The Interactions of (−)-(*R*)-2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) Monohydrate (DWA2114R) and Related Compounds with Deoxyribonucleic Acid

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The interactions of a new antitumor platinum (Pt) complex, (−)-(*R*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate (DWA2114R, 2) and its related compounds, *cis*-diamminedichloroplatinum(II) (CDDP, 1), *trans*-diamminedichloroplatinum(II) (TDDP, 3), (+)-(*S*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate (DWA2114S, 4), (*R*)-2-aminomethylpyrrolidinedichloroplatinum(II) (5) and *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (CBDCA, 6), with calf-thymus deoxyribonucleic acid (DNA) and DNA nucleosides were investigated by ultraviolet (UV) and circular dichroism (CD) spectrometry.

The UV spectra of the DNAs treated with these Pt complexes exhibited both bathochromic shift and hyperchromicity, showing a binding of Pt to the heterocyclic groups of these DNA as well as an alteration in the secondary structure of DNA. The reaction rates of the Pt complexes with DNA, however, differed from one another, and the order was CDDP, TDDP, 5≫DWA2114R, S>CBDCA.

The CD spectra of the DNAs treated with the Pt complexes, except TDDP, at a low Pt ratio (<approximately (*ca.*) 0.1 of Pt bound to DNA/DNA base molar ratio) exhibited an increase of ellipticity at *ca.* 275 nm.

The melting temperature of the DNAs treated with DWA2114R or CDDP were almost the same as the native DNA, while the melting temperature with TDDP was higher by 7—8 °C than that of the native DNA.

All the Pt complexes reacted with 2′-deoxyguanosine (dG), 2′-deoxyadenosine and 2′-deoxycytidine, but none reacted with thymidine. The CD spectral change of the dG was largest. DWA2114R reacted faster with dG than other nucleosides.

Keywords platinum complex; DNA; nucleoside; ultraviolet spectrum; circular dichroism; melting curve; (−)-(*R*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II); *cis*-diamminedichloroplatinum(II); *trans*-diamminedichloroplatinum(II); *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)

Since the discovery of the antitumor activity of *cis*-diamminedichloroplatinum(II) (CDDP, **1**) by Rosenberg *et al.*,[1] many analogous platinum (Pt) complexes have been synthesized and examined for their antitumor activities. We have synthesized various Pt complexes having unsymmetrical bidentate diamines, such as 2-aminomethyl-*N*-heterocycles, as carrier ligands and screened them for antitumor activity.[2] Among these complexes, (−)-(*R*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)-platinum(II) monohydrate (DWA2114R, **2**)[2e,3] proved to be the most promising candidate for development as an antitumor agent with both potent antitumor activity against various tumors and low nephrotoxicity, which is one of the serious side effects of CDDP.

CDDP is believed to exhibit its antitumor activity through interactions with deoxyribonucleic acid (DNA).[4] The binding mode of CDDP to DNA has been studied in various experiments and has been compared with that of *trans*-diamminedichloroplatinum(II) (TDDP, **3**), which has less antitumor effect.[5] In the ultraviolet (UV) spectrum of the DNA treated with CDDP, bathochromic shift and hyperchromicity, which indicate the binding of Pt to the heterocyclic groups of DNA and a change of the secondary structure of DNA, were observed.[6] In a circular dichroism (CD) spectral study, the enhancement of the ellipticity of the positive band at approximately (*ca.*) 275 nm, which indicates a distortion of the double helical conformation of DNA, was seen when DNA was treated with CDDP at molar ratios lower than a 1 : 10 Pt/DNA base ratio.[7] However, enhancement of the ellipticity at *ca.* 275 nm was not observed with TDDP.[7] At a 1 : 25 Pt/DNA base ratio, the melting temperature of the DNA treated with CDDP was almost the same as that of the native DNA, but that with TDDP was higher.[7b] These results suggest that the binding mode of CDDP to DNA must definitely be different from that of TDDP, and that CDDP might form DNA intrastrand crosslinking.

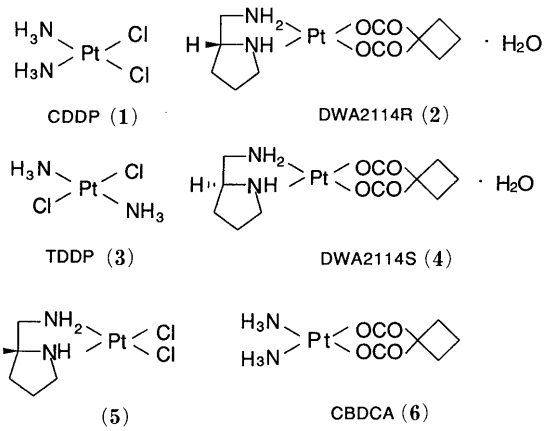

Fig. 1. The Structures of the Pt Complexes Used in the Present Study

Examination of the interaction of DWA2114R with DNA is of great interest because its carrier ligand and leaving group are completely different from CDDP. In this paper, we report the interaction of DWA2114R with calf-thymus DNA using UV and CD spectroscopy, and compare the findings with those of CDDP and TDDP. We also report the interaction of DWA2114R with DNA nucleosides, 2′-deoxyguanosine (dG), 2′-deoxyadenosine (dA), 2′-deoxycytidine (dC) and thymidine by the same means, so as to analyze the Pt–DNA interactions in more detail. The interactions of DWA2114R related compounds, (+)-(*S*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate (DWA2114S, **4**), (*R*)-2-aminomethylpyrrolidinedichloroplatinum(II) (**5**) and *cis*-

diammine(1,1-cyclobutanedicarboxylato)platinum(II) (CBDCA, **6**)[8] with DNA were also examined, in order to establish the relationships between the structure of Pt complexes and their reactivity for DNA.

Experimental

Apparatus UV absorption spectra were recorded on a Hitachi U-3200 spectrophotometer. CD spectra were measured on a JASCO spectropolarimeter J-500A equipped with a data processor. The amounts of Pt were measured by atomic absorption spectrophotometry using a Jarrell Ash AA-8500 Mark II.

