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DNA-binding, spectroscopic and antimicrobial studies of palladium(II) complexes containing 2,2′-bipyridine and 1-phenylpiperazine

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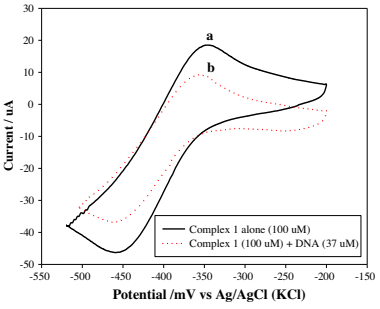
HIGHLIGHTS

- Two new complexes [Pd(bpy)(OH_2] (**1**) and [Pd(Phenpip)(OH_2)] (**2**) have been synthesized.
- The binding properties of the two complexes with CT-DNA were investigated.
- The intrinsic binding constants K_b have been calculated.
- The mode of binding is an electrostatic and/or groove one.
- Our complexes may act as model anticancer agents.

GRAPHICAL ABSTRACT

[Pd(bpy)(OH_2] (**1**) and [Pd(Phenpip)(OH_2) (**2**) where, (bpy = 2,2′-bipyridine; Phenpip = 1-phenylpiperazine) have been synthesized and characterized on the basis of elemental analysis. The interaction of these complexes with calf thymus DNA was extensively investigated by a variety of techniques, *viz.* spectroscopic, electrochemical, ethanol precipitation and gel electrophoresis. All studies showed that both complexes presumably intercalate in DNA by electrostatic and/or groove binding mode. The calculated binding strength (K_b) of the two complexes to CT-DNA was estimated to be of lower magnitude than that of the classical intercalator EB (Ethidium bromide) ($K_b = 1.23(\pm 0.07) \times 105\ M^{-1}$) supporting the electrostatic and/or groove binding mode. The antimicrobial tests showed that both complexes exhibited antimicrobial properties, and they were found to be more active against Gram-negative than Gram-positive bacteria.



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ABSTRACT

With the purpose of evaluating the ability of Pd(II) complex to interact with DNA molecule as the main biological target, two new complexes [Pd(bpy)(OH_2)$_2$] (**1**) and [Pd(Phenpip)(OH_2)$_2$] (**2**), where (bpy = 2,2′-bipyridine; Phenpip = 1-phenylpiperazine), have been synthesized and the binding properties of these complexes with CT-DNA were investigated. The intrinsic binding constants (K_b) calculated from UV–Vis absorption studies were $3.78 \times 10^3\ M^{-1}$ and $4.14 \times 10^3\ M^{-1}$ for complexes **1** and **2** respectively. Thermal denaturation has been systematically studied by spectrophotometric method and the calculated ΔT_m was nearly 5 °C for each complex. All the results suggest an electrostatic and/or groove binding mode for the interaction between the complexes and CT-DNA. The redox behavior of the two complexes in the absence and in the presence of calf thymus DNA has been investigated by cyclic voltammetry. The cyclic voltammogram exhibits one quasi-reversible redox wave. The change in $E_{1/2}$, ΔE_p and I_{pc}/I_{pa} supports that the two complexes exhibit strong binding to calf thymus DNA. Further insight into the binding of complexes with CT-DNA has been made by gel electrophoresis, where the binding of complexes is confirmed through decreasing the intensity of DNA bands. The two complexes have been screened for their antimicrobial activities using the disc diffusion method against some selected *Gram-positive* and *Gram-negative*

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bacteria. The activity data showed that both complexes were more active against *Gram-negative* than *Gram-positive bacteria*. It may be concluded that the antimicrobial activity of the compounds is related to cell wall structure of bacteria.

Introduction

The interaction and reaction of metal complexes with DNA has long been the subject of intense investigation in relation to the development of new reagents for biotechnology and medicine [1]. A number of metal complexes have been used as probes of DNA structure in solution, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents [2–4]. The landmark discovery of cisplatin by Rosenberg in 1965 heralded a new era of anticancer drug research based on metallo-pharmaceuticals [5]. To date, cisplatin and its analogs are some of the most effective chemotherapeutic agents in clinical use as the first line of treatment in testicular and ovarian cancers. Unfortunately, they have several major drawbacks. Common problems include cumulative toxicities of nephrotoxicity and ototoxicity [6–9]. In addition to the serious side effects, the therapeutic efficacy is also limited by inherent or treatment-induced resistant tumor cells. These drawbacks have provided the motivation for alternative chemotherapeutic strategies. Naturally, every therapeutic drug interacts with its bio-targets to remediate undesirable disease condition associated with various side effects and drug resistances. Enhancing the quality of life of patients is generally a prerequisite for proper chemotherapy. Therefore, there is particular interest in the search of anticancer agents that can minimize side effects and enhance the therapeutic effects. Mechanistic investigations of the mechanism of action of Pt(II) anticancer drugs, their Pd(II) analogues are suitable model compounds since they exhibit ca. 10^4–10^5 times higher reactivities, whereas their structural and equilibrium behavior are very similar [10].The similarity between the coordination chemistry of platinum(II) and palladium(II) compounds supports the theory that palladium complexes can act successfully as antitumor drugs. Several studies demonstrate that palladium derivates exhibit a noticeable cytotoxic activity, similarly to standard platinum-based drugs (e.g. cisplatin, carboplatin and oxaliplatin) used as reference, and show fewer side effects relative to other heavy metal anticancer compounds [11]. Moreover, palladium(II) complexes can serve as good models for the understanding of more inert platinum(II) anti-cancer drugs [12]. Also, it has been suggested by Saddler et al. [13] that the palladium complexes may be useful for the treatment of tumors of the gastrointestinal region where cisplatin fails.

