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Thermodynamic characterization of binding of DNA with cisplatin in aqueous solution by calorimetry

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Abstract

The behaviour of *cis*-diamminedichloroplatinum(II) (cisplatin) binding to DNA was studied thermodynamically by calorimetric methods such as flow microcalorimetry and differential scanning calorimetry (DSC). The thermodynamic quantities of binding of cisplatin to DNA were determined from the measurement of the heat of mixing From the results obtained, it was suggested that the complex formation by the interaction of DNA with cisplatin may be influenced by the entropy term as a dominant factor. UV spectral measurement on solutions having a known concentration of DNA and cisplatin solutions of various concentrations was carried out at room temperature, and the difference of absorption, ΔA_{260}, at wavelength 260 nm between DNA solutions with and without cisplatin was estimated. From the results obtained, a hyperchromic effect in the DNA solution containing cisplatin was found to exist The appearance of the hyperchromic effect may be considered to originate from the disturbance of the base stacking between adjacent base pairs of DNA by the interaction of DNA with cisplatin. In addition, the thermal stability of the DNA–cisplatin complex was also studied by DSC method. The binding of cisplatin decreases the thermal stability of DNA; the transition temperature and the heat of the helix–coil transition of DNA decrease accompanying the binding of cisplatin. The decrease of the transition temperature is caused by the kinked DNA(helix′) accompanying the appearance of the hyperchromic effect by binding cisplatin, also, the decrease of the heat of helix–coil transition may be based on the cooperative action between the heat of helix–coil transition of the kinked DNA(helix′) and the heat of dissociation when cisplatin is dissociated from the DNA–cisplatin complex. By taking into consideration these results, the heat of binding of cisplatin to DNA was estimated to be about −106 kJ per mole of cisplatin.

INTRODUCTION

Considerable attention has been given to *cis*-diamminedichloroplatinum(II) (cisplatin) as an antitumour agent, and many workers have investigated the interactions of cisplatin with DNA [1–5], its oligomers [6–10], and/or polynucleotides [11–13]. Especially, it has been reported from the study of X-ray crystal structure that cisplatin binds predominantly to

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d(GpG) sequences in the oligonucleotide–cisplatin system [14]. No information, however, has been obtained on the thermodynamics of the binding process accompanying complex formation in the DNA–cisplatin system, although thermodynamic studies are necessary for understanding the interaction involved at the DNA–cisplatin binding site.

In this paper, in order to obtain thermodynamic information about the interaction of DNA with cisplatin, we measured the heat of mixing for the DNA–cisplatin system by using a flow microcalorimeter at 298 K. Thermodynamic parameters accompanying the interaction of DNA with cisplatin were estimated, and the binding mode of cisplatin to DNA in aqueous solution was considered. In addition, the thermal stability behaviour of the DNA–cisplatin complex was characterized from the measurements with an adiabatic differential scanning calorimeter to observe the influence of cisplatin binding on the DNA double helix.

EXPERIMENTAL

The DNA sample used in this study was calf thymus DNA (guanine–cytosine (GC) content 42%) which was obtained from Sigma Chemical Co. Ltd., USA; *cis*-diamminedichloroplatinum(II) (cisplatin) was also obtained from Sigma. The solvent used to adjust the pH (7.60) was 0.1 mol dm^{-3} Tris–HCl [tris(hydroxymethyl)aminomethane hydrochloride] buffer solution.

Apparatus and procedure

The calorimeter used for measurement of the heat of mixing of DNA and cisplatin was the same as the flow type of microcalorimeter described previously [15]. For the measurement of heat of mixing, a DNA solution (1.0×10^{-3} mol dm^{-3}) at a given flow rate (1.0×10^{-3} cm^3 s^{-1}) and cisplatin solutions (1.0×10^{-3} mol dm^{-3}) with different flow rates were mixed. The procedure for obtaining the net heat of mixing was the same as described previously [15].

The measurement of the enthalpy change accompanying the transition from helical to coiled conformations of DNA solutions containing various concentrations of cisplatin was carried out on an adiabatic differential scanning calorimeter (DSC, DASM-4, USSR) at 1.0 K min^{-1} rate. In this case, the DNA concentration was about 1.2×10^{-3} mol dm^{-3}.