Materials Calf-thymus DNA (type 1) was purchased from Sigma Chemical Co. DNA was put into 10 mM of an aqueous $NaClO_4$ followed by gentle shaking for 2 d in a cold room (4 °C). The concentration of DNA was determined by UV spectrophotometry as $\varepsilon_{260}=6400$ $M^{-1}cm^{-1}$. dG, dA and thymidine were purchased from Tokyo Kasei Kogyo Co., Ltd. dC, CDDP (**1**) and TDDP (**3**) were purchased from Aldrich Chemical Company, Inc. CBDCA (**6**) was synthesized according to the reported method.[9] DWA2114R (**2**), DWA2114S (**4**) and (**5**) were synthesized according to the methods previously described.[2e]

Interaction of DNA with Pt Complexes Reaction Procedure: The Pt complexes were dissolved in 10 mM of an aqueous $NaClO_4$ just before use. An aliquot of the solution was added to the DNA solution so that the final concentration of the DNA was 1×10^{-4} M (P), and the mixture was then incubated at 25 °C. The molar ratio of Pt complex to DNA base was adjusted by changing the Pt complex concentration in the reaction mixture.

Measurements of UV and CD Spectra: UV difference spectra were measured at room temperature with two 1-cm cells for the sample and the reference in a range of 320—220 nm. The sample cell contained the reaction solution, and the reference cell contained the solution of DNA and the Pt complex mixed at the same concentration just before measurement.

CD spectra were measured at room temperature with a 1-cm cell in a range of 310—230 nm. The data are expressed in terms of molar ellipticity, $[\theta]$, in $deg\cdot cm^2/dmol^{-1}$. CD spectra of the DNA treated with the optically active Pt complexes were obtained by subtracting the spectra of the corresponding Pt complexes at the same concentration from the spectra of the reaction solution.

Melting curves were recorded automatically at 260 nm using a spectrophotometer by increasing the temperature at a rate of 0.5 °C/min.

Measurements of Pt Amounts Bound to DNA: After the mixture of DNA and Pt complexes was incubated at 25 °C for a desired period, the reaction solutions were dialyzed against 10 mM of an aqueous $NaClO_4$ at 4 °C using a diffusion shell (Visking Company, Cellulose Tubing) to remove the free Pt complexes. After dialysis, a 60% HNO_3 solution (1 ml) was added to the solution (1 ml) of DNA with the bound Pt complexes and heated at 80 °C until the solution dried up. The obtained residues were dissolved in a 0.1 N HNO_3 solution (1 ml) and the Pt amount in the solution was measured by atomic absorption spectrophotometry.

Interaction of Nucleosides with Pt Complexes Reaction Procedure: The concentrations of stock solutions containing Pt complexes and nucleosides were made so as to be 2×10^{-3} M in H_2O. The stock solution of each Pt complex was mixed with that of the indicated nucleosides in a molar ratio of 1:1 (v/v: at a final concentration of 10^{-3} M, but in the case of TDDP-nucleosides at 5×10^{-4} M). These mixtures were incubated at 25 or 60 °C for a desired period.

Measurement of UV and CD Spectra: All the reaction solutions of the nucleosides and the Pt complexes, except the TDDP-nucleoside solution, were diluted with a ten-fold volume of H_2O just before the measurements. The TDDP-nucleoside solutions were diluted with a five-fold volume of H_2O just before the measurements.

UV spectra were measured as described above. In the measurement of the UV difference spectra, the sample cell contained the diluted reaction solution and the reference cell contained the solution of the nucleoside and the Pt complex (each of the concentrations: 10^{-4} M) mixed just before measurement.

CD spectra were measured as described above. CD spectra of the nucleoside treated with the optically active Pt complexes were obtained by subtracting the spectra of the corresponding Pt complexes from the spectra of the reaction solution.

Results

Interaction of DNA with Pt Complexes 1) UV Spectral Study In Fig. 2 are illustrated the UV difference spectra of the DNA treated with the Pt complexes.

When DNA was treated with CDDP for 72 h, a maximum at *ca.* 270 nm and a minimum at *ca.* 250 nm were

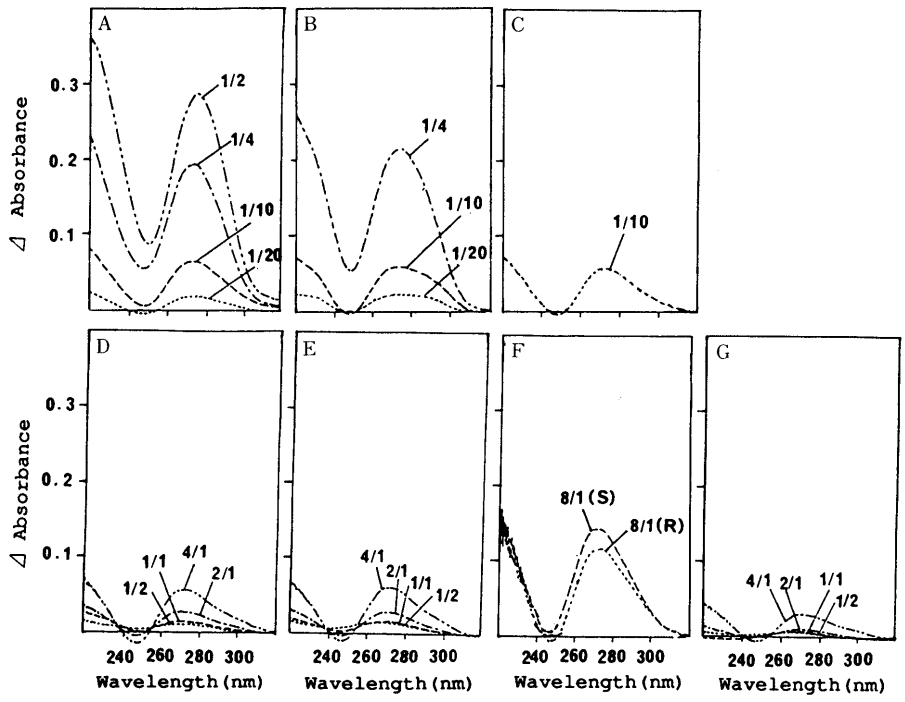

Fig. 2. The Changes of the UV Difference Spectra of Calf Thymus DNA Interacting with the Pt Complexes

The results of the difference spectra are plotted as the absorbance at a given wavelength in a 1-cm path. The numbers indicate moles Pt present/DNA base. DNA (P) concentration: 1×10^{-4} M.