In this paper we have synthesized two model Pd(II) amine complexes, $[Pd(bpy)(OH_2)_2]$ (1) and $[Pd(phenpip)(OH_2)_2]$ (2), where bpy: is 2,2'-bipyridine and phenpip: is 1-phenylpiperazine. In general, the pyridyl compounds operate as bidentate chelating ligands for metal binding. The UV–Vis absorption properties of this class of bipyridyl metal complexes have been utilized to monitor the interaction processes with DNA. In addition, the molecular nature of bipyridyl metal complex provides a square-planar geometry to intercalate with DNA. These evidences suggest that DNA does represent the potent target for many bipyridyl metal complexes. Recent progresses have emphasized the antiproliferative effects and molecular mechanisms from diverse metals, gold(III) [14,15], copper(II/I) [16,17], palladium(II) [18,19], rhodium(III) [20], ruthenium(II) [21,22], zinc(II) [23], and platinum(II) [24,25] with bipyridyl ligands.

The study of Phenyl piperazine complexes were performed because (1) piperazine and many of its derivatives are notable succesfull drugs, piperazine has O_6–N···H [26] and/or phosphate-N···H intramolecular hydrogen bonding [23,24] with the Pt-DNA adduct, favoring interaction with DNA and (2) the piperazine ring may undergo stacking interactions with the sugar group of DNA, again favoring interaction with DNA. The latter effect is similar to that reported for carboplatin, where the stacking interaction between the cyclobutane ring and the sugar group is part of the increased antitumor activity [27]. Also, piperazine is an important pharmic intermediate, its rings are capable of resting in the minor groove of GC base pairs [28,29].

In continuation of our previous work on equilibria of complex formation reactions of cis-(diamine) palladium(II) complexes with DNA, the major target in chemotherapy of tumors [30], and amino acids, peptides, dicarboxylic acids and esters [31,32], in this project we study the mode of binding of the two complexes **1** and **2** with Calf thymus DNA. Their interaction studies are attempted by UV–Vis spectroscopy, electrochemical technique and gel electrophoresis as well as ethanol precipitation. The two complexes have been screened for their antimicrobial activities against some selected *Gram-positive* and *Gram-negative* bacteria.

Experimental

Materials and reagents

Palladium(II) chloride was purchased from Acros organics. The ligands 2,2′-bipyridine and 1-phenylpiperazine were from Aldrich and Fluka. Calf thymus DNA was provided from Sigma. All solvents used were of the analar grade. All DNA-binding experiments were carried out in Tris–HCl buffer solution (50 mM NaCl, 5 mM Tris–HCl, pH 7.1). Tris–HCl buffer was prepared using deionized triple distilled water. Solutions of CT-DNA in buffer gave a ratio of UV–Vis absorbance of 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [33]. The concentration of DNA was determined spectrophotometrically (ε_{260} = 6600 M^{-1} cm^{-1}) [34]. Stock solution of DNA was stored at −20 °C. Concentrated stock solution of the palladium complexes (1.0×10^{-3} M) was prepared by dissolving an appropriate amount of the complex into 50 ml of deionized doubly distilled water and diluted suitably with Tris–HCl buffer to the required concentrations for all the experiments.

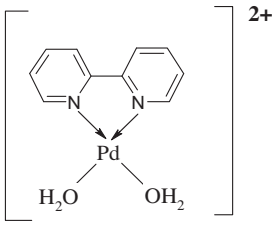
Apparatus and measuring techniques

The microchemical analysis of the separated solid complexes for C, H and N was performed in the microanalytical center, Cairo University. The analyses were performed twice to check the accuracy of the analytical data. Absorption titration experiments were made using TB-85-thermobath Shimadzu model UV spectrophotometer. Cyclic voltammetric measurements were made on an EG&G PAR model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 273 using a three electrode set-up comprising a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE).

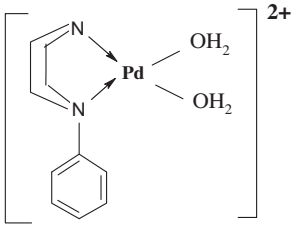
Synthesis of the complexes

$[Pd(bpy)Cl_2]$ and $[Pd(phenpip)Cl_2]$ were prepared by heating $PdCl_2$ (0.177 g, 1.0 mM) in 40 ml H_2O and KCl (0.149 g, 2.0 mM) in 10 mL water to 70 °C with stirring. The clear solution of

$[PdCl_4]^{2-}$ was filtered and the ligands (0.156 g, 1.0 mM) of 2,2′-bipyridine and (0.162 g, 1.0 mM) of 1-phenyl piperazine were dissolved in 10 ml H_2O and added dropwise to the stirred $[PdCl_4]^{2-}$ solution. The pH value was adjusted to 2–3 by the addition of HCl and/or NaOH. A yellowish-brown precipitate of $[Pd(bpy)Cl_2]$ or $[Pd(phenpip)Cl_2]$ is formed. The precipitates were stirred for an additional 30 min at 50 °C. After filtering off the precipitates, they were thoroughly washed with H_2O, ethanol, and diethyl ether. Yellow powders were obtained. For the $[Pd(bpy)Cl_2]$, $C_{10}H_8N_2PdCl_2$: Calc. (%): C, 36.0; H, 2.40; N, 8.40. Found: C, 35.78; H, 2.02; N, 8.1% and for $[Pd(phenpip)Cl_2]$, $C_{10}H_{14}N_2PdCl_2$: Calc.: C, 35.3; H, 4.12; N, 8.2%. Found: C, 35.1; H, 4.02; N, 8.1%. Aqueous solutions of the diaqua analogues of $[Pd(bpy)Cl_2]$ or $[Pd(phenpip)Cl_2]$ complexes were prepared *in situ* by the addition of slightly less than two mole equivalents of $AgNO_3$ to a solution of a known amount of the dichloro complexes and stirred over night. The white precipitate of AgCl that formed was filtered off using a 0.1 lm pore membrane filter. Great care was taken to ensure that the resulting solution was free of Ag^+ ion and that the dichloro complexes have been converted completely into the diaqua species.