UV spectral measurements for DNA solutions with various molar ratios C/P, of cisplatin C to DNA nucleotide P were carried out with a spectrophotometer (220A, Hitachi, Japan) at room temperature. The temperature dependence of UV absorption for DNA solutions with various concentrations of cisplatin was also evaluated by using a spectrophotometer equipped with a temperature controller (SPR-7, Hitachi, Japan), and the temperature

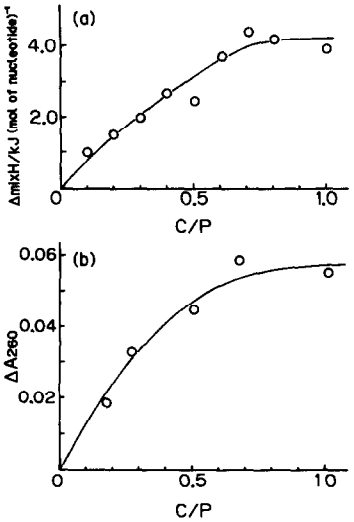


Fig. 1. (a) Heat of mixing $\Delta_{mix}H$ of DNA–cisplatin system at 298 K plotted against the molar ratio C/P of cisplatin to nucleotide of DNA. (b) Difference of absorption ΔA_{260} at 260 nm between DNA solutions with and without cisplatin plotted against C/P.

of the solution in the cell was determined with a copper–constantan thermocouple. The heating rate was about 0.5 K min^{-1}. The DNA concentration for UV spectral measurement was about 1.0×10^{-4} mol dm^{-3}. The DNA concentration was determined by phosphorus analysis [16].

RESULTS AND DISCUSSION

Heat of mixing

In order to obtain the enthalpy change accompanying the interaction between DNA and cisplatin, the heats of mixing of DNA and cisplatin solutions were measured at 298.15 ± 0.005 K for solutions with various concentrations of cisplatin at a given concentration of DNA by using the flow microcalorimeter. The results obtained proved to be endothermic, corresponding to the heat of interaction of DNA with cisplatin, under the assumption that the heats of dilution of DNA and/or cisplatin solutions were negligibly small.

Figure 1(a) shows the plots of the heat of mixing per mole of DNA nucleotide, $\Delta_{mix}H$, against the molar ratio, C/P, of cisplatin to DNA nucleotide, where C and P are the molar concentrations of cisplatin and DNA nucleotide, respectively. As seen in Fig. 1(a), $\Delta_{mix}H$ increases monotonically at first and then levels off at $C/P = 0.6$ with increasing concentration of cisplatin. A constant value of $\Delta_{mix}H$ in the region beyond $C/P = 0.6$ may indicate the termination of the interaction of DNA with cisplatin.

UV spectra

In order to obtain further information about the interaction between DNA and cisplatin, UV spectral measurements on DNA solutions containing various concentrations of cisplatin were carried out at room temperature. The results obtained are shown in Fig. 1(b), where the differences of absorption, ΔA_{260}, between DNA solutions with and without cisplatin at a wavelength of 260 nm are plotted against C/P. As seen in Fig. 1(b), ΔA_{260} increases with increasing C/P and then reaches a definite value at $C/P = 0.6$, demonstrating that a hyperchromic effect exists in DNA solutions containing cisplatin. This effect seems to be based on disturbance of base stacking between adjacent base pairs of DNA by the interaction between DNA and cisplatin [17]. It is also worth noting that the dependence of ΔA_{260} on C/P is comparable with that of $\Delta_{mix}H$ as shown in Fig. 1(a).

Thermodynamic parameters

Assuming that a DNA–cisplatin complex is formed between DNA and cisplatin, the reaction process can be expressed as follows

$$\text{DNA} + \text{cisplatin} \rightleftharpoons (\text{DNA–cisplatin}) \text{ complex} \tag{1}$$

the binding constant of this reaction, K, can be expressed as

$$K = \frac{C_b}{(nP - C_b)(C - C_b)} \tag{2}$$

where n is the number of binding sites per nucleotide molecule of DNA, and C_b is the molar concentration of binding of cisplatin per nucleotide molecule of DNA.

