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Ultraviolet Difference Spectral Study of the Interaction of DNA with Platinum Complexes

KENJI INAGAKI and YOSHINORI KIDANI

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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Ultraviolet difference spectral data provided useful information to differentiate the binding modes between antitumor-active and -inactive platinum complexes. The uv difference spectra for the DNA samples treated with platinum complexes exhibit a band at 295 nm due to the change in electron distribution of nucleic acid bases and a band at 270 nm due to the change in the secondary structure of DNA. The absorption ratio, $\Delta A_{270}/\Delta A_{295}$, is an index for the change of the secondary structure of DNA induced by binding with platinum. Changes in the DNA secondary structure induced by antitumor-active platinum complexes are greater than those induced by antitumor-inactive platinum complexes, and the uv difference spectral behavior of antitumor-active platinum complexes is similar. A tendency for preferential binding toward the guanine residues in DNA was confirmed in this study. Melting profiles for the DNA samples treated with platinum complexes showed a decrease in hyperchromicity and a broadening of transition width. The platinum complexes, other than trans-*$Pt(NH_3)_2Cl_2$, decreased the melting temperature.*

Introduction

It is well known that *cis*-$Pt(NH_3)_2Cl_2$ is effective against various tumor systems, while *trans*-$Pt(NH_3)_2Cl_2$ is inactive. It has been reported that DNA is the main target of *cis*-$Pt(NH_3)_2Cl_2$, which interferes with DNA replication process [1, 2]. The extent of binding of *cis*-$Pt(NH_3)_2Cl_2$ with DNA depends on G–C content in DNA [3–6]. Among the possible binding sites, the guanine residues were shown to be the most preferred site for binding of various platinum complexes [7–9].

The purpose of our present work was to differentiate the mode of the interaction of DNA with antitumor-active and -inactive platinum complexes. We measured the uv difference spectra and the melting profiles for the DNA samples treated with $[Pt(NH_3)_3Cl]Cl$, *trans*-$Pt(NH_3)_2Cl_2$, *cis*-$Pt(NH_3)_2Cl_2$, Pt(RR-dach)Cl_2, Pt(SS-dach)Cl_2, or Pt(RS-dach)Cl_2 (RR-dach, SS-dach, and RS-dach refer to *trans(l)-*, *trans(d)-*, and *cis*-diaminocyclohexane, respectively), and it has become apparent that the conformational perturbations of DNA induced by binding with platinum may allow differentiation of the binding mode between antitumor-active and -inactive platinum complexes.

Very recently, Macquet and Butour carried out a fluorescence study using ethidium bromide as a probe [10] and a circular dichroism study [11] in order to differentiate the binding mode of platinum with DNA. Their results are in good agreement with ours.

Experimental

Materials

According to the method reported in the literature, *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ [12] and $[Pt(NH_3)_3Cl]Cl$ [13] were synthesized. Pt(RR-dach)Cl_2, Pt(SS-dach)Cl_2, and Pt(RS-dach)Cl_2 were synthesized as previously reported [14, 15]. Calf thymus DNA (type 1) was purchased from Sigma Chemical Co., U.S.A. DNA was placed in 70% ethanol–water and then the solvent was decanted in order to remove the salt from DNA. DNA stock solutions were prepared by dissolving DNA in 0.01 *M* phosphate buffer, pH 6.8, containing 0.01 *M* NaCl by gentle stirring in a refrigerator. Concentration of the DNA solution was determined by spectrophotometry, as $\epsilon_{(P)} = 6600$. The platinum complexes were dissolved before use in 0.01 *M* phosphate buffer, pH 6.8, containing 0.01 *M* NaCl. Concentration of the solutions of platinum complexes was determined by atomic absorption spectrophotometry.