(A) CDDP/DNA, 72 h; (B) TDDP/DNA, 72 h; (C) **5**/DNA, 72 h; (D) DWA2114R/DNA, 144 h; (E) DWA2114S/DNA, 144 h; (F) DWA2114R and DWA2114S, 144 h; (G) CBDCA/DNA, 144 h.

observed in the UV difference spectra (Fig. 2A). Almost the same spectral features were observed for both the TDDP–DNA and **5**–DNA systems (Fig. 2B and C) except for a shoulder at *ca.* 295 nm seen in the TDDP–DNA system (Fig. 2B).

On the other hand, the spectral changes of the DNA when treated with the Pt complexes (DWA2114R, DWA2114S, and CBDCA) having 1,1-cyclobutanedicarboxylato as a leaving group were very slow compared with those of the DNAs treated with the Pt complexes having dichloro as a leaving group. The interactions of DNA with these Pt complexes were, therefore examined in high Pt molar ratios and a reaction time of 144 h. These UV difference spectral patterns were similar to those of the CDDP–DNA or **5**–DNA systems (Fig. 2D—G). Based on the UV spectral change at a 8 : 1 Pt/DNA base ratio, the extent of the DNA spectral change in the DWA2114S–DNA system was larger than that in the DWA2114R–DNA system (Fig. 2F). It is also worthy of note that the extents of the spectral changes of the DNA treated with DWA2114R were obviously larger than those with CBDCA at every molar ratio, *i.e.* 1 : 2, 1 : 1, 2 : 1 or 4 : 1 Pt/DNA base ratios (Fig. 2D and G).

2) Measurements of Pt Amounts Bound to DNA The amounts of Pt bound to the DNAs treated at 25°C with the dichloro complexes (CDDP and TDDP) and the dicarboxylato complexes (DWA2114R, DWA2114S and CBDCA) at a 1 : 10 and 4 : 1 Pt/DNA base ratio for 72 and 144 h, respectively, were measured. The difference absorbances (ΔA_{270} and ΔA_{295}) at 270 and 295 nm, and the amounts of Pt bound to DNA are shown in Table I.

TABLE I. UV Spectral Changes in the Mixture of Pt Complexes with DNA and Comparison of the Number of Pt Bound to DNA among Various Pt Complexes

Compound	Reaction condition		Result			
	Pt/DNA base[a]	Time (h)[b]	ΔA_{270}[c]	ΔA_{295}[d]	$\Delta A_{270}/\Delta A_{295}$	Bound Pt/DNA base[a]
CDDP	0.1	72	0.054	0.022	2.45	0.090
TDDP	0.1	72	0.054	0.034	1.59	0.087
DWA2114R	4	144	0.052	0.026	2.00	0.080
DWA2114S	4	144	0.061	0.027	2.26	0.080
CBDCA	4	144	0.027	0.011	2.45	0.048

a) Molar ratio. *b*) Reaction time at 25 °C. *c*) UV difference absorbance at 270 nm between the DNA treated with Pt complexes and uncomplexed DNA. *d*) UV difference absorbance at 295 nm between the DNA treated with Pt complexes and uncomplexed DNA.

The ΔA_{270} in the CDDP–DNA system is the same as that in the TDDP–DNA system, but the ΔA_{295} in the TDDP–DNA system is larger than that in the CDDP–DNA system. In both systems, *ca.* 90% of the applied amount of Pt complex was found to bind to DNA.

On the other hand, only *ca.* 2% of the applied Pt complex bound to the DNA in DWA2114R–DNA and DWA2114S–DNA systems. Interestingly, although the ΔA_{270} with DWA2114S was larger than that with DWA2114R, the amounts of Pt bound to the DNA base were the same. In the CBDCA–DNA system, only *ca.* 1.2% of the applied Pt complexes bound. The ratios, $\Delta A_{270}/\Delta A_{295}$, are larger than 2 for the platinum complexes with *cis*-coordinated carrier ligands (CDDP, DWA2114R, DWA2114S and CBDCA), while the ratio for TDDP is 1.59.

3) CD Spectral Study Figure 3 shows CD spectra of the DNA treated with CDDP, TDDP or **5** at 25°C for 72 h.

In the CD spectra of the CDDP–DNA system, bathochromic shifts and changes of the positive Cotton effects were observed. The positive band at *ca.* 275 nm increased at a 1 : 20 or 1 : 10 Pt/DNA base ratio, but decreased at a 1 : 4 or 1 : 2 Pt/DNA base ratio (Fig. 3A). In contrast, in the CD spectra of the TDDP–DNA system, the bathochromic shifts were observed, but the ellipticities at *ca.* 275 nm show no transient increase and instead continuous decrease (Fig. 3B). These results of the two systems were in good agreement with previously published work.[7a,b] In the CD spectra **5**–DNA system, the ellipticity at *ca.* 275 nm increased at a 1 : 10 Pt/DNA base ratio and was almost the same as that of the CDDP–DNA system at a 1 : 10 Pt/DNA base ratio (Fig. 3C).

The CD spectra of the dicarboxylato Pt complexes, DWA2114R, DWA2114S and CBDCA are shown in Fig. 4.