Complex 1



Complex 2

Absorption titration

Absorption titration experiments were carried out by varying the DNA concentration in the range of (0, 29.2, 43.6, 59.36, 77.28, 89.8, 96 and 128.8 μM) and maintaining the complex concentration constant at (20 μM). Upon measuring the absorption spectra, equal amount of DNA was added to both the palladium complex solution and the reference solution to eliminate the absorbance of DNA itself. The reference solution was the corresponding buffer solution. Absorbance values were recorded after each successive addition of DNA solution and equilibration (ca. 10 min). The sample solution was scanned in the range of 200–400 nm. Each sample was measured three times and an average value was calculated. The absorption data were analyzed for an evaluation of the intrinsic binding constant, K_b, of the complexes with CT-DNA.

Thermal denaturation experiments were carried out by monitoring the change in the absorption of CT-DNA at 260 nm at various temperatures to evaluate the melting temperature (T_m). Whereas, T_m is defined as the temperature at which 50% of double stranded DNA becomes single stranded. Tm was measured in absence and in presence of the complexes in *Tris*–HCl buffer pH 7.1 containing a mixture of 60 μM CT-DNA and 20 μM of the complex. The mixture was stirred continuously and the temperature was elevated gradually from 30 to 90 °C with a reading of absorbance taken every 5 °C. All experiments were made using TB-85-thermobath Shimadzu model UV spectrophotometer equipped with cell-temperature controller. The denaturation temperature (T_m) was taken as the midpoint of the hyperchromic transition. All measurements of T_m were repeated three times and the data presented are the average values.

Ethanol precipitation

Palladium(II) complexes (175 μM of $[Pd(bpy)(OH_2)_2]$ and 247 μM of $[Pd(phenpip)(OH_2)_2]$ were interacted with DNA (50 μm) in 5–10 mL of the above-mentioned Tris–HCl buffer of pH 7.1. To this interacted solution, an excess of absolute ethanol was added. The precipitated DNA was separated and washed with alcohol. This precipitate was redissolved in the same buffer and the solution was monitored spectrophotometrically for DNA at 258 nm and Pd(II) complexes at their λ_{max} (nm).

DNA gel electrophoresis

The binding of palladium complexes with CT-DNA was monitored using agarose gel electrophoresis. The reaction mixture containing (16 μM) CT-DNA and different concentrations of complexes (5, 10, 15, 20 and 25 μM) in tris-buffer solution was incubated for 90 min at 37 °C followed by the addition of (3 μl) loading buffer containing (bromophenol blue 0.25%, SDS 20%, 0.5 M EDTA (PH:8) and glycerol 30%). Electrophoresis was performed at (50 V) for 2 h. in TBE buffer (10.8% tris, (Tris (hydroxymethyl) aminomethane), 5.5% boric acid, (0.5 M) EDTA (pH 8)) using 0.8% agarose gel containing (2 μl) 1.0 mg/ml ethidium bromide. Bands were visualized using UV light and photographed. The binding ability was observed from the intensity of bands.

Cyclic voltammetric measurements

All the electrochemical measurements were carried out at room temperature in a 15 ml electrolytic cell by using (5 mM Tris–HCl/50 mM NaCl buffer (pH 7.1) as the supporting electrolyte. The GCE surface was freshly polished to a mirror prior to each experiment with 0.05 mm a-Al_2O_3-paste and then cleaned in water. The working electrode was cleaned after every electrochemical assay. The voltammogram of 15 ml of the solution of the complexes, [complex] = 100 μM, was recorded in absence of DNA. The procedure was then repeated for systems of 15 ml of a mixture containing constant concentration of the complexes ([complex] = 100 μM) and varying the concentration of DNA. For complex **1**, 100 μM of the complex was mixed with ([DNA] = 37, 80 and 125 μM) in the molar ratios (1:0.4, 1:0.8 and 1:1.25) respectively. For Complex **2** the concentration of the DNA used was ([DNA] = 100 and 150 μM); in the molar ratios (1:1 and 1:1.5).