Methods

An aliquot of the solution of platinum complexes was added to the DNA solution, and the mixture was incubated at 30 °C for 4 days. The amount of platinum complexes added to the DNA solution was expressed as r, the molar ratio of platinum to phosphorus in DNA. In the measurement of uv difference

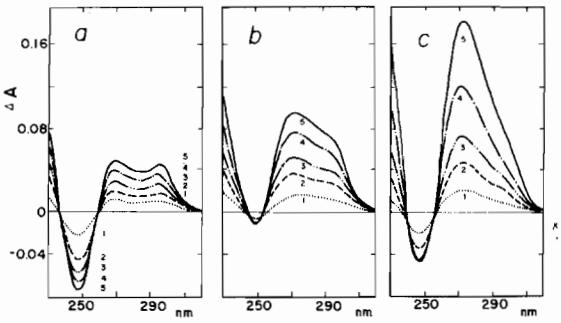

Fig. 1. Ultraviolet difference spectra as a function of r for the platinum–DNA systems at pH 6.8.

a) $[Pt(NH_3)_3Cl]$ Cl–DNA system: [DNA] = 2.25×10^{-4} M, 1) $r = 0.029$, 2) $r = 0.059$, 3) $r = 0.088$, 4) $r = 0.118$, 5) $r = 0.176$.

b) *trans*-$Pt(NH_3)_2Cl_2$–DNA system: [DNA] = 2.25×10^{-4} M, 1) $r = 0.024$, 2) $r = 0.062$, 3) $r = 0.095$, 4) $r = 0.142$, 5) $r = 0.190$.

c) *cis*-$Pt(NH_3)_2Cl_2$–DNA system: [DNA] = 2.25×10^{-4} M, 1) $r = 0.018$, 2) $r = 0.037$, 3) $r = 0.056$, 4) $r = 0.092$, 5) $r = 0.185$.

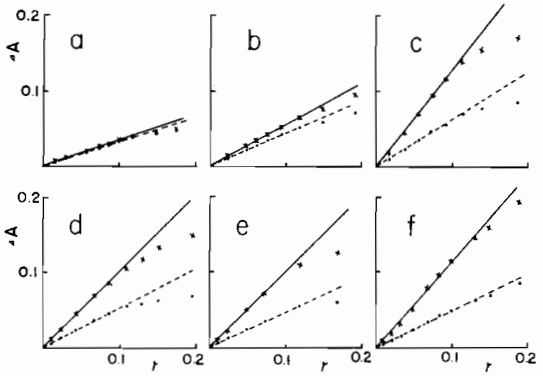

Fig. 2. Variation of ΔA as a function of r for the platinum–DNA system at pH 6.8; solid line: 270 nm, broken line: 295 nm. a) $[Pt(NH_3)_3Cl]Cl$–DNA system: [DNA] = 2.25×10^{-4} M; b) *trans*-$Pt(NH_3)_2Cl_2$–DNA system: [DNA] = 2.25×10^{-4} M; c) *cis*-$Pt(NH_3)_2Cl_2$–DNA system: [DNA] = 2.25×10^{-4} M; d) Pt(RR-dach)Cl_2–DNA system: [DNA] = 1.29×10^{-4} M; e) Pt(SS-dach)Cl_2–DNA system: [DNA] = 1.29×10^{-4} M; f) Pt(RS-dach)Cl_2–DNA system: [DNA] = 1.29×10^{-4} M.

spectra, the sample cell contained the reaction solution of various r, and the reference cell contained DNA solution. The uv spectra were recorded on a Shimadzu UV-200 spectrophotometer. Melting profiles were recorded automatically at 260 nm with a Shimadzu Tm Analyzer, attached to a Shimadzu UV-200 spectrophotometer. The melting temperature, Tm, is defined as the temperature at half denaturation. The width of transition, 2σ, is conventionally defined as the temperature range corresponding to 15.8% and 84.2% denaturation.