The net heat of interaction, ΔH, between DNA and cisplatin can be related to the heat of mixing, $\Delta_{mix}H$, estimated from calorimetry as follows

$$\Delta H = \frac{\Delta_{mix}H}{C_b/P} \tag{3}$$

From eqns. (2) and (3), $\Delta_{mix}H$ will be expressed as follows

$$\Delta_{mix}H = \frac{\Delta H}{2}\left(n + \frac{1}{KP} + \frac{C}{P} - \sqrt{\left(n + \frac{1}{KP} + \frac{C}{P}\right)^2 - \frac{4nC}{P}}\right) \tag{4}$$

where P should be assigned a fixed value under the experimental conditions, but ΔH, K and n must be treated as variable parameters. The values for ΔH, K and n which give the best fit of the values calculated by eqn. (4) to the experimental values can be determined with a non-linear least-squares treatment. The values of ΔH, K and n obtained in such a way are summarized in Table 1, together with the values of the free energy change, ΔG, estimated from $\Delta G = -RT \ln K$ and the entropy change, ΔS, calculated by $\Delta S = (\Delta H - \Delta G)/T$.

TABLE 1

Thermodynamic parameters of binding of cisplatin to DNA at 298 K estimated from the heat of mixing $\Delta_{mix}H$ by using eqn. (4)

K ($dm^3\ mol^{-1}$)	ΔG ($kJ\ mol^{-1}$) [a]	ΔH ($kJ\ mol^{-1}$) [a]	ΔS ($J\ mol^{-1}\ K^{-1}$) [a]	n
2.2×10^4	−25	7 6	110	0.6

[a] Here, mol means moles of cisplatin.

As seen in Table 1, from the free energy change of interaction, ΔG, the complex formed by the interaction of DNA with cisplatin seems to be stable, although ΔH is endothermic. However, the endothermic ΔH value seems to be caused by the enthalpy change (endothermic) accompanying the conformational change of DNA due to addition of cisplatin rather than by the enthalpy change (exothermic) of the interaction of DNA with cisplatin.

Thermal stability of complex

In order to obtain further information about the heat of interaction between DNA and cisplatin demonstrated from the heat of mixing, the interaction of DNA with cisplatin was also studied from the standpoint of the helix–coil transition by means of the temperature dependences of UV absorption spectroscopy and DSC measurements. Figure 2(a) shows the differential transition curves of UV absorption obtained for DNA solutions with and without cisplatin. As seen in Fig. 2(a), it is interesting to note that the differential transition curve of UV absorption for DNA solution without cisplatin shows peaks with double shoulders at higher temperature. These double shoulders seem to correspond to the transition of the GC base pair region in the double-stranded helical structure of DNA, since the transition temperature of the GC base pair is higher than that of the adenine–thymine (AT) base pair [18]. The melting temperature of DNA solutions with cisplatin decreases with increasing concentration of cisplatin, and it is worth noting that the double shoulders of DNA at higher temperature disappear with increasing concentration of cisplatin, demonstrating that cisplatin binds predominantly to the d(GpG) sequence in DNA.

Figure 2(b) shows the typical DSC curves obtained for DNA solutions with and without cisplatin. The DSC curve of DNA solution without cisplatin ($C/P = 0$) shows an endothermic peak with double shoulders at higher temperature, similar to the differential transition curve 1 of UV absorption as shown in Fig. 2(a). It is suggested that the endothermic peak corresponds to the transition from the helical to the coiled conformation of DNA with increasing temperature. The double shoulder on the DSC curve begins to disappear on adding cisplatin, and the transition temperature

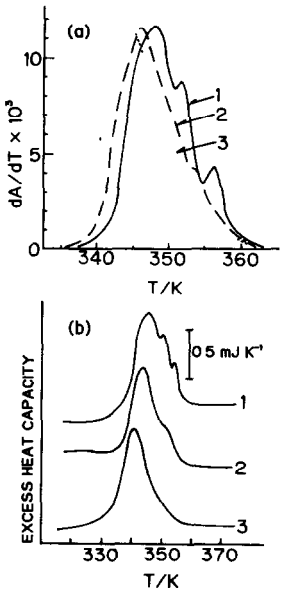


Fig 2. (a) Differential transition curves of UV absorption obtained for DNA solutions with and without cisplatin, 1 $C/P = 0$, 2. $C/P = 0.500$, 3· $C/P = 1.000$ (b) Typical DSC curves for DNA solutions with and without cisplatin; 1: $C/P = 0$, 2· $C/P = 0.062$, 3: $C/P = 0.200$ Here, C/P means molar ratio of cisplatin to nucleotide.

shifts to a lower temperature than that of the DNA solution without cisplatin.

