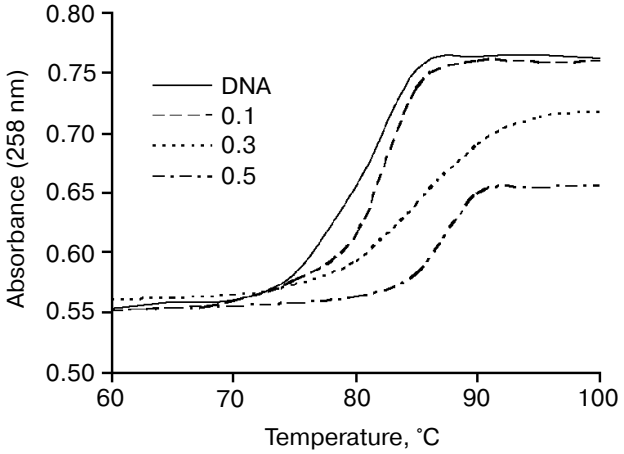

Fig. 3. Melting curves of CT-DNA ($8.18 \cdot 10^{-5}$ M) in the absence and in the presence of Pd complex at various r = [complex]/[DNA] = 0.0, 0.1, 0.3, 0.5 in 10 mM Hepes buffer, pH 7.0.

metal complexes. The increase in melting temperature (Fig. 3) of the studied complex is much larger than that for $[Pt(en)(py)_2]^{2+}$ and lower than that for $[Pt(bpy)(py)_2]^{2+}$, whose intercalating properties are well-established [42]. These results suggest that the Pd complex partially intercalates with the double helix.

Circular dichroism. The CD technique is very sensitive for detecting minor conformational changes in DNA conformation produced by ligand binding.

The observed CD spectrum of calf thymus DNA consists of a positive band at 275 nm (UV: λ_{max}, 260 nm) due to base stacking and a negative band at 245 nm due to helicity, which is characteristic of DNA in right-handed B-form [43].

The interaction of the complex with DNA induces a change in the CD spectrum of B-DNA (Fig. 4) in two ways. The presence of two different types of interaction as a function of [complex]/[DNA] ratio is clearly shown also by circular dichroism. At low ratios (r = 0.05, 0.08, 0.11, 0.12), the intensities of both the negative and positive bands increase. But at high ratios (0.2, 0.3, 0.5, 0.7, 1), because of the existence of high concentrations of the complex, the spectrum of DNA was perturbed strongly (complex concentration in comparison with DNA is high) and the intensities of both the negative and positive bands decrease significantly (shifting to zero levels, data not shown), which are a result of denaturation of the DNA duplex. These findings are indicative of deep conformational changes of the DNA double helix following the interaction of the DNA macromolecule with metal complexes. The interaction of the complex effectively screens the negative charge on N(7) base sites as well as phosphate oxygens simultaneously, both along the deoxyribophosphate backbone and in the groove of the helix, to promote a conformational change from a more B-like to a more C-like structure at high complex concentrations. Further transformation of DNA structure proceeds by removal of water from the base sites and the grooves of the helix [44].

The investigation of input voltage to the detector and its resulting peak was very interesting. In the CD instrument, the magnitude of the CD peak (ellipticity) was measured because of the presence of chiral species. Another application of CD is measuring the difference between source output voltage and detector input voltage.

In addition to the CD peak, the interaction between absorbing species and light produces variation in input voltage of the detector. The value of the input voltage of the detector and its difference with output voltage from the source is dependent upon the degree of interaction between chiral species and polarized light. In the CD technique, the difference between source and detector voltage versus wavelength is recorded, showing that with increase in this difference the intensity of the peak also increased. When complex added to sample included DNA, the intensity of the high tension peak at 260 nm increases, which is indicative of interaction between DNA and the complex (Fig. 5).

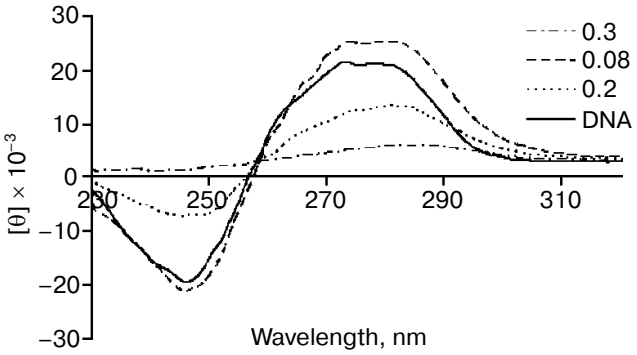

Fig. 4. Circular dichroism spectra of CT-DNA ($6.0 \cdot 10^{-4}$ M) in 10 mM Hepes in the presence of increasing amounts of $PdCl_2$(LL) at the following stoichiometric ratios of r = [complex]/[DNA] = 0.0, 0.08, 0.2, 0.3.