At 1 : 1, 2 : 1, 4 : 1 and 8 : 1 Pt/DNA base ratios, the ellipticity at *ca.* 275 nm of the DNA treated with

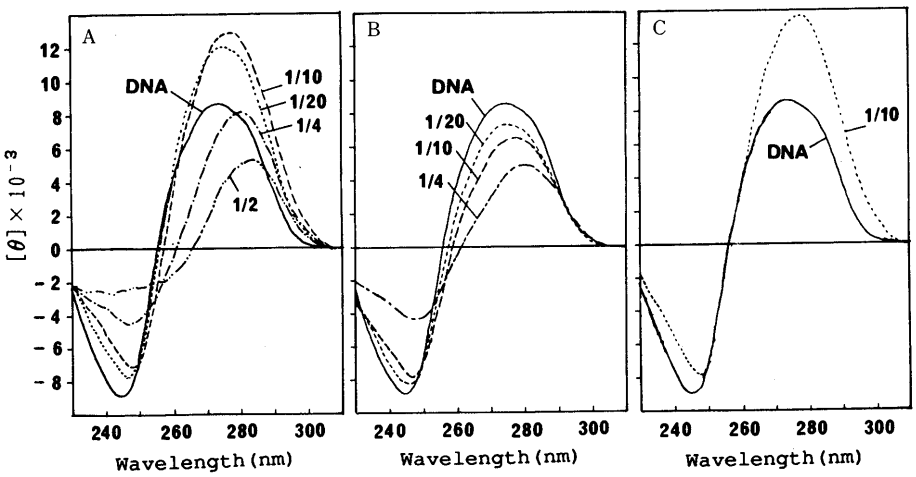

Fig. 3. The Effect of the Pt Complexes on the CD Spectra of Calf Thymus DNA

The numbers indicate moles Pt present/DNA base. DNA (P) concentration: 1×10^{-4} M.
(A) CDDP/DNA, 72 h; (B) TDDP/DNA, 72 h; (C) **5**/DNA, 72 h.

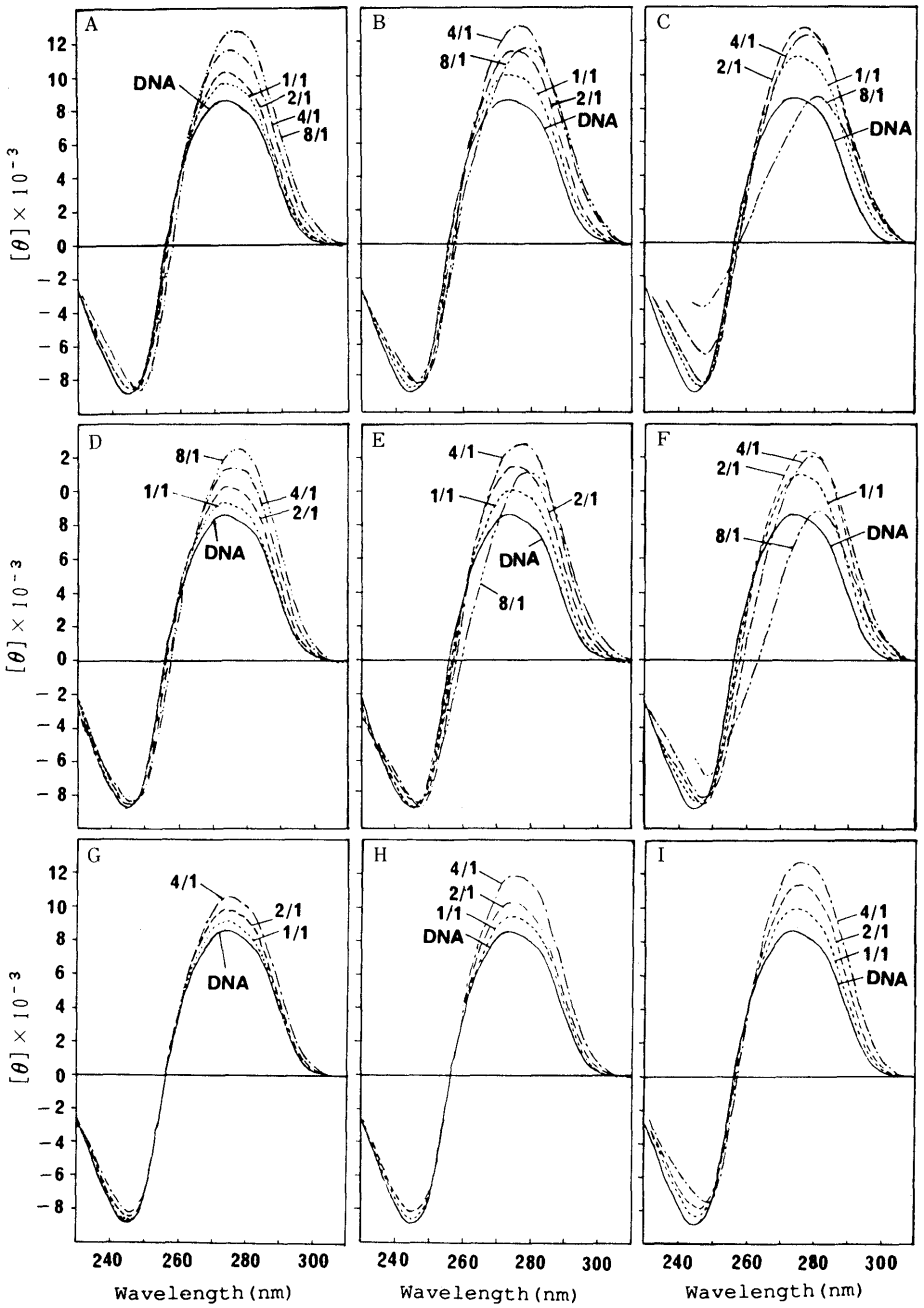

Fig. 4. The Effect of the Pt Complexes on the CD Spectra of Calf Thymus DNA

The numbers indicate moles Pt present/DNA base. DNA (P) concentration: 1×10^{-4} M.
(A) DWA2114R/DNA, 72 h; (B) DWA2114R/DNA, 144 h; (C) DWA2114R/DNA, 240 h; (D) DWA2114S/DNA, 72 h; (E) DWA2114S/DNA, 144 h; (F) DWA2114S/DNA, 240 h; (G) CBDCA/DNA, 72 h; (H) CBDCA/DNA, 144 h; (I) CBDCA/DNA, 240 h.