The heat of the helix–coil transition, ΔH_t, and the transition temperature, T_t, which can be estimated from the endothermic peak area and temperature

TABLE 2

Helix–coil transition enthalpy ΔH_t and enthalpy change per mole of cisplatin $\Delta\Delta H'$ for DNA solutions containing various concentrations of cisplatin

C/P	C_b/P [a,b]	ΔH_t (kJ mol^{-1}) [c]	$-\Delta\Delta H'$ (kJ mol^{-1}) [d]
0	0	21.0 [e]	0
0 031	0.029	18 0	103.4
0.062	0.059	15 5	93 2
0 128	0.119	14.1	58.0
0 200	0.182	15.2	31 9
0.356	0.304	13.8	23 7
0.578	0 417	13.8	17.3
1 000	0.555	10 3	19.3

[a] Here, C_b means molar concentration of cisplatin bound DNA

[b] P here means molar concentration of DNA as phosphorus.

[c] Mol here means mole of nucleotide.

[d] Mol here means mole of cisplatin

[e] This value corresponds to the heat of helix–coil transition, $\Delta H_t°$, for DNA without cisplatin.

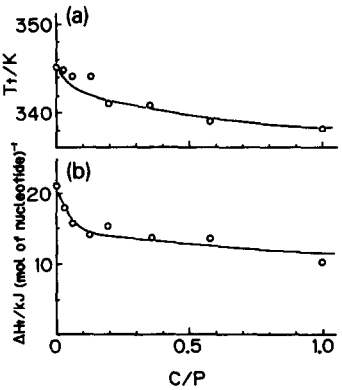
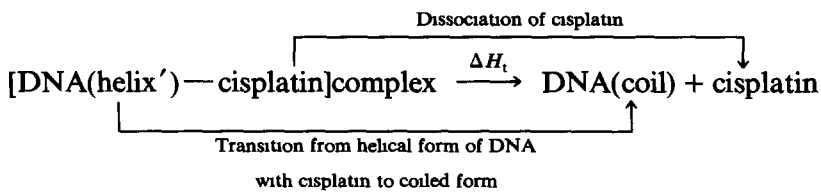


Fig. 3 Plots of (a) the transition temperature, T_t, and (b) the heat of helix–coil transition ΔH_t estimated from the endothermic peak and areas of DSC curves for DNA solutions with and without cisplatin, against C/P.

respectively, were obtained for DNA solutions with various concentrations of cisplatin. The results obtained are listed in Table 2 and plotted against C/P in Fig. 3. As seen in Fig. 3(a) and (b), both T_t and ΔH_t decrease with increasing concentration of cisplatin, demonstrating that cisplatin may play an important role in the conformational change of DNA accompanying the interaction with cisplatin.

Binding enthalpy of cisplatin

Assuming that the endothermic peaks of DSC curves 2 and 3 as shown in Fig. 2(b) are based on the heat of helix–coil transition of DNA on binding with cisplatin, the observed heat of transition corresponds to the sum of the heat of helix–coil transition of bound DNA and the heat of dissociation of cisplatin from the DNA complex accompanying the transition of bound DNA, as shown in the following reaction process.

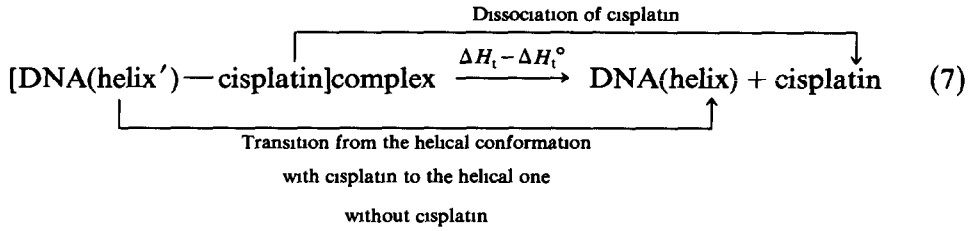


(5)