Antimicrobial activity

The Pd(II) diaqua complexes **1** and **2** were evaluated for their antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (as Gram-positive bacteria) and *Escherichia coli* and *Neisseria gonorrhoeae* (as Gram-negative bacteria) using the disc diffusion technique [35] as described in British Pharmacopoeia (2000). Paper discs of Whatman filter paper (No. 42) of uniform diameter (2 cm) were sterilized in an autoclave. The paper discs, soaked in the desired concentration of the complex solutions, were placed aseptically in the petri dishes containing nutrient agar media (agar 15 g + beef extract 3 g + peptone 5 g) seeded with *S. aureus* and *B. subtilis* bacteria separately. The petri dishes were incubated at 37 °C and the inhibition zones were recorded after 24 h of incubation. The antibacterial activities are calculated as a mean of three replicates. The antibacterial activity of a common standard antibiotic Tetracycline was also recorded using the same protocol as above and at the same concentration and solvent. The antibacterial results of the compounds were compared with the standard and the% activity index for the complexes was calculated by using the formula:

$$\%\,\text{Activity index} = \frac{\text{Zone of inhibition by test compound(diameter)}}{\text{Zone of inhibition by standard(diameter)}} \times 100$$

Results and discussion

DNA-binding studies

Absorbance titration experiments

One of the most common techniques in DNA-binding studies of metal complexes is electronic absorption spectroscopy. The magnitude of spectral perturbation is an evidence for DNA-binding [36]. The absorption titrations were carried out by using a fixed amount of each metal complex (20 μM) with increasing concentrations of DNA in the range from (0–160 μM). The reference solution was the corresponding buffer solution. While measuring the absorption spectra, equal amount of DNA was added to both the compound solution and the reference solution to eliminate the absorbance of DNA itself. Each sample solution was scanned in the range of 200–400 nm. The absorption spectra of the title complexes with increasing concentrations of CT-DNA are given in Figs. 1 and 2 respectively. On increasing the concentration of CT-DNA, the absorption bands of the complexes at 253 nm for complex **1** and at 251 nm for complex **2** were affected, resulting in hyperchromicity and slight red shift. The absorption intensity is increased due to the fact that the purine and pyrimidine DNA-bases are exposed because of binding of the complexes to DNA.

Metal complexes may bind to DNA with several different binding modes, namely intercalation or non intercalation, such as groove binding and binding to the phosphate group [37]. In the present study it is assumed that the positively charged diaqua complexes **1** and **2** would electrostatically interact with the negatively charged phosphate backbone at the periphery of the double helix CT-DNA. This electrostatic binding mode of the two complexes to CT-DNA is also evidenced by the strong hyperchromism obtained for both complexes suggesting tight binding to CT-DNA and stabilization. The observed strong hyperchromism is most pronounced in the π–π^* transition, indicating that the complexes are actively in associating with the DNA. This agrees with the previous study that mentioned that piperazine rings and bipyridine rings are capable of resting in the minor groove of GC base pairs [28,29,38]. As far as the pd(II) is concerned, it is likely that the ligands facilitate the formation of the van der Waal's contacts within the walls of groove or hydrogen bonds in DNA grooves when interact with DNA in Tris–HCl buffer.

In order to further illustrate the binding strength of the palladium(II) complexes with CT-DNA, the intrinsic binding constant K_b was determined from the spectral titration data. It can be calculated by monitoring the changes in absorbance at the corresponding λ_{max} with increasing concentrations of CT-DNA, using the following Eq. (1) [39]:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad (1)$$

Where [DNA] is the concentration of DNA in base pairs, ε_f, ε_a, and ε_b correspond to the extinction coefficient, respectively, for the free palladium diaqua complexes (II), for each addition of DNA to the palladium(II) complex and for the palladium(II) complex in fully bound form.

A plot of $\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)}$ versus [DNA] gives K_b, insets in Figs. 1 and 2, as the ratio of the slope to the intercept. From the $\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)}$ versus [DNA] plots, the intrinsic binding constant K_b for complex **1** was $3.78 \times 10^3\ M^{-1}$ ($R^2 = 0.98$ for eight .points) and that for complex **2** was $4.15 \times 10^3\ M^{-1}$ ($R^2 = 0.99$ for eight points). The higher K_b value obtained for complex **2** may plausibly be due to more base pairs available for binding than in complex **1.** The calculated K_b values for complexes **1** and **2** are of lower magnitude than that of the classical intercalator EB (Ethidium bromide) ($K_b = 1.23\ (\pm 0.07) \times 10^5\ M^{-1}$) [40] and both reveal a strong binding to CT-DNA.

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.

Previous studies have revealed that when a cationic species interact with double helix the stability increases and so does the DNA melting temperature. In general, groove binding or electrostatic binding along the phosphate backbone of DNA gives rise to

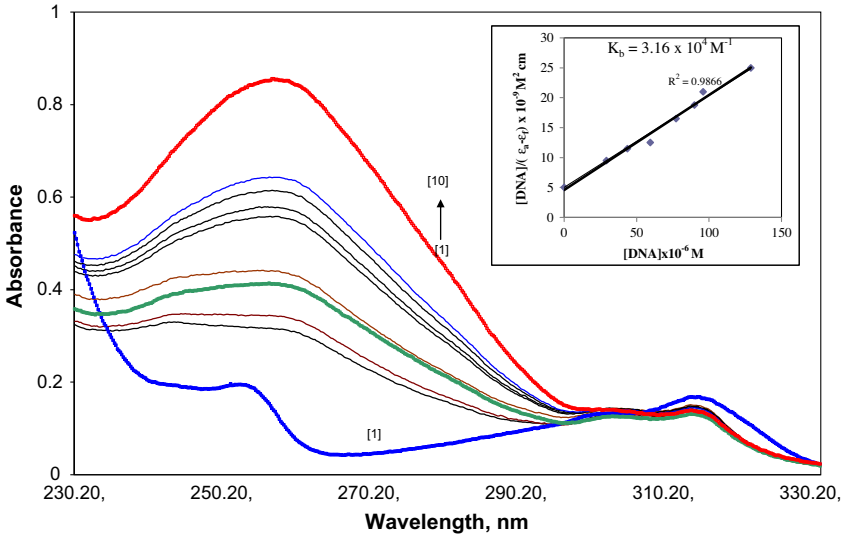


Fig. 1. Absorption spectra of [Pd(bpy)(OH2)2](1) in Tris–HCl buffer upon addition of CT-DNA. [complex] = 20 μM, [DNA] = (0) [1], (43.6) [2],(45.6) [3],(59.3) [4],(60.3)[5],(79.8) [6], (82.9) [7],(89.8) [8], (96) [9],(128.9) [10] μM. Arrow shows the absorbance changing upon the increase of DNA concentration. Inst: Pot of [DNA]/(ε_a–ε_f) versus [DNA] for the titration of CT-DNA with the complex.