DWA2114R for 72 h continuously increased as the ratio of Pt/DNA base increased (1 through 8) (Fig. 4A). When these reaction solutions stood for 144 h, the ellipticity increased further with the ratios of 1 : 1, 2 : 1 and 4 : 1, but decreased for the ratio of 8 : 1 (Fig. 4B). After 240 h of standing, the ellipticity further increased with 1 : 1 and 2 : 1 Pt/DNA base ratio, but decreased for the 4 : 1 and 8 : 1 Pt/DNA base ratios (Fig. 4C). The positive band with DWA2114S at *ca.* 275 nm changed in a similar fashion as those observed in the DWA2114R–DNA system (Fig. 4D—F). The ellipticity at *ca.* 275 nm continuously increased with CBDCA in all cases, with time and in a dose-dependent manner (Fig. 4G—I).

4) Denaturation Study In Fig. 5 are illustrated the melting curves of the calf thymus DNA treated with CDDP or TDDP at a 1 : 20 Pt/DNA base ratio at 25 °C for 72 h and with DWA2114R at a 2 : 1 Pt/DNA base ratio at 25 °C for 144 h. The amounts of Pt bound to the DNA are apparently almost the same since the amplitudes of the UV difference spectra under these conditions were almost the same (Fig. 2A, B and D).

Although the melting temperatures of the DNAs treated with CDDP or DWA2114R were lower by 2—3 °C than that of the uncomplexed DNA, the melting curves of these DNAs resemble that of the uncomplexed DNA. The melting curve of the DNA treated with TDDP, however, shifted to a much higher temperature region, and the melting temperature was higher by 7—8 °C than that of

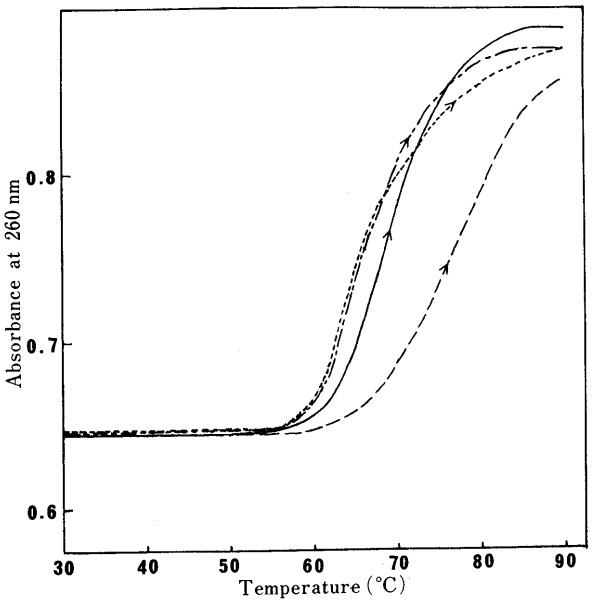

Fig. 5. Melting Curves of Calf Thymus DNA Incubated with the Pt Complexes at Room Temperature

The results of the UV spectra are plotted as the absorbance at 260 nm at a given temperature in a 1-cm path. DNA (P) concentration: 1×10^{-4} M.
Pt/DNA base ratio: ——, 0; – – –, 1/20 (CDDP, 72 h); ——, 1/20 (TDDP, 72 h); —·—, 2/1 (DWA2114R, 144 h).

uncomplexed DNA.

Interaction of DNA Nucleosides with Pt Complexes
1) UV Spectral Study In Fig. 6 are illustrated the UV difference spectra of the nucleosides treated with the Pt complexes at a 1 : 1 molar ratio at 25 °C. The spectral changes of thymidine with the Pt complexes were not observed at all in any instance (data not shown).

The difference spectra exhibited the maxima at *ca.* 260 nm and *ca.* 295 nm when dGs were treated with CDDP, TDDP or **5** for 72 h, and these spectral patterns were similar to each other (Fig. 6A). Almost the same spectral features were also observed with DWA2114R, DWA2114S or CBDCA for 144 h (Fig. 6B), although the amplitude, particularly that with CBDCA, was very small. No difference between the DWA2114R–dG and DWA2114S–dG systems was found in these UV difference spectra.

The difference spectra exhibited the maximum at *ca.* 280 nm when dAs were treated with CDDP, TDDP or **5** for 72 h, and these spectral patterns were similar (Fig. 6C). The spectral features of the dAs treated with DWA2114R, DWA2114S or CBDCA for 144 h were also almost the same (Fig. 9D). However, these difference spectra were very small. No difference between the DWA2114R–dA and DWA2114S–dA systems was found in these UV difference spectra.

Difference spectra exhibiting an absorption maximum at *ca.* 290 nm were obtained when dCs were treated with CDDP, TDDP or **5** for 72 h, and the spectral patterns were similar (Fig. 6E). The difference spectra of the dCs treated with DWA2114R, DWA2114S or CBDCA for 144 h changed little (Fig. 6F).

