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DNA Interaction with $PtCl_2(LL)$ (LL = Chelating Diamine Ligand: *N*,*N*-Dimethyltrimethylendiamine) Complex

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Abstract The $[PtCl_2(LL)]$ complex, as a cisplatin derivative, which LL is diamine chelate ligand (*N*,*N*-dimethyltrimethylendiamine), was synthesized and characterized by elemental analysis (CHN) mass, ^1H, and ^{13}C nuclear magnetic resonance techniques. Then the binding of this complex to calf thymus DNA was investigated by various physicochemical methods such as spectrophotometric, circular dichroism, spectrofluorometric, melting temperature, and viscosimetric techniques. Upon addition of the complex, important changes were observed in the characteristic UV–Vis bands (hypochromism) of calf thymus DNA, increase in melting temperature and some changes in specific viscosity. Also, the fluorescence spectral characteristics showed an increase in the fluorescence intensity of methylene blue–DNA solutions in the presence of increasing amounts of metal complex, indicating $PtCl_2(LL)$ is able to displace the methylene blue bound to DNA but not as complete as intercalative molecules. The experimental results showed that the platinum complex is bound to DNA non-intercalatively, and an outside binding is the preferred mode of interaction.

Keywords Pt (II) complex · CT-DNA · Outside binding · DNA melting

Introduction

In the last decades, much attention was paid to the binding of small molecules with DNA, as a result of decided advantages of these molecules as potential drugs. Many natural or synthetic drugs serve as analogs in the research of protein–nucleic acid recognition and provide site-specific reagents for molecular biology. Therefore, the investigation of drug–DNA interaction is important to understand the molecular mechanisms of the drug action

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and designing specific DNA-targeted drug. Since the concept of intercalation into DNA was first formulated by Lerman in 1961, it has become widely recognized that many compounds of pharmacological interest, including anticancer drugs and antibiotics, correlate their biological and therapeutic activities with the ability of intercalative interaction with DNA. This noncovalent binding has an important function in life phenomena at the molecular level, indicating the interaction specificity of drug with DNA [1].

The square-planar complexes of platinum (II) has been of increasing importance since the discovery of the antitumor property of cis-dichlorodiammineplatinum(II) or cisplatin. Cisplatin chemistry has provided a fertile ground for exciting bioinorganic chemistry research. It is very important to note that similar coordination complexes from other groups of metals do not yield active compounds [2]. As a consequence, thousands of cisplatin analogs have been synthesized by varying the nature of the leaving groups and the carrier ligands, but only four platinum compounds (cisplatin, carboplatin, oxaloplatin, and nedaplatin) are currently registered for clinical use. Nevertheless, there are several candidates under clinical trials. Precise mechanism of antitumor action of platinum drugs is not completely understood, but DNA is believed to be the main target of this kind of compounds [3]. Biological studies suggest that DNA is one of the primary intracellular targets of the metallocene dihalides, but their mechanism of action is poorly understood [4].

Today, there is a great interest in understanding how DNA recognizes metal complexes, particular attention being devoted to noncovalent modes especially to external binding which is mainly governed by electrostatic effects [5].

In this study, the platinum complex of the [$PtCl_2$(LL)] type (LL = chelating diamine ligand: *N*,*N*-dimethyltrimethylendiamine; Fig. 1) was synthesized and used as a model for platinum and its other complex derivatives with five- and six-membered chelate rings to study their interaction with calf thymus DNA (CT-DNA) by circular dichroism , UV–Vis absorption , thermal denaturation, fluorescence, and viscosity measurements. It should be mentioned that the interactions have been carried out in 4-(2-hydroxyethyl)-1-piperazinee-thanesulfonic acid (HEPES), which is one of the Good's buffers, in order not to produce misleading results due to organic solvents [6, 7].