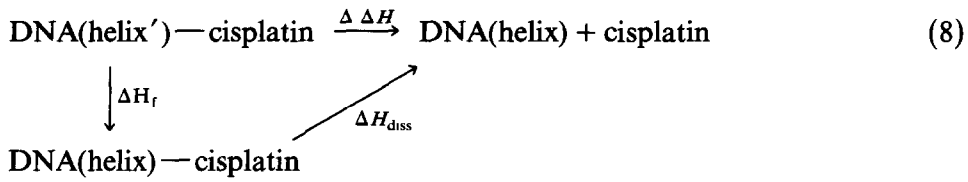
Here, DNA(helix′) means the helical conformation of DNA accompanying the interaction between a usual helical conformation (DNA (helix)) of DNA and cisplatin. The heat of the usual helix–coil transition of DNA without cisplatin, ΔH_t°, can be expressed as

$$\text{DNA(helix)} \xrightarrow{\Delta H_t^\circ} \text{DNA(coil)} \qquad (6)$$

Subtraction of reaction (6) from reaction (5) gives



The enthalpy change, $\Delta\Delta H(=\Delta H_t - \Delta H_t^\circ)$, represented by reaction (7) is the sum of the apparent heat of dissociation, ΔH_{diss}, and the apparent heat of conformational change, ΔH_f, accompanying the transition from the helical conformation [DNA(helix′)] binding to cisplatin to the helical form [DNA(helix)] which does not bind to cisplatin. That is, the enthalpy change, $\Delta\Delta H$, in the reaction process of reaction (7) can be taken as confirming the following enthalpy cycle as



The following relation from the enthalpy cycle mentioned above is obtained

$$\Delta\Delta H = \Delta H_f + \Delta H_{diss} \quad (9)$$

Dividing $\Delta\Delta H$ by the molar ratio, C_b/P of bound cisplatin to the nucleotide of DNA, the apparent enthalpy change per mole of cisplatin, $\Delta\Delta H'$, can be calculated; C_b can be calculated from eqn. (2) with K and n values estimated from the heat of mixing as given in Table 1.

Values of $\Delta\Delta H'$ calculated in this manner are summarized in Table 2 and also plotted against C_b/P as shown in Fig. 4. $\Delta\Delta H'$ must have a definite

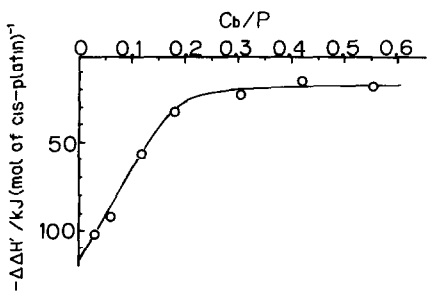


Fig. 4. Plots of enthalpy change $\Delta\Delta H'$ per mole of cisplatin accompanying the transition of DNA–cisplatin complex against the molar ratio, C_b/P, of bound cisplatin to the nucleotide of DNA The value (−115 kJ) estimated by extrapolating to an infinitely dilute solution of cisplatin ($C_b \to 0$) corresponds to the net heat of conformational change, ΔH_F

value which is nearly independent of the concentration of cisplatin; however, $\Delta\Delta H'$ as seen in Fig. 4 seems to depend on the cisplatin concentration as follows. In the region of $C_b/P > 0.3$, $\Delta\Delta H'$ has a specific value of about -20 kJ mol^{-1}, but in the region of $C_b/P < 0.3$, $\Delta\Delta H'$ decreases appreciably with decreasing cisplatin concentration. From this C_b/P dependence, the net heat of conformational change, ΔH_F, may be estimated as follows. Since ΔH_{diss} is zero at $C_b \to 0$, the net heat of conformational change, ΔH_F, determined by graphically extrapolating at $C_b \to 0$ is estimated to be about -115 kJ per mole of cisplatin from Fig. 4. In the region of $C_b/P > 0.3$, since $\Delta\Delta H' = -20$ kJ per mole of cisplatin, the net heat of dissociation, ΔH_D, can be estimated to be about 95 kJ per mole of cisplatin according to eqn. (9) using the ΔH_F value ($= -115$ kJ) estimated above.