Next, the time–course of the changes in the UV diffference spectra of the DWA2114R–dG, –dA and –dC systems were examined. Each nucleoside was treated with DWA2114R at a 1 : 1 molar ratio at 60°C, and the diffference absorbance

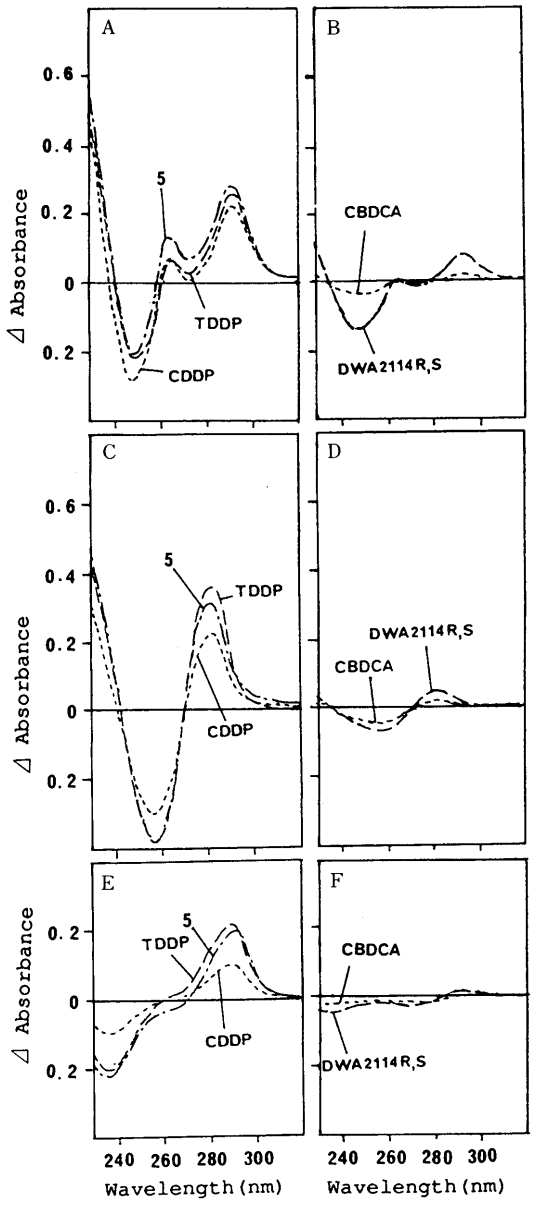

Fig. 6. The UV Difference Spectra of Nucleosides Interacting with the Pt Complexes

The results of the difference spectra are plotted as the absorbance at a given wavelength in a 1-cm path. The Pt: nucleoside molar ratio was 1 : 1 in every case. Pt complexes and nucleoside concentration: 1×10^{-4} M.
(A) Pt (CDDP, TDDP, **5**)–dG, 72 h; (B) Pt (DWA2114R, DWA2114S, CBDCA)–dG, 144 h; (C) Pt (CDDP, TDDP, **5**)–dA, 72 h; (D) Pt (DWA2114R, DWA2114S, CBDCA)–dA, 144 h; (E) Pt (CDDP, TDDP, **5**)–dC, 72 h; (F) Pt (DWA2114R, DWA2114S, CBDCA)–dC, 144 h.

(ΔA_{295}) at 295 nm for the DWA2114R–dG system, that (ΔA_{280}) at 280 nm for the DWA2114R–dA system, and that (ΔA_{290}) at 290 nm for the DWA2114R–dC system, were measured and are shown in Fig. 7. The ΔA_{295} of DWA2114R–dG is seen to reach a constant value after 120 h, while the ΔA_{280} of DWA2114R–dA and the ΔA_{290} of DWA2114R–dC did not become constant even after 192 h.

2) CD Spectral Study The CD spectra of the nucleoside treated with the Pt complexes in a molar ratio of 1 : 1 at 25 °C are shown in Fig. 8. No spectral changes were found at all on treatment of thymidine with each Pt complex (data not shown).

The CD spectra of dG were changed greatly with

CDDP, TDDP or **5** for 72 h, and the resultant spectra depended on the Pt complex structures (Fig. 8A). Also, when dG was treated with DWA2114R or DWA2114S for 144 h, the obtained spectra were different from each other (Fig. 8B). The spectral change of the dG with CBDCA was very small (Fig. 8B). These results suggest that the three-dimensional structure of dG in the Pt–dG adduct is affected by the structures of the carrier ligands.

The extents of the spectral changes of the dAs treated with CDDP, TDDP or **5** for 72 h, and with DWA2114R, DWA2114S or CBDCA for 144 h, were much less than those of the Pt–dG interactions (Fig. 8C and D).

Bathochromic shifts were observed in the CD spectra of the dCs treated with CDDP, TDDP or **5** for 72 h (Fig. 8E). However, these spectra were almost the same as uncomplexed dC. The spectra of the dC, in contrast, changed little when the dCs were treated with DWA2114R, DWA2114S or CBDCA for 144 h (Fig. 8F).

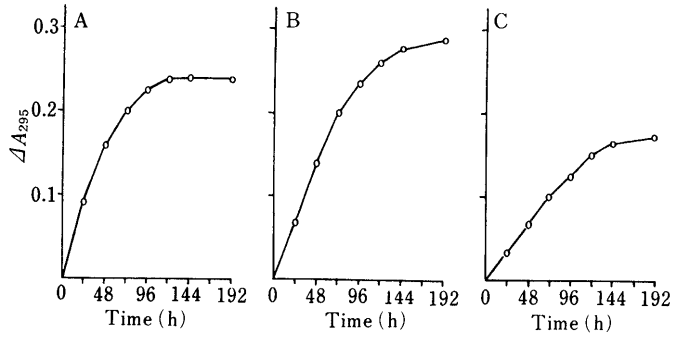

Fig. 7. The Time-Dependent Changes of the Difference UV Absorption (ΔA) of dG (A), dA (B) and dC (C) Interacting with DWA2114R at 60 °C at a Molar Ratio of 1 : 1

The difference absorption was measured at 295 nm about DWA2114R–dG, at 280 nm about DWA2114R–dA, and measured at 290 nm about DWA2114R–dC in a 1-cm path. The DWA2114R: nucleoside molar ratio was 1 : 1 each case. DWA2114R and nucleoside concentration: 1×10^{-4} M.