Materials and Methods

Material

Commercially pure chemicals such as *N*,*N*-dimethyltrimethylendiamine (Merck) and HEPES (Sigma, Madrid Spain) were used as purchased. Experiments were carried out in

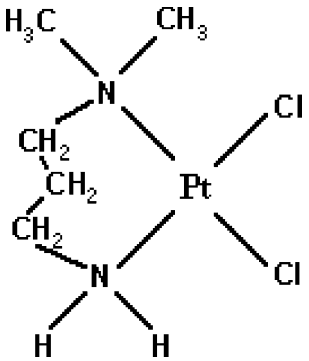

Fig. 1 Schematic structure of $PtCl_2$(LL) (LL = (chelating diamine ligand: *N*, *N*-dimethyltrimethylendiamine)

HEPES buffer (10 mM, pH 7.0). Solutions were prepared with distilled water. Highly polymerized calf thymus-DNA was purchased from Sigma.

The stock solution of DNA was prepared by dissolving DNA in 10 mM of the HEPES buffer at pH 7.0. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein [8].

The DNA concentration (monomer units) of the stock solution was determined by UV spectrophotometery, in properly diluted samples, using the molar absorption coefficient 6,600 M^{-1} cm^{-1} at 260 nm [9]. The stock solution was stored at 4 °C.

To study the interaction of platinum complex with DNA, stock solutions were prepared by dissolving the complex in HEPES buffer to a final concentration of 0.5 mg mL^{-1} and incubated for 24 h at 37 °C after addition of DNA.

Synthesis of Platinum Complex

Synthesis of $PtCl_2$(LL)

N,*N*-Dimethyltrimethylendiamine ligand was added dropwise to the solution of $K_2[PtCl_4]$ until the pH of this solution was constantly adjusted to 9.5–10. In basic medium, no complex formation could be observed, and irreversible hydrolysis processes interfered. The solution was light red, and then it was cooled for 48 h at 4 °C. Then, the precipitate was formed, filtered off, and thoroughly washed with water. The product appeared as pink powders with a high yield (92–95%). *Anal.Calc*. for $C_5H_{14}N_2Cl_2Pt$: C, 16.3; H, 3.8; N, 7.6. Found: C, 16.1; H, 3.7; N, 7.5. The mass spectra of the complex recorded at room temperature showed a molecular ion peak at *m*/*z* 367 which confirms the structure of platinum complex.

$PtCl_2$(LL) complex shows an intense absorption band at about 221 nm and suggests complexation. The ^{1}H nuclear magnetic resonance (NMR) spectrum of the complex showed a quintet at 1.8 ppm caused by the protons of the inner methylene group, a singlet at 2.05 ppm arising from the amine group (NH_2), two singlet signals at 2.52 ppm and 2.6 ppm that arise from the three protons of $N(CH_3)_2$, and two triplet signals observed at 2.75 ppm and 2.86 ppm corresponding to the outer methylene groups. In ^{1}H NMR spectrum of Pt(II) complex, the signals due to various protons of diamine ligand are seen to be shifted (0.3 ppm) with corresponding free ligand and suggest complexation. ^{13}C NMR spectrum showed four signals at 29.56, 38.8, 43.55, and 54.62 ppm which were assigned to four carbon atoms of diamine ligand.

Methods

The complex obtained was characterized by elemental analysis, UV–Vis, and ^{1}H and ^{13}C NMR spectroscopy and mass spectroscopy. The elemental analysis was performed using a Heraeus CHN elemental analysis. The NMR spectra were recorded with a Bruker Avance DPX 200 MHz (4.7 Tesla) spectrometer using d_6-dimethyl sulfoxide as solvent and sodium 3-(trimethylsilyl) tetradeuteriopropionate as internal standard.

Absorbance spectra were recorded using an hp spectrophotometer (Agilent 8453) equipped with a thermostated bath (Huber polystat cc1). The absorbance measurements were performed by keeping the DNA concentration constant (4.33×10^{-5}M) while varying the complex concentration (from 4.33×10^{-6} to 8.66×10^{-5}M). The samples were incubated at 37 °C for 24 h, and the spectra were recorded in the range of 200–400 nm.

Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter. Solutions of DNA and platinum, which were prepared as described previously,

were scanned in 0.5 cm (1 mL) quartz cuvette. The spectra were recorded after incubation at 37 °C for 24 h.

For viscosity measurements, a viscosimeter (SCHOT AVS 450) which thermostated at 25 °C by a constant temperature bath was used. Flow time was measured with a digital stopwatch; the mean values of three replicated measurements were used to evaluate the viscosity $\acute{\eta}$ of the samples. The data were reported as $(\acute{\eta}/\acute{\eta}^{\circ})^{1/3}$ vs. $1/R$ that R=[DNA]/[$PtCl_2$(LL)] ratio [10], where $\acute{\eta}^{\circ}$ is the viscosity of the DNA solution alone.

The thermal denaturation temperature of complex–DNA mixtures was determined in 10 mM HEPES buffer (pH 7) containing 5×10^{-5}M of DNA and 1.5×10^{-5} to 10^{-4}M of the complex. Melting curves were recorded at 260 nm on an Agilent 8453 spectrophotometer interfaced with an IBM computer.

All fluorescence measurements were carried out with a JASCO spectrofluorometer (FP6200). The stock solutions of methylene blue (MB), DNA, and the Pt (II) complex were 10^{-5}, 4.24×10^{-4}, and 1.26×10^{-3}mol/L in concentration, respectively. The buffer solution of pH 7.0 contained 10 mM/L of HEPES buffer. All above solutions were further diluted as required. Xenon arc lamp (450 W) was used as the excitation light source (λ_{ex}=630 nm). The stock solution of DNA (236 μL) and MB solution (1,000 μL) were transferred to several 2 ml sample tubes. A known volume of Pt (II) complex solution was also added to these sample tubes at r_i=0.4, 0.8, 1.2, 1.6, 2.0 (r_i=[complex]/[DNA]). These solutions were then mixed, diluted to the volume with buffer, and incubated for 8 h. Pure MB and MB–DNA complex solutions were also prepared in a similar manner as above.

Results and Discussion

Electronic Spectra

Electronic absorption spectra are initially employed to study the binding of complexes to DNA.

In the present study, the interaction of Pt (II) complex with CT-DNA has been monitored in aqueous solution. Aliquots of the DNA solution at a constant concentration equal to 4.33×10^{-5} incubated with Pt complex at r_i values of 0.1–2.0 in 10 mM HEPES, (pH=7) at 37 °C.

The r_i values were calculated from the following equation: r_i = [complex]/[DNA].

The UV band of DNA at about 260 nm was monitored at the absence and presence of different amounts of Pt (II) complex. Hypochromic effects are generally observed, but no bathochromic shift occurred (Fig. 2), which concur with the data reported for Cp_2TiCl_2 and Et_2SnCl_2 [11] which could be interpreted as follows: Hydrolyzed complex, in which Cl atoms are replaced by H_2O molecules, contain positive charges which facilitate their outside binding with negative charges of DNA.

The UV studies reveal that the Pt complex causes “hypochromic effect” of DNA at different concentrations (r_i=0.0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.3, 1.5, 2.0; see Fig. 3). Hypochromism results from the contraction of DNA in the helix axis, as well as from the change in the DNA conformation, while hyperchromism results from the damage of DNA double-helix structure. As it is obvious from Fig. 3, $PtCl_2$(LL) can only cause DNA hypochromism but not hyperchromism, indicating that binding of the Pt complex with DNA occurs through its phosphate groups [11] which concurs with our outside binding findings.