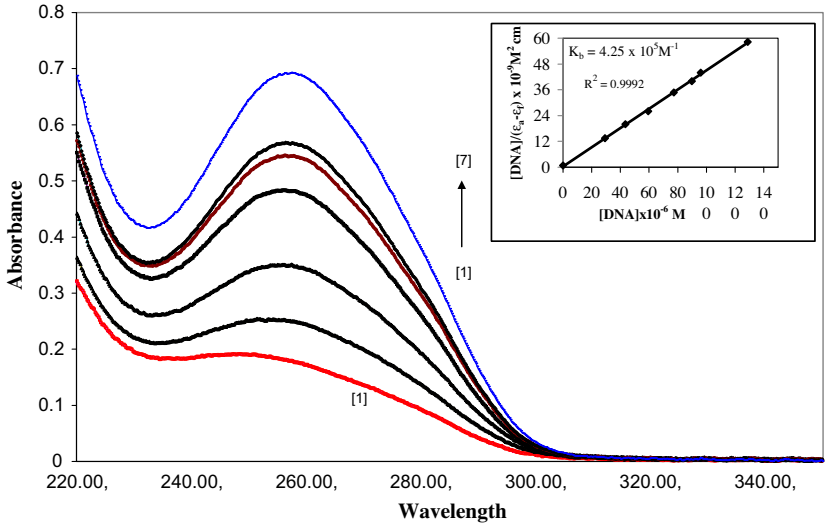


Fig. 2. Absorption spectra of [Pd(phenpip)(OH2)2]2+(2) in Tris–HCl buffer upon addition of CT-DNA. [complex] = 20 μM, [DNA] = (0) [1], (29.2) [2],(43.6) [3],(59.3) [4],(59.3) [5],(77.8) [6], 160 [7] μM. Arrow shows the absorbance changing upon the increase of DNA concentration. Inst: plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] for the titration of CT-DNA with the complex.

only a small change in thermal denaturation temperature, while intercalation binding mode leads to a significant rise in thermal denaturation temperature of DNA due to the stabilization of the Watson–Crick base paired duplex [41,42]. The DNA melting studies for the present complexes show a moderate positive shift in the melting temperature (ΔT_m) of nearly 5 °C for each complex, Fig. 3. Here $\Delta T_m = T_m - T_m^0$, where, T_m and T_m^0 refer to the melting temperature of DNA in presence and absence of complexes respectively. The extent of ΔT_m is in the interface between the value induced by electrostatic and intercalative binding and it is relatively lower as compared to those observed for common organic intercalators such as ethidium (13 °C) [43] and some derivatives of porphyrins (≈15 °C) [44–46]. This indicates primarily electrostatic and/or groove binding nature of the complexes in preference to an intercalative mode of binding to DNA [47], which is in accordance with the results obtained from the absorption titration experiments and from the calculation of K_b.

Cyclic voltammetry

Cyclic voltammetry is one of the most important electroanalytical techniques employed for interaction of metal complexes with biomolecules due to the similarity between various redox chemical and biological processes [48]. It is extremely useful in probing the nature and mode of DNA binding of metal complexes and it provides a useful complement to the above method of investigation such as UV spectra [49]. The redox behavior of the two studied complexes **1** and **2** in absence and presence of CT-DNA was studied at room temperature within potential range of −550 to −150 mV for complex **1** and −400 to 400 mV for complex 2 at a scan rate of 200 mVs^{-1}. The results indicate that, the two complexes are redox active and show quasi-reversible cyclic voltammetric response. The cyclic voltammogram of complex **1** exhibits a quasi-reversible redox wave with E_{pc} and E_{pa} values of −344 and −454 mV, respectively. The ratio of cathodic to anodic peak currents I_{pc}/I_{pa} was 0.413. The formal electrode potentials $E_{1/2}$, ΔE_p (difference in cathodic E_{pc} and anodic E_{pa} peak potentials) were −399.4 and 109.3 mV respectively. At constant temperature, the addition of CT-DNA resulted in the shift in $E_{1/2}$ = −407 mV and ΔE_p = 106 mV, Fig. 4, respectively. The ratio of I_{pc}/I_{pa} is 0.25 for CT-DNA bound metal complex **1**. The shift in potentials and decrease in current ratio suggest the binding of complex **1** to CT-DNA [50]. The cyclic voltammogram of complex **2** at scan rate 200 mVs^{-1} features a quasi-reversible redox wave with $E_{1/2}$, ΔE_p

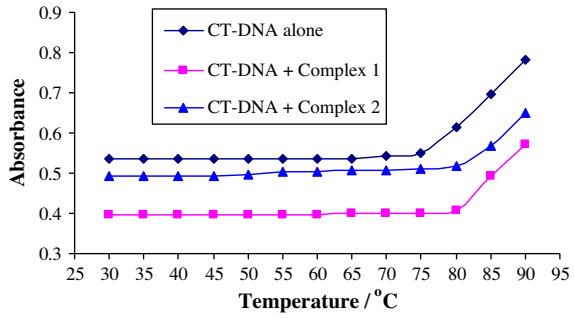


Fig. 3. Melting curves of CT-DNA in Tris–HCl buffer in the absence and presence of complexes, [DNA] = 60 uM, [complex] = 20 uM, showing an increase in Tm due to binding.