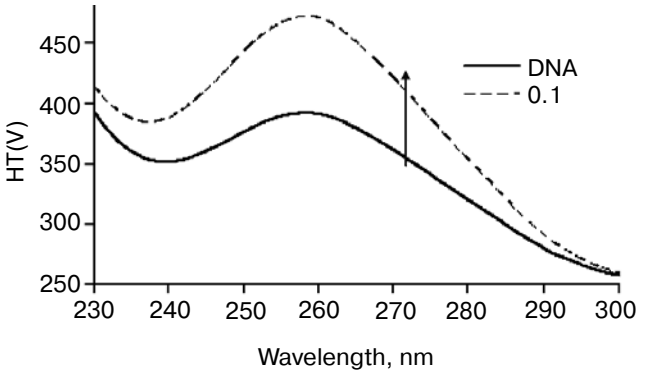

Fig. 5. High tension (HT) spectra of DNA in the presence of complex (r = [complex]/[DNA] = 0.1).

Viscosity measurements. Viscosity measurements were used to clarify the interaction between the complex and DNA. Optical photophysical probes provide necessary but not sufficient clues to support the binding model. Hydrodynamic measurements that are sensitive to length change (i.e. viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data.

The classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to increase in DNA viscosity [45]. In contrast, a partial and/or non-classical intercalation ligand could bend (or kink) the DNA helix, reduce its effective length, and concomitantly its viscosity, while ligands that bind exclusively in the DNA grooves (e.g. netropsin, distamycin), under the same conditions, typically cause less pronounced changes (positive or negative) or no changes in DNA solution viscosity [29].

The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$ (here η_0 and η are the specific viscosity contributions of DNA in the absence and in the presence of the present complexes) were plotted against r (r = [complex]/[DNA]) (Fig. 6).

In the viscosity curve, the observed decrease rules out the intercalative mode of binding, and is indicative of an "outside binding" that leads to condensation of DNA, shifting it to a form similar to A-DNA. In summary, the palladium complex decreases the specific viscosity of DNA possibly due to a number of factors including a change in conformation (it is somehow expected to shift from a more B-like to a more A-like structure), flexibility, or solubility of DNA molecules. However, in view of the tendency of DNA toward particulate formation, it is most likely that extensive aggregation of DNA on binding to the complex would sharply reduce the number of independently moving DNA molecules in solution, which is mainly due to the diffusion of molecules into each other. Thus, this decrease has been ascribed to DNA aggregation [44]. Furthermore, decrease in viscosity was explained by a model in which ligands were bound by a partial, non-classical intercalation mode. There, the planar complex could not be fully inserted between base pairs. The result of such binding was proposed to be a static bend (or kink) in the DNA helix, which would reduce its effective length and, concomitantly, its viscosity.

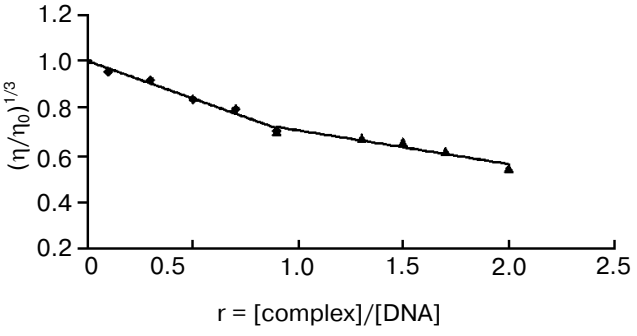

Fig. 6. Effect of increasing amounts of complex on the viscosity of CT-DNA ($5\cdot10^{-5}$ M) in 10 mM Hepes (r = 0.1, 0.3, 0.5, 0.7, 0.9, 1.3, 1.5, 1.7, 2).

Also, partial intercalation may occur, which would however act as a "wedge" to put apart one side of a base pair stack but not fully separate the stack as required by the classical intercalation model as observed for Δ-$[Ru(phen)_3]^{2+}$ [46, 47]. The possible result is a static bend or kink in the helix. Since viscosity is exquisitely sensitive to length changes, a reduction in end-to-end distance of a helix resulting from bending is readily measured.

Fluorescence studies (competitive binding studies). Since no luminescence was observed for the $PdCl_2$(LL) complex, it is hard to monitor the interaction of this complex with DNA by direct fluorescence emission methods, but it is possible by using a fluorescence assay of an organic molecular probe. Intercalation interaction of DNA with phenothiazinium dyes such as MB has been suggested by several spectroscopic methods [48-50]. In this contribution, we report spectroscopic studies of the interactions between Pd(II) complex and DNA using MB as a fluorescent probe. Upon binding to DNA, the emission of the fluorescence probe is efficiently quenched by the DNA bases with no apparent shift in the emission maximum (Fig. 7).

This emission-quenching phenomenon also reflects changes in the excited-state electronic structure because of electronic interactions in the MB–DNA complexes [51].

