A Thermodynamic Study of New Designed Complex of Ethylendiamine 8-Hydroxyquinolinato Palladium(II) Chloride with Calf Thymus DNA

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A Thermodynamic study on the interaction of bovine calf thymus DNA with new designed Pd(II) complex (Ethylendiamine-8-hydroxyquinolinato Palladium(II) chloride) was studied by using isothermal titration calorimetry (ITC) at 27 °C in Tris buffer solution at pH = 7.5. The enthalpies of Pd(II) complex + DNA interaction are reported and analysed in terms of the new solvation theory. It was indicated that there are three identical and non-cooperative sites for Pd(II) complex. The binding of a Pd(II) complex is endothermic with association equilibrium constants of 428.03 mM^{-1} at 27 °C. The binding of Pd(II) complex can cause some changes in the stability of the DNA at low and high Pd(II) complex concentrations. Our results suggested that this complex might interact with DNA as an intercalator, thus interfering with DNA replication and cell proliferation.

Key Words: DNA, Pd(II) complex, Isothermal titration calorimetry

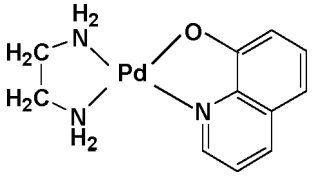
Introduction

DNA plays an important role in the life process, because it bears heritage information and instructs the biological synthesis of proteins and enzymes through the replication and transcription of genetic information in living cells.[1] The main target of anti-tumor chemotherapies is DNA.[1,2] Alteration of DNA structure affects its synthesis and function which usually leads to disruption of cell proliferation and can eventually elicit cell death *via* apoptosis. These effects are currently being exploited to develop novel biologically active drugs with potential applications as anti-proliferative therapies, e.g. ligands that will form ternary complexes with DNA and the enzyme (s) topoisomerase.[3-5] Several experiments have also demonstrated that DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death.[4-6] In addition, the apoptosis can also cause the cell death.[7] The ability of small molecules to interfere with transcription and DNA replication makes it a major target for drug interaction studies.[7]

Transition metal complexes have attracted considerable attention as catalytic systems for use in the oxidation of organic compounds,[8] probes in electron-transfer reactions involving metallo-proteins, and intercalators with DNA.[3,9] During recent years, the interest for metal complexes containing planar extended polyaromatic ligands has increased tremendously, mainly for their usage as probes capable to utilize the nucleic acid structures[1,2] and as DNA-molecular light switches.[10] There are several types of sites in the DNA molecule where binding of metal complexes can occur: (I) between two base pairs (intercalation), (II) in the minor groove, (III) in the major groove, and (IV) on the outside of the helix.[11]

Among these complexes, the role of the Pt(II) and Pd(II) complexes is crucial since they can bind to DNA covalently as well as noncovalently. Several reports have shown that palladium complexes are expected to have lower kidney toxicity than cisplatin due to the inability of proteins in the kidney tubules to replace the tightly bound chelate ligands of Pd(II) with sulfydril groups.[12-15] Concerning the noncovalent interactions between transition-metal complexes and DNA, they can occur by intercalation, groove binding, or external electrostatic binding.[10-11] Many anticancer drugs are known to interact with DNA to exert their biological activities. Generally, DNA-acting anticancer drugs can be classified into three categories. Drugs of the first category form covalent linkages with DNA while drugs of the second category form noncovalent complexes with DNA by either intercalation or groove-binding. Drugs of the final category cause DNA backbone cleavages.[8]

A search for new, low-molecular weight ligands which can specifically bind to DNA has been conducted for many years in the hope that new therapeutics could selectively modulate aberrant gene expression.[1] Since design of new drugs that directly interacted with DNA is very important, in present study, we have investigated the effect of the new designed Pd(II) complex (Ethylendiamine 8-hydroxyquinolinato Palladium(II) chloride) (Scheme 1) on the stability of the calf thymus DNA, in addition to some investigations on the binding parameters of complex to the DNA has been considered. Information obtained