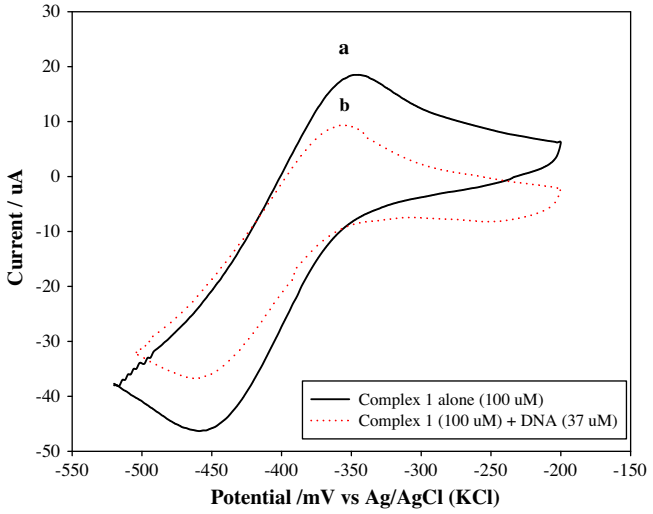


Fig. 4. Cyclic voltammograms of complex (1) in Tris–HCl buffer in the absence (a) and presence (b) of CT-DNA. v = 200 mV s^{-1}, [complex] = 100 μM; [DNA]: (a) 0, (b) 37 μM.

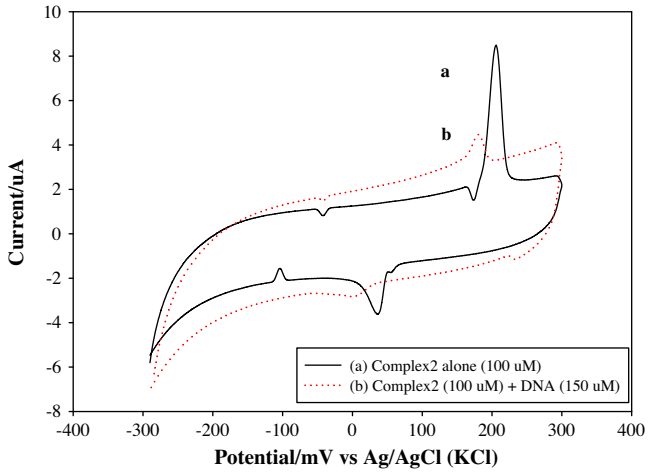


Fig. 5. Cyclic voltammograms of complex (2) in Tris–HCl buffer in the absence (a) and presence (b) of CT-DNA. ν = 200 mV s^{-1}, [complex] = 100 μM; [DNA]: (a) 0, (b) 150 μM.

and I_{pc}/I_{pa} values of 122 mV, 168 mV and 0.43 respectively. Upon addition of CT-DNA under the same recording conditions complex **2** experience a shift in $E_{1/2}$ (103 mV), ΔE_p (182 mV) and ratio of anodic to cathodic peak currents I_{pa}/I_{pc} is 0.38 (Fig. 5).

The mode of the drug vs DNA interaction can be judged from the variation in formal potential. In general, it was reported that the positive shift (anodic shift) in formal potential is caused by the intercalation of the cationic drug into the double helical structure of DNA [51], while negative shift is observed for the electrostatic interaction of the cationic drug with the anionic phosphate of DNA backbone [52,53]. So the obvious negative peak potential shift (cathodic shift) in the CV behavior of both complexes **1** and **2** by the addition of DNA supports our previous assumption for an electrostatic interaction of the positively charged diaqua complexes with the polyanionic DNA. The effect of concentration of CT-DNA on potential and current peaks of the complexes **1** and **2** was also studied using a constant concentration of the complex (100 μM) and varying the concentration of DNA. With complex **1**: ([DNA] = 37, 80 and 125 μM) and with complex **2**: ([DNA] = 100 and 150 μM), Figs. 6 and 7. The results showed that the incremental addition of CT-DNA to both complexes causes a diminution of

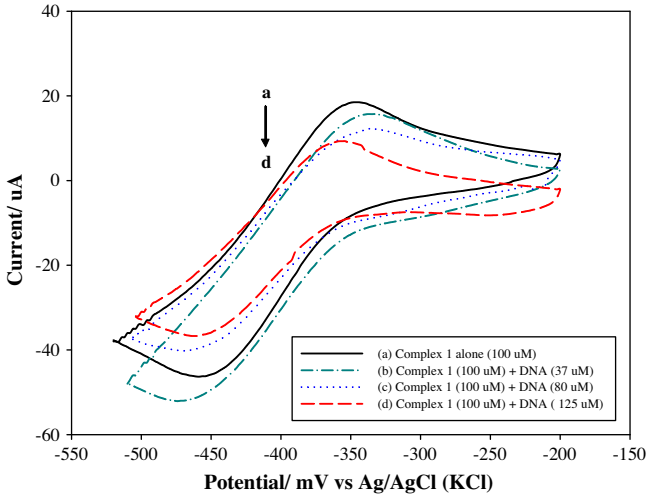


Fig. 6. Cyclic voltammograms of 100 μM complex (1) in Tris–HCl buffer in the absence (a) and presence of 37 μM DNA (b), 80 μM DNA (c) and 125 μM DNA (d). ν = 200 mV s^{-1}.