Discussion

The patterns of the UV and CD spectral changes of the DNA treated with DWA2114R were very similar to those treated with CDDP, but differed from those treated with TDDP in many respects. This suggests that the binding mode of DWA2114R with DNA might be almost the same as that of CDDP, but must be quite different from that of TDDP.

The first point in favor of the similarity between DWA2114R and CDDP, and a difference between DWA2114R and TDDP, is the UV difference spectral patterns (Fig. 2). The UV difference spectra of the DNA treated with TDDP exhibited a maximum at *ca.* 270 nm with a shoulder at *ca.* 295 nm in a range of 250—320 nm (Fig. 2B), but a definite shoulder at *ca.* 290 nm was not observable with DWA2114R and CDDP (Fig. 2A and D). Consequently, the absorption ratios, $\Delta A_{270}/\Delta A_{295}$, were larger than 2 for DWA2114R and CDDP, while this ratio was about 1.6 for TDDP (Table I). The transient increase of

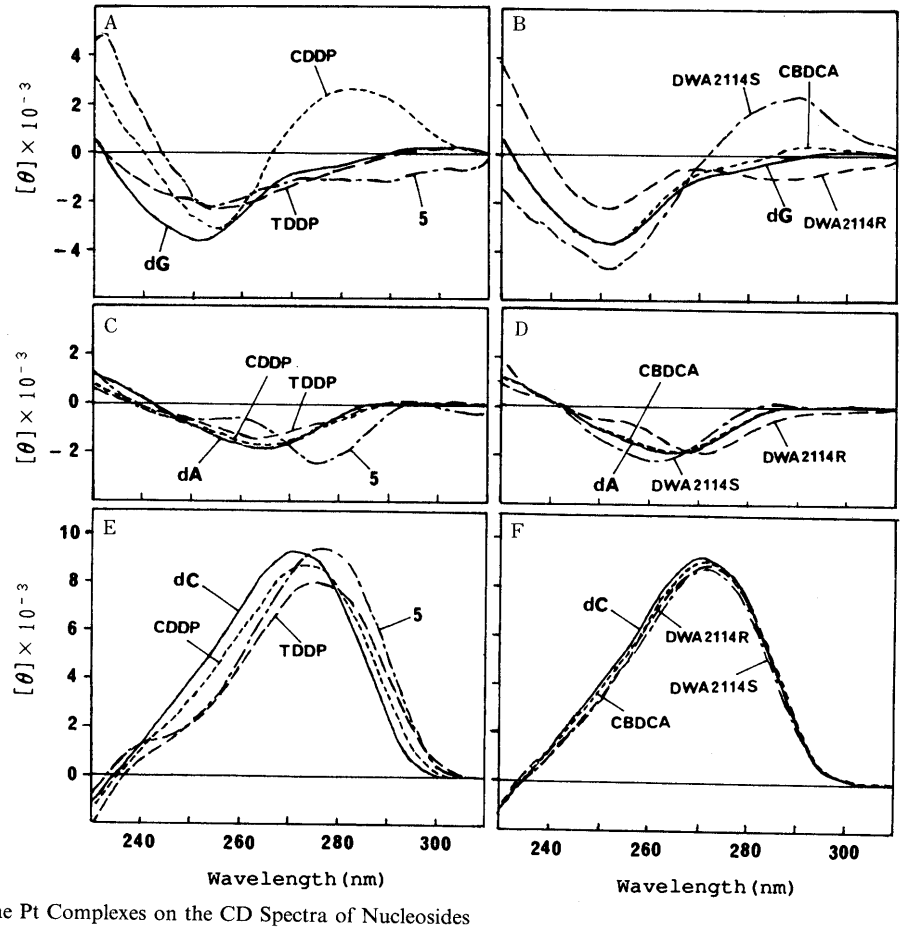

Fig. 8. The Effect of the Pt Complexes on the CD Spectra of Nucleosides

The Pt: nucleoside molar ratio was 1 : 1 in each case. Pt complexes and nucleoside concentration: 1×10^{-4} M.
(A) Pt (CDDP, TDDP, **5**)–dG, 72 h; (B) Pt (DWA2114R, DWA2114S, CBDCA)–dG, 144 h; (C) Pt (CDDP, TDDP, **5**)–dA, 72 h; (D) Pt (DWA2114R, DWA2114S, CBDCA)–dA, 144 h; (E) Pt (CDDP, TDDP, **5**)–dC, 72 h; (F) Pt (DWA2114R, DWA2114S, CBDCA)–dC, 144 h.

the absorbance at 295 nm in the Pt–DNA seems to be considerably affected by the increase in the absorbance of the dG on the binding with Pt complexes, because the increases of the absorbance at *ca.* 295 nm were observed in the UV difference spectra of the dG with the Pt complexes (Fig. 6). On the other hand, the transient increases in the absorbance at *ca.* 270 nm are attributable to the loss of base stacking of DNA, because the increments of this absorbance were very small or negligible for each DNA nucleoside with the Pt complexes (Fig. 6). This interpretation concerning the ΔA_{270} and the ΔA_{295} has already been proposed by Inagaki and Kidani,[6b] and it has been pointed out that 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 Pt, and that the antitumor-active Pt complexes have a ratio larger than 2. The absorption ratios, $\Delta A_{270}/\Delta A_{295}$, in the DNA treated with antitumor-active DWA2114S, **5** or CBDCA were also larger than 2.