Scheme 1. The molecular structure of ethylendiamine 8-hydroxyquinolinato palladium(II) chloride (Pd(II) complex)

Table 1. Enthalpies of Pd(II) complex + DNA interactions, Q, at 300 K. Precision is ± 0.400 μJ or better

[Comp] / μM	[DNA] / μM	Q / μJ	Q_{dilut} / μJ
9.12	119.57	12.415352	12.654532
27.26	118.72	26.972077	28.189544
45.27	117.88	41.85016	31.155128
63.16	117.04	57.94362	33.266028
80.91	116.21	75.381995	35.118395
98.53	115.38	93.560856	36.950907
116.02	114.56	112.81619	38.325542
133.38	113.74	131.72873	39.449586
150.61	112.93	151.46544	40.469381
167.7	112.13	171.60718	41.464513
184.67	111.33	191.72924	41.63489
201.51	110.54	211.51649	41.078532
218.22	109.75	232.22287	40.544077
234.8	108.97	251.88747	41.532564
251.24	108.19	271.58906	41.243349
267.56	107.42	291.01553	39.283598
283.74	106.65	310.73163	38.883865
299.8	105.89	329.45623	38.391126
315.73	105.14	347.97058	37.755725
331.52	104.38	367.03017	37.065105
347.18	103.64	385.46936	36.398395
362.72	102.9	403.54151	35.797562
378.12	102.16	421.72952	35.642693
393.39	101.43	439.89584	34.82776
408.54	100.7	457.01633	33.951757
423.55	99.98	474.5883	33.888054

from these studies will be helpful to understand the mechanisms of the interaction between the metal anticancer complexes and nucleic acids.

Materials and Method

Calf thymus DNA was obtained from Sigma. For the synthesis of ethylenediamine-8-hydroxyquinolinatopalladium(II) chloride, Pd(II) complex (Scheme 1), material required: 8-hydroxyquinolin, ethylenediamine, potassium tetrachloropalladate(II), sodium bicarbonate and sodium chloride were purchased from Aldrich (U. K). The first stage in this synthesis is the preparation of ethylenediamine palladium(II) dichloride [Pd(en)Cl_2], from which the titled compound is obtained. Place 1.63 g (mmol) K_2PdCl_4 in a 250 mL flask containing 200 mL distilled water and stirred for 10 ~ 15 min at 0 °C in an ice bath. 99% ethylenediamine (0.34 mL, 5 mmol) was added dropwise while stirring vigorously. Stirring continued for another 2 h at room temperature. The crude yellowish brown precipitate so obtained was filtered and washed several times with water, ethyl alcohol and diethyl ether and dried at 40 °C. Yield was 1.05 g (89%). To obtain the titled compound from the chloride, a well suspension of [Pd(en)Cl_2] (0.237 g, 1 mmol) in 20 mL water was treated with a solution of $NaHCO_3$ (0.084 g, 1 mmol) and 8-hydroxyquinoline (0.145 g, 1 mmol) in 20 mL water. The mixture was stirred at 45 ~ 50 °C for 2 h. The yellow obtained solution was filtered and evaporated at 45 ~ 50 °C to complete dryness. Recrystallization was carried out by stirring the crude precipitate in 20 mL methanol-acetonitryl (1:1 v/v) mixture and filtering out the undissolved particles. Diffusion of diethyl-ether into this filtrate gave yellow needle-like crystals, which were filtered off, washed with diethyl-ether, and dried in an oven at 45 ~ 50 °C. Yield: 0.242 g (78%). Anal. calcd. for $C_{11}H_{14}N_3O$ Pd (310): C, 42.58; H, 4.52; N, 13.55%. Found: C, 42.59; H, 4.50; N, 13.51%. Solid state FT-IR spectroscopy of the above complex shows four characteristic bands at 1110, 3071, 3209, and 3414 cm^{-1} assigned to ν (C−O stretching), ν (C−H aromatic), ν (C−H aliphatic) and (N−H stretching) modes respectively. ^{1}H NMR (500 MHz, DMSO-d_6, ppm): protons of ethylenediamine moiety resonate at 2.6 (s, 4H, C_2H_4), 5.6 (s, 2H, NH_2), and 5.8 (s, 2H, NH_2) and the protons of quinoline moiety resonate at 6.8 (doublet), 7.07 (doublet), 7.42 (triplet), 7.60 (quarter), 8.38 (doublet) and 8.56 (doublet). Electronic absorption spectrum of the Pd(II) complex shows two bands at 260 and 375 nm which are tentatively be assigned to the transition of n → δ* of phenolic C−O and π → π* of C = N aromatic ring respectively.