Results and Discussion

The uv spectrum of DNA exhibits a bathochromic shift accompanied by hyperchromicity by reaction with platinum complexes. This behavior has been interpreted as an evidence for the interaction of platinum complexes with DNA bases. We used uv difference spectral and melting techniques to examine the interaction of platinum complexes with calf thymus DNA at pH 6.8. The uv difference spectrum for the DNA solution treated with $[Pt(NH_3)_4]Cl_2$ did not change at all, indicating no reaction with DNA bases. This result is in agreement with the reported data [7]. Figure 1 shows uv difference spectra for the DNA solutions treated with $[Pt(NH_3)_3Cl]Cl$, *trans*-$Pt(NH_3)_2Cl_2$ or *cis*-$Pt(NH_3)_2Cl_2$. These spectral patterns are different. The uv difference spectrum obtained for $[Pt(NH_3)_3Cl]Cl$–DNA system exhibited absorption maxima at 269 and 295 nm, and an absorption minimum at 248 nm. ΔA at 269 and 295 nm increased with increasing r, the molar ratio of platinum complex added to DNA, while ΔA at 248 nm decreased with increasing r. Isosbestic points were observed at 237 and 259 nm. The uv difference spectra obtained for *trans*-$Pt(NH_3)_2Cl_2$–DNA or *cis*-$Pt(NH_3)_2Cl_2$–DNA system exhibited absorption maximum at 272 nm, with a shoulder at 295 nm. ΔA at both wavelengths also increased with increasing ν. ΔA at 248 nm decreased with increasing r until 0.06 and then it became constant. Comparison of ΔA at 270 nm shows that increment due to *cis*-$Pt(NH_3)_2Cl_2$ is larger than that due to *trans*-$Pt(NH_3)_2Cl_2$. The uv difference spectral pattern for the DNA samples treated with Pt(RR-dach)Cl_2, Pt(SS-dach)Cl_2, or Pt(RS-dach)Cl_2 was almost the same as that obtained for *cis*-$Pt(NH_3)_2Cl_2$–DNA.

Figure 2 shows the change of ΔA as a function of r. There is a linear relationship between ΔA and r ($r < 0.1$). In $[Pt(NH_3)_3Cl]Cl$–DNA system, ΔA at 270 nm was almost the same as that at 295 nm, while the former is slightly larger than the latter in *trans*-$Pt(NH_3)_2Cl_2$–DNA. Both $[Pt(NH_3)_3Cl]Cl$ and *trans*-$Pt(NH_3)_2Cl_2$ have no antitumor acitivity. Binding of antitumor-active platinum complex (*cis*-$Pt(NH_3)_2Cl_2$, Pt(RRdach)Cl_2, Pt(SS-dach)Cl_2, and Pt(RS-dach)Cl_2) to DNA is characterized by the absorption ratio, $\Delta A_{270}/\Delta A_{295}$. ΔA at 270 nm is about twice as much as that at 295 nm. This behavior is commonly observed for all antitumor-active platinum complexes.

It is of interest to compare the uv difference spectral patterns obtained in this work with those obtained with nucleic acid constituents. Figure 3 shows the uv difference spectra obtained by the interaction of $[Pt(NH_3)_3Cl]Cl$ with dGMP, dAMP, or dCMP. The uv difference spectrum obtained for $[Pt(NH_3)_3Cl]Cl$–dGMP exhibited absorption maxi-

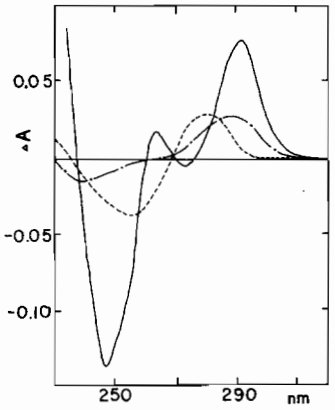

Fig. 3. Ultraviolet difference spectra resulting from [Pt(NH_3)$_3$Cl]Cl–deoxynucleotides interaction; ———: [Pt(NH_3)$_3$Cl]Cl–dGMP system ([dGMP] = 8.0 × 10^{-5} *M*, [Pt(NH_3)$_3$Cl] Cl = 8.0 × 10^{-5} *M*, 0.01 *M* phosphate buffer (pH 6.8) + 0.01 *M* NaCl, temperature = 40 °C, incubation time = 65 hr); -------: [Pt(NH_3)$_3$Cl]Cl–dAMP system ([dAMP] = 8.0 × 10^{-5} *M*, [Pt(NH_3)$_3$Cl] Cl = 8.0 × 10^{-5} *M*, 0.01 *M* phosphate buffer (pH 6.8) + 0.01 *M* NaCl, temperature = 40 °C, incubation time = 65 hr); – · –' [Pt(NH_3)$_3$(OH_2)](NO_3)$_2$–dCMP system ([dCMP] = 2.35 × 10^{-4} *M*, [Pt(NH_3)$_3$(OH_2)](NO_3)$_2$ = 1.50 × 10^{-5} *M*, 0.01 *M* phosphate buffer (pH 6.5), incubation time = 84 hr).