This complex has a six-membered chelate ring that is conformationally similar to the corresponding cyclohexane derivative [12]. By introducing steric hindrance on the

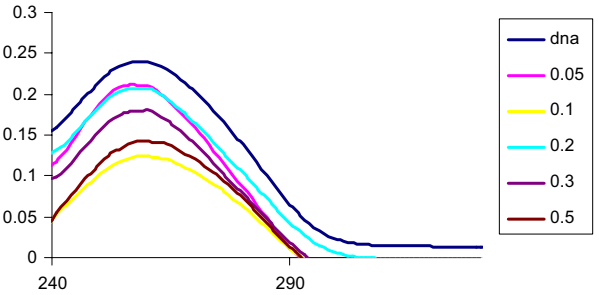

Fig. 2 Electronic absorption spectra for the titration of 4.33×10^{-5}M DNA with Pt complex at r_i values of 0, 0.05, 0.1, 0.2, 0.3, 0.5, pH 7, 10 mM HEPES

ethylenediamine-type ligands, the lability of the metal center can be tuned to control its reactivity in possible catalytic and biological applications [13].

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 offer information about the interaction strength of complexes with DNA. The stabilization of CT-DNA through the hydrogen bonding and electrostatic interactions of the noncovalent complexes was further assessed by measuring the melting temperature.

Our experiments were carried out for CT-DNA in the absence and presence of different amounts of Pt (II) complex. The melting plot of DNA (27 μg/mL) was monitored by plotting the UV maximum absorption of DNA at 260 nm vs. temperature at various binding ratios (r_i=0.0, 0.3, 0.7, 1.0, 1.5; Fig. 3). In the present case, melting temperature (T_m) of DNA in the absence of any added complexes has been found to be 78±0.5 °C. An increase in the DNA melting temperature by 3, 5, 7, 10, and 10.5 °C for the above-mentioned concentrations were observed. These values clearly show this complex is able to stabilize DNA helix [14, 15].

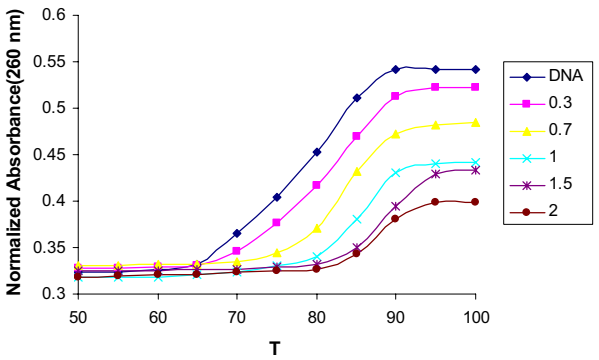

Fig. 3 Melting curves of CT-DNA (5×10^{-5}M) in the absence and in the presence of different amounts of Pt complex in HEPES buffer

Circular Dichroism

The CD technique is indeed very sensitive to detect minor conformational changes of the DNA conformation produced by ligand binding.

The observed CD spectrum of calf thymus DNA consists of a positive band at 275 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 [16]. The interaction of the mentioned complex with DNA induces a change in the CD spectrum of B DNA (Fig. 4). The intensities of both the negative and positive bands decrease significantly (shifting to zero levels). This suggests that the DNA binding of the complex induces certain conformational changes, such as the conversion from a more B-like to a more C-like structure within the DNA molecule [9]. The complex interaction effectively screens the negative charge on N (7) base sites as well as phosphate oxygen atoms simultaneously, both along the deoxyribophosphate backbone and in the groove of the helix to promote a transconformational change from a more B-like to a more C-like structure. Further transformation of DNA structure proceeds by removal of water from the base sites and the grooves of the helix [17]; also, the only one isodichroic point at 258 nm indicates the existence of a single binding mode [18]. Molar ellipticity values were calculated according to the formula [19]:

$$[\theta]_\lambda = \frac{100\theta_{(\lambda)}}{l \times C}$$

Where $[\theta]_\lambda$ is the molar ellipticity value at a particular wavelength expressed in deg cm^2 $dmol^{-1}$, C the concentration in moles of nucleotide phosphate per liter, l the length of the cell in dm, and θ_λ is the observed rotation in degrees.