The buffer solution used in the experiments was 50 mM Tris, pH = 7.5, which was obtained from Merck. All other materials and reagents were of analytical grade.

The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra sensitive titration calorimeter (MicroCal, LLC, Northampton, MA). The microcalorimeter consists of a reference cell and a sample cell of 1.8 mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with DNA solution (120 μM) and the reference cell contained buffer solution. The solution in the cell was stirred at 307 rpm by the syringe (equipped with micro propeller) filled with Pd solution (2 mM) to ensure rapid mixing. Injections were started after baseline stability had been achieved. The titration of DNA with Pd solution involved 30 consecutive injections of the ligand solution, the first injection was 5 μL and the remaining ones were 10 μL. In all cases, each injection was done in 6 s at 3-min intervals. To correct the thermal effects due to Pd dilution, control experiments were done in which identical aliquots were injected into the buffer solution with the exception of DNA. In the ITC experiments, the enthalpy changes associated with processes occurring at a constant temperature are measured. The measurements were performed at a constant temperature of 27.0 ± 0.02 °C and the temperature was controlled using a Poly-Science water bath.

Results and Discussion

It has been shown previously[16-26] that the enthalpies of interactions of biopolymers with ligands (DNA + Pd(II) complex in this case) in the aqueous solvent (Pd(II) complex + water in the present case) mixtures, can be reproduced *via* the following equation.

$$Q = Q_{max}x'_B - \delta^{\theta}_A(x'_A L_A + x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A + x'_B L_B)x'_B \quad (1)$$

The parameters δ^{θ}_A and δ^{θ}_B are the indexes of the DNA sta-

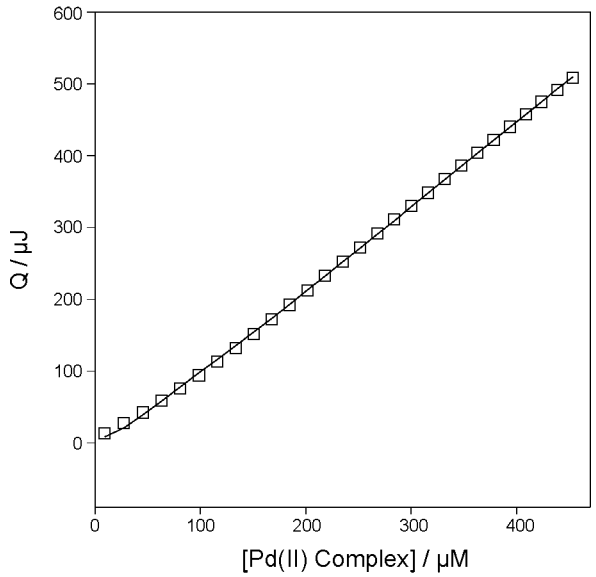


Figure 1. Comparison between the experimental enthalpies for Pd(II) complex + DNA interactions at 300 K and calculated data (lines) *via* equation 1.