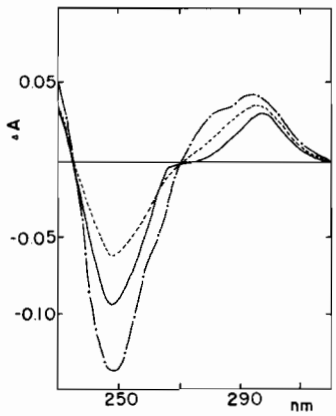

Fig. 4. Ultraviolet difference spectra calculated from the spectra of the platinum–DNA system (r = 0.1) by subtracting the absorbance due to the loss of base stacking; ———: [Pt(NH_3)$_3$Cl]Cl–DNA system; ------: *trans*-Pt(NH_3)$_2$$Cl_2$–DNA system; – · –: *cis*-Pt(NH_3)$_2$$Cl_2$–DNA system.

mum and minimum at 290 and 247 nm, respectively. In Fig. 3, ΔA around 270 nm shows little change by coordination of [Pt(NH_3)$_3$Cl]Cl to nucleotides (dGMP, dAMP, and dCMP). This fact shows that changes in the electron distribution of the conjugate system through coordination of [Pt(NH_3)$_3$Cl]Cl to nucleotides affect the absorbance around 270 nm very little. Therefore, it seems reasonable to consider that the increment of ΔA at 270 nm in the [Pt(NH_3)$_3$Cl]Cl–DNA system is attributable to the change of the secondary structure of DNA induced by the binding of platinum with DNA, being indicative of the hyperchromicity caused by the loss of base stacking. Figure 4 shows the spectrum which was calculated from the spectrum of [Pt(NH_3)$_3$Cl]Cl–DNA (Fig. 3) by subtracting the absorbance due to the loss of base stacking. The spectral pattern thereby obtained is satisfactorily similar to that obtained for [Pt(NH_3)$_3$Cl]Cl–dGMP system, considering the difference between DNA and dGMP. This result suggests that [Pt(NH_3)$_3$Cl]Cl binds to the N(7) site of the guanine residues in DNA. It has been reported that [Pt(dien)Cl]Cl (dien stands for diethylenetriamine) binds preferentially to the N(7) site of guanine [7, 16]. Macquet and Theophanides [7, 10] proposed that *trans*-Pt(NH_3)$_2$$Cl_2$ interacted with DNA on one site (N(7) site of the guanine residue) when the ratio [Cl^-]/[*trans*-Pt(NH_3)$_2$$Cl_2$] was higher than one (if ionic chlorine atoms were absent in the solution, *trans*-Pt(NH_3)$_2$$Cl_2$ liberated two chlorine atoms and reacted with DNA as a bifunctional reagent). The spectral pattern obtained for *trans*-Pt(NH_3)$_2$$Cl_2$ in Fig. 4 is also considerably similar to that obtained for [Pt(NH_3)$_3$Cl]Cl.

The spectrum for *cis*-Pt(NH_3)$_2$$Cl_2$ in Fig. 4 was obtained by the same treatment as that for [Pt(NH_3)$_3$Cl]Cl. Scovell and O'Connor [17] used uv difference spectroscopy to determine the conditional formation constants of *cis*-Pt(NH_3)$_2$$Cl_2$ with guanosine, adenosine, and cytidine at pH 6.5. The uv difference spectral patterns reported by them were found to be quite similar to those of [Pt(NH_3)$_3$Cl]Cl–nucleotide system (Fig. 3). The uv spectral pattern obtained for *cis*-Pt(NH_3)$_2$$Cl_2$–DNA system in Fig. 4 is approximately similar to that obtained for [Pt(NH_3)$_3$Cl]Cl–DNA system, though its spectrum has a shoulder at about 280 nm. These results suggest that a tendency for preferential binding with guanine residues exists in all platinum complexes.