Generally speaking, CD results are indicative of deep conformational changes of the DNA double helix following the interaction of the DNA macromolecule with metal complexes.

Viscosity Measurements

Spectroscopic data are necessary but not sufficient to support a binding mode. As a means for further clarifying the binding of this complex, viscosity measurements were carried out on DNA by varying the concentration of the added complex.

A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in

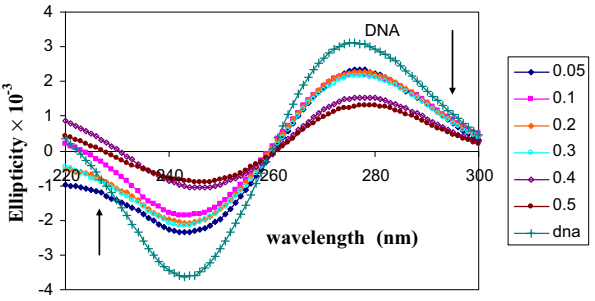

Fig. 4 Circular dichroism spectra of CT-DNA in the presence of increasing amounts of Pt complex at the following stoichmetric ratios: R = [Pt complex]/[DNA] = 0.0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5

overall DNA length. By contrast, complexes that bind exclusively in the DNA grooves by partial and/or nonclassical intercalation, under the same conditions, typically cause less positive or negative or no change in DNA solution viscosity [20].

Hydrodynamic measurements being sensitive to length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and most critical tests for a classical intercalation model in solution in the absence of crystallographic structural data. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity. In contrast, a partial, nonclassical intercalation of ligand could bend (or kink) the DNA helix, reducing its length and, concomitantly, its viscosity [3].

Relative viscosity curve is reported in Fig 5. The DNA intercalating binding mode is definitely excluded after viscosity measurements of 5×10^{-5}M DNA solutions in the presence of increasing amounts of the complex (r_i=0.1, 0.3, 0.5, 0.7, 1.3).

As it is observed from Fig. 5 in the presence of the platinum complex, viscosity of DNA exhibits a dependence on the concentration of complex. The results showed that the specific viscosity of DNA in HEPES buffer was increased. Under appropriate conditions, intercalation of classical compounds such as ethidium bromide (EthBr) causes a significant increase in the viscosity of DNA solutions due to increase in separation of base pairs at intercalation sites and hence an increase in the overall DNA contours length. Since the increase of viscosity is less than that observed for an intercalator like EthBr, interaction involving classical intercalation is ruled out for the present complex, and outside binding can be suggested as the binding mode. Liu et al. [21] have hypothesized that such viscosimetery results could be explained in terms of bridged structure to promote extension of duplexes. It is assumed that the complex exhibits the electrostatic binding and involves inner sphere complex formation via phosphate oxygen negative charge with the positive charge of the complex [9].

Fluorescence Studies (Competitive Binding Studies)

No luminescence is observed for the $PtCl_2LL$ complex; it is hard to monitor the interaction of this complex with DNA by employing direct fluorescence emission methods but actually possible by using a fluorescence assay of the organic molecule probe. Phenothiazinium

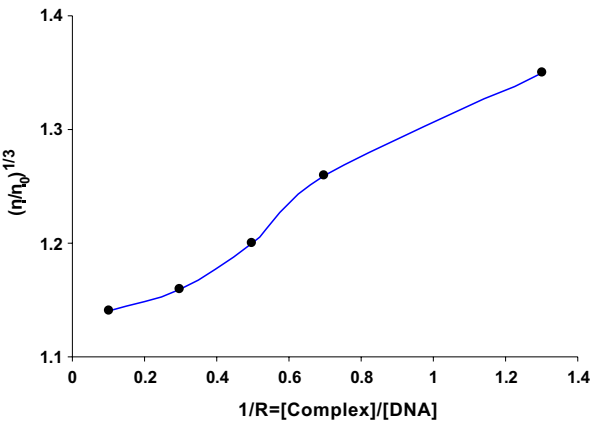

Fig. 5 Effect of increasing amounts of complex on the viscosity of CT-DNA (5×10^{-5}M) in 10 mM HEPES