Table 2. Binding parameters for Pd(II) complex + DNA interactions *via* equations 1 and 15. $p = 1$ shows the overall non-cooperativity for the interaction of Pd(II) complex with DNA. The big association equilibrium constant values show that there is strong interaction between Pd(II) complex and DNA

parameters	T = 300 K
K_1 / mM^{-1}	428.03 ± 0.02
K_2 / mM^{-1}	428.03 ± 0.02
K_3 / mM^{-1}	428.03 ± 0.02
p	1.000 ± 0.002
δ_A^θ	0.615 ± 0.077
δ_B^θ	0.849 ± 0.052
ΔH_{max} / kJ mol^{-1}	1.481 ± 0.034

bility as a result of interaction with Pd(II) complex in the low and high Pd(II) complex concentrations respectively. Cooperative binding requires that the macromolecule have more than one binding site, since cooperativity results from the interactions of identical binding sites with some similar ligands. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity. Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, the protein exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{px_B}{x_A + px_B} \tag{2}$$

$p < 1$ or $p > 1$ indicate positive or negative cooperativity of macromolecule for binding with ligand respectively; $p = 1$ indicates that the binding is non-cooperative. x_B is the fraction of bounded Pd(II) complex with DNA, and $x_A = 1 - x_B$ is the fraction of unbounded Pd(II) complex. We can express x_B fractions, as the total Pd(II) complex concentrations divided by the maximum concentration of the Pd(II) complex upon saturation of all DNA as follows:

$$x_B = \frac{[Pd(II)\ complex]_T}{[Pd(II)\ complex]_{max}} \quad x_A = 1 - x_B \tag{3}$$

[Pd(II) complex]$_T$ is the total concentration of the Pd(II) complex and [Pd(II) complex]$_{max}$ is the maximum concentration of the Pd(II) complex upon saturation of all DNA. L_A and L_B are the relative contributions of unbounded and bounded Pd(II) complex to the enthalpies of dilution with the exclusion of DNA and can be calculated from the enthalpies of dilution of Pd(II) complex in buffer as follows:

$$L_A = Q_{dilut} + x_B\left(\frac{\partial Q_{dilut}}{\partial x_B}\right), \quad L_B = Q_{dilut} - x_A\left(\frac{\partial Q_{dilut}}{\partial x_B}\right) \tag{4}$$

The enthalpies of Pd(II) complex + DNA interactions, Q, were fitted to Eq. 1 over the whole Pd(II) complex compositions. In the fitting procedure, p parameter varied in the course of an iterative process until the best fit between experimental and calculated data was approached (Fig. 1). δ_A^θ and δ_B^θ parameters have been also optimized to fit the data. The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2 values (0.99999) support the method. The binding parameters for Pd(II) complex + DNA interactions recovered from Eq. 1 were listed in Table 2. The agreement between the calculated and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. 1. Φ is the fraction of DNA molecule undergoing complexation with Pd(II) complex which can be expressed as follows:

$$\Phi = \frac{Q}{Q_{max}} \tag{5}$$

Q_{max} represents the heat value upon saturation of all DNA. The appearance equilibrium constant values, K_a, as a function of free concentration of Pd(II) complex, [Pd(II) complex]$_F$, can be calculated as follows:

$$K_a = \frac{\Phi}{(1-\Phi)[Pd(II)complex]_F} = \frac{\Phi}{(1-\Phi)[Pd(II)complex]_T(1-x_B)} \tag{6}$$

The Gibbs free energies as a function of Pd(II) complex concentrations can be obtained as follows:

$$\Delta G = -RT\,LnK_a \tag{7}$$

Gibbs energies, ΔG, at different temperatures calculated from Eq. 7 have shown graphically in Fig. 2. ΔS values were calculated using ΔG values at different temperatures and have shown in Fig. 3.