The emission-quenching phenomenon and the hypochromic and red shift effects in the absorption spectra fit the intercalative mode of MB to DNA.

Obvious spectroscopic changes of the MB (5 μM)/DNA (50 μM) system have been observed after adding Pd(II) complex. The emission spectra of the MB–DNA complexes in the presence of the increasing Pd(II) complex concentrations are shown in Fig. 7. This figure clearly reveals an increase in the fluorescence intensity of the probe molecule on adding the Pd(II) complex. In the case of the highest concentration Pd(II) complex, the emission intensity of the MB–DNA complex could not approach that of pure MB, as shown in Fig. 7.

The increase in fluorescence intensity should be due to a greater amount of free MB molecules in solution. Considering MB molecules have been already intercalated into the DNA helix, these results indicate that some MB molecules are released from the DNA nucleobases after addition of the Pd(II) complex. That is, the formation of metal complex–DNA prevents the binding of MB.

The extent of MB release and following increase in fluorescence intensity is applied to compare the strength

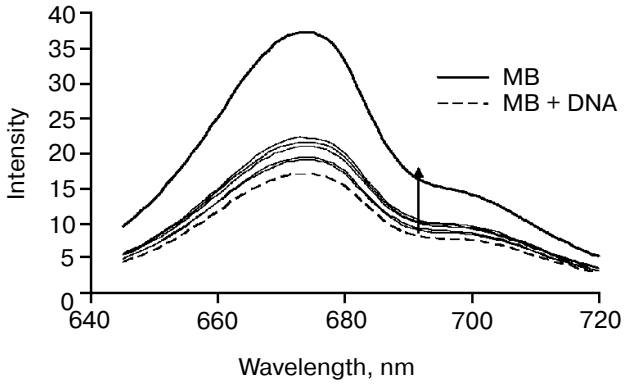

Fig. 7. Emission spectra of MB–DNA complexes in the presence of increasing Pd(II) complex concentrations in aqueous solution at room temperature. r = [complex]/[DNA] = 0.0 (MB + DNA), 0.2, 0.6, 0.8, 1.0, 1.2.

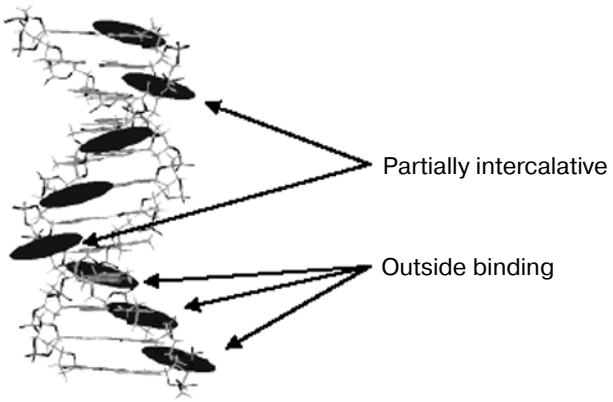

Fig. 8. A representative model for two modes of interactions.

of binding. Figure 7 shows, that our complex could not release MB completely. In this sense, we suggest that base to inner-sphere coordination on partial insertion of the complex can release some but not all the MB molecules. Therefore, this experiment confirms our previous theories and reconfirms our prior evidence based conclusions.

$PdCl_2$(LL) strongly interacts with native DNA, presumably by partially intercalative and outside binding mechanisms. Hydrolyzed complexes, in which Cl atoms are replaced by H_2O molecules, contain positive charges that facilitate their outside binding with negative charges of DNA.

The occurrence of interaction is supported by the following findings:

– the UV hypochromism and small bathochromic shift of the absorption bands at 221 nm, which is followed by hyperchromicity in the presence of increasing amounts of DNA, confirm two predicted modes of interaction. The low value of K_b, $2.69 \cdot 10^3$ M^{-1}, is related to outside binding of DNA;

– the increase in DNA melting temperature, T_m, of about 7°C when the molar ratio of [$PdCl_2$(LL)]/[DNA] is 0.5, which is intermediate between that of intercalative and non-intercalative modes of binding;

– the pronounced changes in the DNA CD spectra in the presence of increasing amounts of complex in two steps are indicative of deep conformational changes of the DNA double helix;

– the viscosity decrease is indicative of a non-intercalative mode of binding;

– the increase of MB–DNA solution fluorescence band by adding increasing amounts of Pd complex that could not completely replace intercalated MB.

Finally, we propose Fig. 8 as a representation of our predicted model.

In the end, it should be stated that because antitumor properties attributed to *cis*-DDP (cisplatin) has already been proved, considering the similarity of Pd(II) complexes to *cis*-DDP and its higher solubility in comparison with that of Pt(II), prompted us to synthesize this Pd(II) complex and investigate its interaction with CT-DNA. It is hoped that this complex and others that are similar will be useful for the development and design of more available antitumor drugs.

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