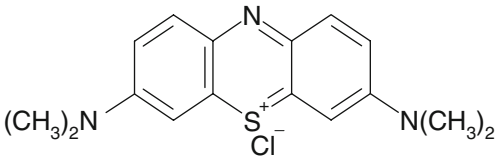

Fig. 6 The molecular structure diagram of methylene blue

dyes, such as methylene blue (MB, Fig. 6), that can interact with DNA by intercalation, have been testified with several spectroscopic methods [22–24]. In this contribution, we report the spectroscopic studies of the interactions between Pt (II) complex and DNA by using MB as a fluorescent probe. Upon binding to DNA, the fluorescence probe is efficiently quenched by the DNA bases with no apparent shifts in the emission maximum (Fig. 7). This emission-quenching phenomenon also reflects the changes in the excited-state electronic structure in consequence of the electronic interactions in the MB–DNA complexes [25]. The emission-quenching phenomenon, the hypochromic and red-shift effects in the absorption spectra, fit the intercalative binding mode of MB to DNA.

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

The increase of the 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 Pt (II) complex. That is, the formation of metal complex–DNA prevents the binding of MB.

The extent of MB releasing and following increase of the fluorescence intensity is applied to compare the strength of binding. Figure 7 shows that our complex could not release MB completely. Incomplete recovery of MB fluorescence is indicative of a non-intercalative mode of binding. This experiment confirms our previous evidences.

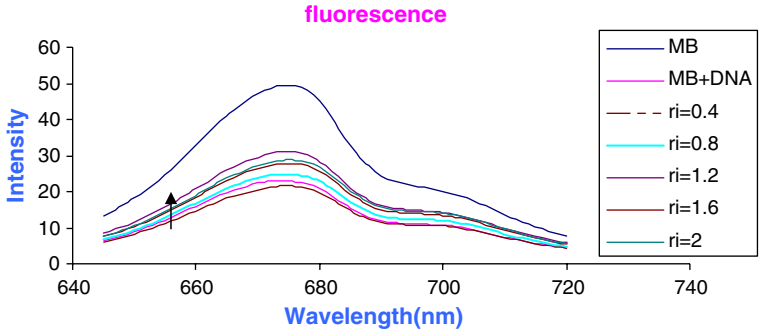

Fig. 7 Emission spectra of the MB-DNA complexes in the presence of the increasing Pt(II) complex concentrations in aqueous solution at room temperature

Conclusion

$PtCl_2(LL)$ (LL = chelating diamine ligand: *N*,*N*-dimethyltrimethylendiamine) strongly interacts with native DNA, presumably by outside binding mechanisms.

Hydrolyzed complexes, in which Cl atoms are replaced by H_2O molecules, contain positive charges which facilitate their outside binding with negative charges of DNA.

The interaction occurrence is supported by the following findings:

1. The UV hypochromism of the absorption bands confirms outside binding interaction with DNA.
2. The increase of the DNA melting temperature, of about 10 °C, which is less than classical intercalators when molar ratio of $[PtCl_2(LL)]/[DNA]$ is 1.5, is indicative of a non-intercalative mode of interaction.
3. The pronounced alterations of the DNA CD spectra in the presence of increasing amounts of the complex are indicative of deep conformational changes of the DNA double helix (B-DNA to C-DNA).
4. The viscosity increase is indicative of an outside binding mode of interaction.
5. The incomplete recovery of MB fluorescence can be considered as a non-intercalative mode of binding.

At the end, it should be stated that, albeit antitumor properties attributed to platinum (II) complexes have already been proved, several side effects due to currently used complexes, e.g., kidney failure in the case of cisplatin, prompted us to design a different platinum (II) complex and investigate its interaction with CT-DNA. It is hoped that this complex and suchlike are capable of paving the way for the development and designing of safer antitumor drugs.

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