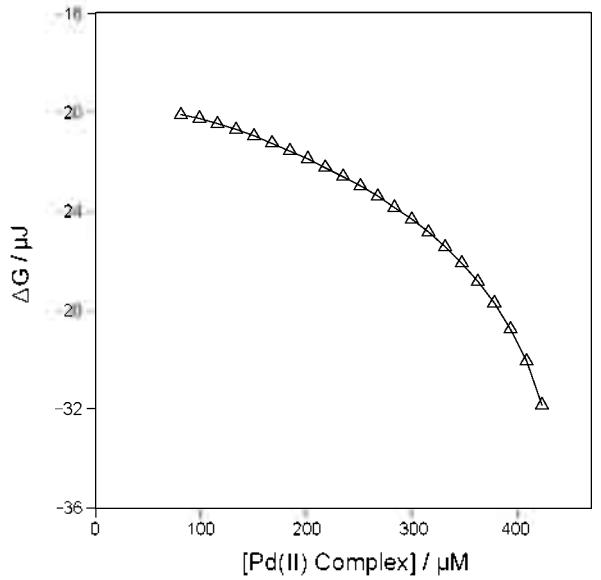


Figure 2. Comparison between the experimental Gibbs free energies at 300 K for Pd(II) complex + DNA interactions and calculated data (lines) *via* equation 7. The linearity of ΔG against Pd(II) complex concentrations indicates that the structural effects compensate each other in the free energy with support the solvation model.

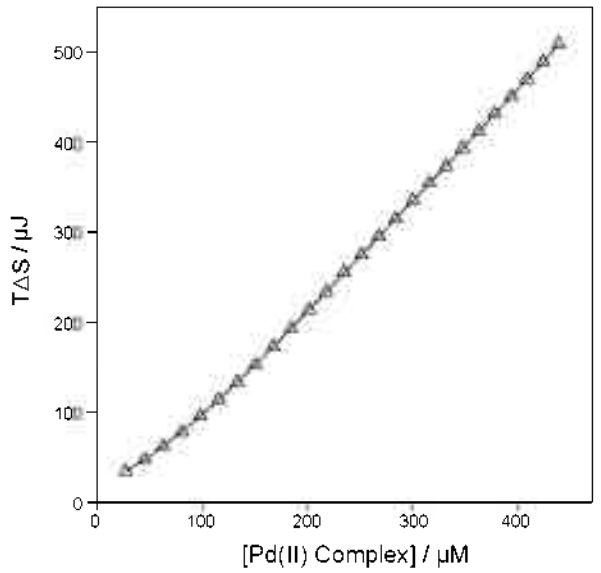


Figure 3. Comparison between the experimental entropies at 300 K for Pd(II) complex + DNA interactions in and calculated data (lines) *via* equations 1 and 7.

Consider a solution containing a ligand (Pd(II) complex) and a macromolecule (DNA_g) that contains "g" sites capable of binding the ligand. If the multiple binding sites on a macromolecule are identical and independent, the ligand binding sites can be reproduced by a model system of monovalent molecules ($DNA_g \rightarrow gDNA$) with the same set of dissociation equilibrium constant, K_d, values. Thus, the reaction under consideration can be written:

$$M + L \Leftrightarrow ML \quad K_d = \frac{[M][L]}{[ML]} \tag{8}$$

If α is defined as the fraction of free binding sites on the biomacromolecule, M_0 is the total biomacromolecule concentration, and L_0 is the total ligand concentration, then the free concentrations of monovalent molecule [M] and ligand [L] as well as the concentration of bound ligand [ML] can be deduced as follows:

$$[ML] = g(1 - \alpha)M_0 \tag{9}$$

$$[L] = L_0 - [ML] = L_0 - g(1 - \alpha)M_0 \tag{10}$$

$$[M] = gM_0 - [ML] = gM_0 - g(1 - \alpha)M_0 = \alpha gM_0 \tag{11}$$

Substitution of free concentrations of all these components in Eq. 8 gives:

$$K_d = (\frac{\alpha}{1 - \alpha})L_0 - \alpha gM_0 \tag{12}$$

or

$$\alpha M_0 = (\frac{\alpha}{1 - \alpha})\frac{1}{g}L_0 - \frac{K_d}{g} \tag{13}$$

The value of 1 – α as the fraction of occupied binding sites on the biomacromolecule:

$$1 - \alpha = \frac{Q}{Q_{max}} \tag{14}$$

where q represents the heat value at a certain L_0 and Q_{max} represents the heat value upon saturation of all biomacromolecules. The combination of Eqs.13 and 14 yields:

$$\frac{\Delta Q}{Q_{max}}M_0 = (\frac{\Delta Q}{Q})L_0 \ \frac{1}{g} - \frac{K_d}{g} \tag{15}$$

Where $\Delta Q = Q_{max} - Q$ Therefore, the plot of $\frac{\Delta Q}{Q_{max}}M_0$ versus $\frac{\Delta Q}{Q}L_0$ should be a linear plot with a slope of $\frac{1}{g}$ and a vertical intercept of $\frac{K_d}{g}$.