The second point in favor of a similarity between DWA2114R and CDDP and a difference between DWA2114R and TDDP is the CD spectral patterns. In the CD spectral changes of the DWA2114R–DNA system (Fig. 4A—C), the magnitude of the positive band at *ca.* 275 nm of the DNA continuously increased as the amount of Pt bound to DNA up to a fixed amount of Pt, and gradually decreased when the amount of bound Pt exceeded this fixed amount. This pattern is very similar to that for the DNA–CDDP system (Fig. 3A) in respect of having the two phases of ellipticity increase and decrease at *ca.* 275 nm. The turning point from the increase to the decrease might be at the molar ratio of Pt bound to the DNA/DNA base of *ca.* 0.08, because the ellipticity at *ca.* 275 nm showed the maximum when the reaction mixture of DWA2114R–DNA at 4:1 Pt/DNA base was standing at 25 °C for 144 h. In CDDP, the turning point might be at the molar ratio of Pt bound to DNA/DNA base of *ca.* 0.1 based upon the results of the present study as well as those of reports published earlier.[7a,b] The two sets of results on the turning point almost coincide in the amount of Pt bound to DNA. The increase of the ellipticity at *ca.* 275 nm observed for the DNA treated with DWA2114R seems not to be due to the change in the ellipticity of each nucleoside with Pt complexes, since the ellipticity in the range of 270—300 nm was decreased for all the nucleosides with DWA2114R (Fig. 8B, D and F); rather, it seems due to a change in the secondary structure of the DNA, probably a considerable distortion of the double helical conformation. It seems that the DNA double helix remains essentially intact during the increase of ellipticity at *ca.* 275 nm, but the local defect is brought about during the decrease, since no ellipticity increase at *ca.* 275 nm is seen for denatured DNA–CDDP.[7a]

The local distortion of the double helical conformation could be caused mainly by the binding of the Pt complexes to the dG, since CDDP[10] binds to dG preferentially and DWA2114R reacts faster with dG than other nucleosides (Fig. 7). The transient increases of the ellipticity at *ca.* 275 nm were also observed in DWA2114S–DNA, **5**–DNA and CBDCA–DNA systems. Kidani and his colleagues[7c] have reported that the transient increase in the ellipticity at *ca.* 275 nm was observed for DNA treated with the Pt complexes having 1,2-diphenylethylenediamine as a carrier ligand. These results suggest that the transient increase of the ellipticity at *ca.* 275 nm might be a common phenomenon observed for the interaction between DNA and the Pt complexes which have a *cis*-coordinated carrier ligand.

The third point in favor of a similarity between DWA2114R and CDDP and a difference between DWA2114R and TDDP is the change in the melting curve of the DNA with these Pt complexes. The melting points with DWA2114R or CDDP fell by 2—3 °C compared with the uncomplexed DNA (Fig. 5). The reason for this decrease may be as follows: the change in the interactions between neighboring base pairs of DNA enhances the destruction of the secondary structure of DNA, and helps to destroy the secondary structure of DNA on heating. The DWA2114R–DNA or CDDP–DNA binding does not contribute to the stabilization of the double helix of DNA, and so their Pt complexes can be expected to bind with neighboring bases in the same strand. In contrast, the melting curve with TDDP shifted to a higher temperature region than that of uncomplexed DNA, and the melting temperature of that DNA increased by 7—8 °C (Fig. 5). This TDDP–DNA binding does contribute to the stabilization of the double helix, and therefore TDDP might form interstrand crosslinking or binding to both the guanine and phosphate residue.[11]

The results discussed above suggest that the binding mode of DWA2114R to DNA might be quite similar to that of CDDP, and this result agrees with the fact[12] that the mechanism of the inhibition of DNA synthesis by DWA2114R is quite similar to that by CDDP.

On the other hand, a definite difference between DWA2114R and CDDP is shown in reactivity toward DNA. The reactivity of DWA2114R with DNA is about 80 times lower than that of CDDP since the CD spectra of the DNA treated with DWA2114R at a 4:1 Pt/DNA base ratio at 25 °C for 72 h is almost the same as that of the DNA treated with CDDP at a 1:20 Pt/DNA base ratio at 25 °C for 72 h (Figs. 3A and 4A). The difference in the reactivity toward DNA between DWA2114R and CDDP is due to the difference in the leaving group, since the reactivity of **5** for DNA is almost the same as that of CDDP and much higher than that of DWA2114R. The leaving group of 1,1-cyclobutanedicarboxylato is expected to have the reactivity lower than dichloro due to the stabilization effect by chelation. This may be related to the fact that the effective dose in tumor therapy of DWA2114R is much higher than that of CDDP.[2e,3b]

It is worthy noting that the reactivity of DWA2114R for DNA is much higher than that of CBDCA, and this difference should be considered to be due to the difference of the nature of the carrier ligands. This difference can be ascribed to the "*trans*-effect".[13] According to this rule, a Pt–ligand bond in the position *trans* to a ligand bound tightly to Pt tends to be weakened. The Pt–N (carrier ligand) bond in DWA2114R[14] may be stronger than the Pt–N (carrier ligand) bond in CBDCA because the pKa of the pyrrolidine (11.11) is larger than that of ammonia (9.26). Therefore, the leaving group of DWA2114R tends to be more easily substituted with DNA bases than that of CBDCA. Indeed, it has been demonstrated that the

reaction rate of DWA2114R with guanosine is faster than that of CBDCA.[15]

A difference between DWA2114R and DWA2114S in the UV spectra of Pt–DNA interactions was observed, and the transient increase in the UV absorbance at *ca.* 270 nm with DWA2114S was larger than that with DWA2114R (Fig. 2F). This suggests that the extent of the change in the DNA secondary structure for the DWA2114S–DNA system might be larger than that in the DWA2114R–DNA, and may be related to the fact that DWA2114S exhibits a slightly more potent antitumor effect *in vivo* than DWA2114R at the same dose.[2e,3a] However, the difference in their UV spectra is small, and there was no difference in the amounts of Pt bound to DNA between DWA2114R and DWA2114S when treated with DNA under the same conditions; nor have differences been observed in the local denaturation of the DNAs treated with these Pt complexes.[16] For these reasons, the difference between the antitumor effects seen *in vivo* for DWA2114R and DWA2114S should be viewed as being due to other factors (*e.g.*, the difference of uptake in tumor cells[3a]), rather than to the interactions with DNA.

Acknowledgement The authors are indebted to Professor I. Harada of Tohoku University for his helpful advice throughout this work.

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