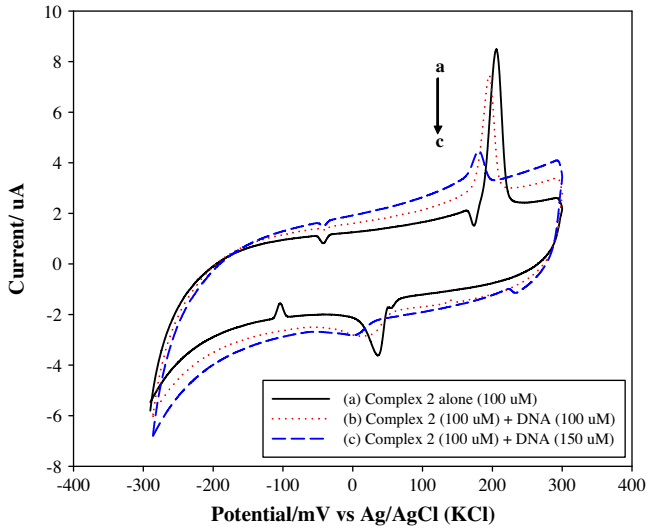


Fig. 7. Cyclic voltammograms of 100 μM complex (2) in Tris–HCl buffer in the absence (a) and presence of 100 μM DNA (b) and 150 μM DNA (c). ν = 200 mVs^{-1}.

the peak currents due to variation of the binding state and slowing the mass transfer of the complex after binding to CT-DNA fragments as well as a shift in the $E_{1/2}$. The decrease of the anodic and cathodic peak currents of the complexes in presence of DNA is due to formation of slowly diffusing complex-DNA supramolecular complex, which in turn lowers the concentration of the free complex (mainly responsible for the transfer of current).

The shift in the value of the formal potential (ΔE^0) can be used to estimate the ration of equilibrium binding constants (K_R/K_0) according to the model of interaction described by Bard and Carter [54]. From this model one can obtain that:

$$\Delta E^0 = E_b^0 - E_f^0 = 59.15 \log(K_R/K_O) \quad (2)$$

Where E_b^0 and E_f^0 are the formal potentials of the bound and free complex forms, K_R and K_0 are the corresponding binding constants for the binding of reduction and oxidation species to CT-DNA, respectively. The K_R/K_O values for complex **1** and **2** are 0.9 and 0.48 respectively, suggesting the stronger binding affinity of the reduced form of complex **1** compared to the oxidized form. The lower value obtained for complex **2** (0.48) suggests that complex **2** is strongly bounded to CT-DNA in its oxidized form. This is further confirmed by the higher cathodic peak potential shift observed for complex **2**, which indicates that the reduced state of complex **2** is easier to oxidize in presence of DNA because its oxidized form is more strongly bound to DNA than its reduced form. Complex **1** shows only small cathodic peak shift indicating that the more strongly bound species is the reduced form.

Ethanol precipitation

The binding between DNA and metal complexes was also studied by precipitating the DNA from the interacted DNA–metal complexes with absolute ethanol. If the interaction between DNA and metal complex is very weak, the DNA should be precipitated separately and all free metal complexes will remain in the supernatant [55]. In this experiment, the precipitated DNA was separated out and washed several times with alcohol. This precipitate was re-dissolved in Tris–HCl buffer, and the solution was monitored spectrophotometrically for DNA at 258 nm, for complex **1** at 253 nm and for complex **2** at 251 nm. The spectral data indicated that the band corresponding to free DNA was not detected at 258 nm, and the bands observed for complexes **1** and **2** are shifted from their previously recorded values at 253 and 251 respectively. This supports

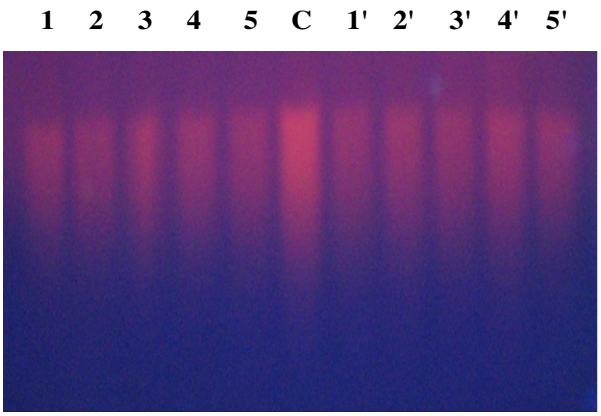


Fig. 8. Agarose gel electrophoresis of CT-DNA samples showing the interaction between the two complexes 1 and 2 and CT-DNA in TBE buffer. Lane C: untreated CT-DNA. Lanes 1–5: 16 μM CT-DNA + (5, 10, 15, 20 and 25 μM) complex 1 respectively. Lanes 1′–5′: 16 μM CT-DNA + (5, 10, 15, 20 and 25 μM) complex 2 respectively.

the strong binding ability of both complexes to CT-DNA, and the inability of ethanol to separate between them. Similar results were obtained for another series of Pt(II) and Pd(II) complexes of 2,2′-bipyridine and amino acids [56] .