If the increment of ΔA at 270 nm is attributable to the loss of base stacking and the increment of ΔA at 295 nm to the change in electron distribution of the conjugate system due to base moieties, the absorption ratio, $\Delta A_{270}/\Delta A_{295}$, can be taken as an index of the relative effect on the secondary structure of DNA caused by binding with platinum. The ratio was about 2 for the antitumor-active platinum complexes, while it was about 1 for the antitumor-inactive platinum complexes (Table I). Therefore, the extent of relative change of the secondary structure of DNA induced by the coordination of platinum complexes to DNA is in the order of: *cis*-Pt(NH_3)$_2$$Cl_2$, Pt(RR-dach)$Cl_2$, Pt(SS-dach)$Cl_2$, Pt(RS-dach)$Cl_2$ ≫ *trans*-Pt(NH_3)$_2$$Cl_2$ ≳ [Pt(NH_3)$_3$Cl]Cl. Butour and Macquet [10] carried out a fluorescence study using ethidium bromide as a probe and examined the change of the secondary structure of DNA induced

TABLE I. Data Obtained from Melting Profiles and UV Difference Spectra of DNA–Platinum Systems ($r = 0.10$)[a].

	Tm (°C)	2σ (°C)	h (%)	h′ (%)	$\Delta A_{270}/\Delta A_{295}$
DNA	70.6	12.4	39.7	0	–
DNA + $[Pt(NH_3)_3Cl]Cl$	68.4	14.4	37.1	2.4	1.1
DNA + *trans*-$Pt(NH_3)_2Cl_2$	74.5	17.6	35.3	3.8	1.2
DNA + *cis*-$Pt(NH_3)_2Cl_2$	66.6	16.8	31.9	8.6	2.2
DNA + Pt(RS-dach)Cl_2	61.0	15.3	29.5	12.2	2.3
DNA + Pt(RR-dach)Cl_2	64.2	17.1	31.2	11.4	2.0
DNA + Pt(SS-dach)Cl_2	63.8	22.0	30.7	11.5	2.0

[a]Tm: Melting temperature is defined as the temperature at half denaturation. 2σ: width of transition is defined as the temperature range corresponding to 15.8% and 84.2% denaturation. h: hyperchromicity by heating. h′: hyperchromicity induced by the platinum binding.

by platinum binding. Binding of the platinum complexes to DNA inhibited the intercalation of ethidium bromide and resulted in the decrease of fluorescence. The fluorescence decreased in the order: *cis*-$Pt(NH_3)_2Cl_2$, $Pt(en)Cl_2 \gg$ *trans*-$Pt(NH_3)_2Cl_2 \gtrsim$ [Pt(dien)Cl]Cl, and $[Pt(NH_3)_3Cl]Cl$, and this order is the same as that found in this work. Tobias *et al.* [9] reported that *cis*-$Pt(NH_3)_2Cl_2$ binds with high selectivity to the guanine residues in calf thymus DNA at $r < 0.2$, using Raman difference spectroscopy, and that *cis*-$Pt(NH_3)_2Cl_2$ distorted greatly the secondary structure of DNA compared to *trans*-$Pt(NH_3)_2Cl_2$.