On the other hand, the binding process accompanying the mixing of DNA with cisplatin as shown in Fig. 1(a) can be assumed as the reverse of the reaction represented in reaction (7) as follows

$$\begin{array}{ccc} & \overset{\Delta H_{bind}}{\longrightarrow} & \\ \text{DNA(helix)} + \text{cisplatin} & \xrightarrow{\Delta_{mix}H} & \text{DNA(helix}')\text{—cisplatin} \\ & \underset{\Delta H_f'}{\longrightarrow} & \end{array} \qquad (10)$$

The heat of mixing per mole of nucleotide, $\Delta_{mix}H$, can be taken as the sum of the heat of conformational change, $\Delta H_f'$, accompanying the interaction of DNA with cisplatin and the heat of binding of DNA with cisplatin, ΔH_{bind}, as follows

$$\Delta_{mix}H = \Delta H_f' + \Delta H_{bind} \qquad (11)$$

Since $\Delta H_{bind} = 0$ at $C_b \to 0$, $\Delta_{mix}H = \Delta H_f'$, and $\Delta H_f'$ can be considered to be about 115 kJ, with the opposite sign to the ΔH_F value at $\lim_{C_b \to 0} \Delta\Delta H' = 0$ from DSC measurement estimated above. Then ΔH_{bind} can be estimated by subtracting the $\Delta H_f'$ value (115 kJ) from $\Delta_{mix}H'$ converted per mole of cisplatin for each C_b/P as shown in the third column of Table 3; and the results obtained are listed in the last column of Table 3. As seen in Table 3, the average ΔH_{bind} value ($= -106$ kJ) corresponds to the net heat of binding between DNA and cisplatin, suggesting that cisplatin binds strongly to DNA. Also, ΔH_{bind} ($= -106$ kJ) has a reasonable value in comparison with the net heat of dissociation ($\Delta H_D = 95$ kJ) estimated from DSC measurement. This large ΔH_{bind} value seems to be in support of the experimental results as follows. The binding mode of cisplatin with DNA in aqueous solution may correspond to the chelation of cisplatin to N_7–N_7 of two adjacent guanines from the X-ray diffraction studies of the d(GpG)–cisplatin complex [14]. Furthermore, d(GpG)–platinum chelation may be responsible for the large value of ΔH_{bind}. However, an endothermic ΔH value estimated from eqn. (4), as shown in Table 1 does not agree with the

TABLE 3

The heat of binding ΔH_{bind} between DNA and cisplatin estimated from the heat of mixing $\Delta_{mix}H'$ converted for each value of C_b/P

C_b/P [a,b]	$\Delta_{mix}H$ [c] (kJ mol^{-1}) [d]	$\Delta_{mix}H'$ [e] (kJ mol^{-1}) [f]	ΔH_{bind} (kJ mol^{-1}) [f]
0 09	1.01	11.22	−104
0 18	1.53	8 50	−106
0.26	1.98	7.61	−107
0.34	2 73	8.03	−107
0.38	2.44	6.42	−108
0.42	3.70	8.81	−106
0.47	4.44	9.44	−105
0 50	4.20	8.40	−106
0 55	3 88	7 05	−107
		average	−106

[a] C_b here means molar concentration of cisplatin bound DNA
[b] P here means molar concentration of DNA as phosphorus.
[c] $\Delta_{mix}H$ is the observed heat of mixing estimated from calorimetry
[d] Mol here means mole of nucleotide.
[e] $\Delta_{mix}H'$ is the heat of mixing converted per mole of cisplatin.
[f] Mol here means mole of cisplatin

heat of binding of −106 kJ. The situation is very difficult to analyze exactly owing to a lack of some information in the present work, but one possible explanation is considered to be as follows. The existence of the hyperchromic effect for DNA solutions with cisplatin may indicate that bending of the DNA has occurred. Additionally, the endothermic evolution accompanying the bending of DNA on adding cisplatin, referring to the result reported by McCarthy and coworkers [19], seems to be larger than that accompanying the interaction between DNA and cisplatin.

The mode of binding of DNA with cisplatin was studied thermodynamically, under the assumption that the interaction between DNA and cisplatin can be treated as a reaction equilibrium [reaction (1) in this text]. However, since ΔH_{bind} (= −106 kJ) has a large value, the interaction between DNA and cisplatin may be demonstrated covalently.

Further work is in progress to obtain information on the dependence of the GC content of DNA on the concentration of cisplatin in order to confirm the binding mode of the d(GpG)–cisplatin complex.

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