The linearity of the plot has been examined by different estimated values for Q_{max} to reach the best value for the correlation coefficient. The best linear plot with the correlation coefficient value ($r^2 \approx 1$) was obtained using 320 μJ (equal to 1.481 kJ/mol). The values of g and K_d, obtained from the slope and vertical-intercept plot, are listed in Table 2. The calorimetric method described recently allows obtaining the number of binding sites (g), the molar enthalpy of binding site ($\Delta H_{bin} = \frac{Q_{max}}{g}$) and the dissociation equilibrium constant (K_d) for a set of biomacromolecule binding sites. The lack of a suitable value for Q_{max} to obtain a linear plot of $(\Delta Q/Q_{max})M_0$ *vs.* $(\Delta Q/Q)L_0$ may be related to the existence of non-identical binding sites or the interaction between them. Using this method shows that there is a set of three identical and non-interacting binding sites for Pd(II) complex. Binding parameters for Pd(II) complex + DNA inter actions using the new model are listed in Table 2.

The positive value for δ_A^θ (0.616) in the low concentration of Pd(II) Complex, indicates that DNA structure is stabilized as a result of binding to Pd(II) Complex. The more positive value of δ_B^θ (0.849) reflects stabilization of the calf thymus DNA structure in the high concentration of Pd(II) complex too. In other words, the positive values of δ_B^θ suggest that complex binds preferentially to the native state of DNA. p value ($p = 1$) shows the overall non-cooperativity for the interaction of Pd(II) complex with DNA including both specific and non-specific interactions. Hossain and Huq[27] have studied the interactions between Pd(II) complex ions and DNA and they believed that Pd(II) complex covalently bind into adenine and guanine in DNA. The big association equilibrium constant values (K_a = 428.03 ± 0.02 mM^{-1}) show that there is strong interaction between Pd(II) complex and DNA, which is in agreement with the above interpretation. Also, other reports have revealed that small organic compounds and transition metal complexes could bind non-covalently (including as intercalators and groove binders) to nucleic acids. These interactions have been shown to disrupt the replication and/or transcription culminating in a cellular death.[27] Intercalators, by definition, bind into DNA by intercalating the flat aromatic ring between the base pairs of the DNA duplex.[28] An intercalator makes contact with two base-pairs whereas a groove binder can span many more, making interactions with several reactive sites along DNA grooves, e.g. phosphate groups, electron acceptors and donors, hydrophobic sites, and acidic hydrogen's.[3] Since the new synthesized Pd(II) complex is a planar system, then, the planar moieties of this complex might be inserted between the DNA base-pairs, parallel to their aromatic rings and perpendicular to the helical axis. As a consequence, this complex might interact with DNA as an intercalator, thus interfering with DNA replication and cell proliferation.

Conclusion

The enthalpies of Pd(II) complex + DNA interaction were fitted to Eq. 1 over the whole Pd(II) complex compositions. In the procedure, the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). The optimized δ_A^θ and δ_B^θ values are recovered from the coefficients of the second and third terms of Eq. 1. The agreement between the calculated and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. 1. $p = 1$ indicates that the binding of Pd(II) complex with DNA is non-cooperative. A suitable value for Q_{max} to obtain a linear plot of $(\Delta Q/Q_{max})M_0$ *vs.* $(\Delta Q/Q)L_0$ indicates that there are a set of three identical and non-interacting binding sites on DNA for Pd(II) complex.

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