Gel electrophoresis

The ability of binding of the synthesized complexes to CT-DNA was investigated by gel electrophoresis through examination of the effect of different concentrations of the Pd(II) complexes on CT- DNA. The results are presented in Fig. 8, it is clear that the intensity of the bands recorded for both complexes after binding to CT-DNA were decreased, as compared to the band of the free CT-DNA. The decrease in the intensity of bands observed after binding of the complexes to CT-DNA is believed to be due to degradation of the DNA double helix and /or quenching of fluorescence from DNA-intercalated ethidium bromide. Previous work hypothesized that DNA degradation may rather have occurred via backbone cleavage due to a nucleophilic attack of basic residues [57]. It was reported that the intensity of bands in gel electrophoresis visualized by the fluorescence of ethidium bromide intercalate to DNA base pairs depends not only on the number of molecules, but also on DNA length [58]. Previous report mentioned that the fluorescent light could be quenched by the addition of a second molecule [59]. Therefore, the reduction in the intensity of electrophoretic bands of CT-DNA upon interaction with Pd(II) complexes could be due to masking of complex upon interaction between the stacked bases within the helix and/or surface binding at the reactive nucleophilic sites on DNA double helix.

Antimicrobial activity

Pd(II) amine complexes are well known to have enhanced antitumor activity. Therefore it seems interesting to screen the biological potential of the synthesized diaqua complexes in vivo against different species of bacteria as well. In testing the antimicrobial activity of these compounds, we used more than one test organism to increase the chance of detecting antibiotic principles in tested materials. The tested complexes show a remarkable biological activity against Gram-positive (G^+) and Gram-negative (G^-) bacteria. The in vitro antimicrobial activity of the complexes was tested against *B. subtilis* and *S. aureus* (as Gram-positive bacteria), *E. coli* and *N. gonorrhoeae* (as Gram-negative bacteria) and then compared with the Standard tetracycline antibacterial agent. The results are listed in Table 1. The results obtained show the following:

1- [Pd(phenpip)$(OH_2)_2$] was found to have higher reactivity against the different strains of bacteria more than [Pd(bpy)$(OH_2)_2$] which was found to be moderately active against them.
2- [Pd(phenpip)$(OH_2)_2$] exhibits relatively higher reactivity against Gram-negative bacteria than Gram-positive ones.

It was reported that Gram-negative bacteria have a thin cell wall consisting of few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. Whereas, *Gram*-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only *Gram*-positive bacteria and are ineffective against *Gram*-negative pathogens [60]. Therefore the interest in these results is that the two studied complexes **1** and **2** were found to be effective against both *Gram-negative* and *Gram-positive* bacteria, in addition increasing interests are now directed to the line of antibiotics affecting Gram-negative bacteria, since there are certain organisms which have proved difficult to treat and most of them are Gram negative rods [61–63]. It may be concluded that the antibacterial activity of the compounds is related to cell wall structure of the bacteria. This is possible because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan [64].

Conclusion

The study of the interaction of Pd(II) amine complexes with CT-DNA is of special interest for pharmaceutical and biomedical research. The present study describes the interaction of two Pd(II) complexes; $[Pd(bpy)(OH_2)_2]^{2+}$ (**1**) and $[Pd(phenpip)(OH_2)_2]^{2+}$ (**2**) with CT-DNA. The introduction of heteroaromatic nitrogen base such as bipyridine and /or phenylpiperazine rings into the Pd(II) coordination sphere results in decreasing the electron density on the metal center, making it more electrophilic. As a consequence, the acidity of the coordinated water molecule in the diaqua complexes would increase and this would favor the binding with CT-DNA. The binding of two complexes with CT-DNA has been studied by UV–Vis spectroscopy and electrochemical techniques, revealing their strong binding ability. The calculated binding strength (K_b) of

Table 1
Antimicrobial and antifungal activities of [Pd(bpy)(OH_2)] and [Pd(phenpip)(OH_2)].

Microorganism	Gram reaction	Inhibition zone diameter (mm/mg sample)		
		[Pd(bpy)(OH_2)]	[Pd(phenpip)(OH_2)]	Standard
Bacillus subtilis	(G^+)	15	23	28
Staphylococcus aureus	(G^+)	14	21	29
Streptococcus faecalis	(G^+)	13	18	31
Escherichia coli	(G^-)	13	19	30
Neisseria gonorrhoeae	(G^-)	14	25	31
Seudomonas aeruginosa	(G^-)	14	25	31

the two complexes to CT-DNA suggests an electrostatic and/or groove binding mode. The relatively higher K_b value observed for complex (**2**) could be attributed to the possibility of piperazine rings for resting in the minor groove of GC base pairs as reported previously [28,29], an aspect that could be of significance for the actual binding ability of this complex with DNA.

The small change in thermal denaturation temperature of DNA (ΔT_m) after binding of both complexes also supports the electrostatic and/or groove binding mode. Redox couple of the two complexes (**1**) and (**2**) was assigned as quasi-reversible from their cyclic voltammetric data. The obvious negative peak potential shift observed by the addition of DNA also supports the electrostatic interaction of the positively charged complexes with the polyanionic DNA. The results obtained from gel electrophoresis also supported the binding of our complexes with CT-DNA. The antimicrobial tests showed that both complexes exhibited antimicrobial properties, and they were found to be more active against Gram-negative than Gram-positive bacteria.

We concluded that the two complexes exhibits strong binding ability to DNA which points to the promising properties of the Pd(II) amine complexes as model anticancer agents .

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