In order to obtain more information about the change in the secondary structure of DNA, melting profiles of DNA samples treated with platinum complexes were examined, and the results are summarized in Table I. Decrease in hyperchromicity (h) and broadening of transition width (2σ) were commonly observed. Platinum complexes, other than *trans*-$Pt(NH_3)_2Cl_2$, decreased the melting temperature (Tm). Binding of platinum complexes with DNA distorts the secondary structure of neighboring base pairs in DNA and helps to destroy the secondary structure of DNA on heating. Binding of antitumor-active platinum complexes with DNA causes a large decrease in the melting temperature when compared with that obtained for $[Pt(NH_3)_3Cl]Cl$–DNA. In contrast, *trans*-$Pt(NH_3)_2Cl_2$ increases the melting temperature. Increase in melting temperature for *trans*-$Pt(NH_3)_2Cl_2$ has been also reported by Harder [18]. This suggests that the binding mode of *trans*-$Pt(NH_3)_2Cl_2$ with DNA is quite different from that of *cis*-$Pt(NH_3)_2Cl_2$ or $[Pt(NH_3)_2Cl]Cl$. *trans*-$Pt(NH_3)_2Cl_2$ contains two labile sites, but it behaves like a monofunctional reagent in the presence of ionic chlorine atoms [7, 10]. Increase in the melting temperature cannot be explained by the monofunctional binding mode (only a N(7) binding in the guanine residues). The second reaction, in which the second chlorine atom is liberated, may take place through the heating process, and there may be a possibility for binding of the second labile site to the phosphate residues in DNA. The binding mode of *trans*-$Pt(NH_3)_2Cl_2$ to both the guanine and phosphate residues has already been proposed by Butour and Macquet [10]. This binding mode may stabilize the double helix against heat denaturation, though there is no such evidence.

If binding of platinum complexes to DNA causes the loss of base stacking, it can be expected that hyperchromicity due to heat denaturation would decrease when compared with that of native DNA. Decrease in hyperchromicity (h), which was observed in the melting profiles, suggests that the interaction with platinum complexes removes base stacking in DNA. The h′ values in Table I are hyperchromicity caused by the binding with platinum, and are calculated from ΔA at 270 nm. The sum of h and h′ is almost consistent with the hyperchromicity of native DNA. These results support the idea that the increment of ΔA at 270 nm may be attributed to the loss of base stacking. Heat denaturation affected the absorbance at 295 nm very little. This result also supports the idea that the increment of ΔA at 295 nm may be attributed to the change in electron distribution of base moieties induced by binding with platinum. That is, the results obtained from melting profiles are in good agreement with those obtained from the uv difference spectra.

Macquet and Theophanides have shown that *cis*-$Pt(NH_3)_2Cl_2$ contains two labile sites and reacts with DNA through displacement of two chlorine atoms even if ionic chlorine atoms are coexistent. In EXAFS study on the *cis*-$Pt(NH_3)_2Cl_2$–DNA compound, it was reported that four Pt–N (or –O) bonds comprised the square planar platinum coordination sphere [19]. Considering the geometry of *cis*-$Pt(NH_3)_2Cl_2$, the distance between the two leaving groups (two chlorine atoms) is similar to the interplanar base distance of DNA (3.4 Å), or the distance between

N(7) and O(6) of guanine. As to the binding mode of *cis*-$Pt(NH_3)_2Cl_2$ to DNA, an interbase crosslink [4, 20, 21], which corresponds to the intrastrand crosslink, and a chelation due to two donor atoms within one base (N(7)–O(6) atoms of the guanine residues [2, 7, 10, 11] have been proposed. In the spectrum obtained for *cis*-$Pt(NH_3)_2Cl_2$ in Fig. 4, appearance of the shoulder around 280 nm may be correlated with the binding mode mentioned above (chelation or interbase crosslink).

It may be concluded that (1) the uv difference spectral method can be used to distinguish the binding modes between antitumor-active and -inactive platinum complexes, (2) changes in the secondary structure of DNA induced by binding with antitumor-inactive platinum complexes are greater than those induced by antitumor-inactive platinum complexes, and this became apparent from the absorption ratio, $\Delta A_{270}/\Delta A_{295}$, (3) there is a linear relationship between ΔA and r, (4) the uv difference spectral patterns obtained for antitumor-active platinum complexes are similar, (5) a tendency for preferential binding with guanine residues exists in all platinum complexes, and (6) binding of platinum complexes to DNA leads to the loss of base stacking and results in decrease of hyperchromicity on heating.

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