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Premelting Phenomenon in DNA Caused by the Anti-Tumor Drug *cis*-dichlorodiammineplatinum

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Introduction

Premelting in deoxyribonucleic acid (DNA) has been demonstrated by an increase in the ellipticity of the positive band of circular dichroism (CD) with increasing temperature, when the ultraviolet (UV) absorbance remains unchanged prior to thermal melting of DNA in aqueous solution [1, 2]. Premelting conformational changes may have considerable biological significance in recombination, transcription and replication processes since DNA functions *in vivo* far below the temperature of melting. Premelting phenomenon has also been demonstrated, when local structural changes in DNA are caused by processes such as superhelical turns in closed circular DNA, pyrimidine dimers induced by UV-light and damage to the bases caused by moderate doses of gamma-radiation [2]. We report here that *cis*-$Pt(NH_3)_2Cl_2$ (*cis*-platinum), which is gaining widespread clinical use as an antitumor drug [3, 4], causes DNA premelting at low doses of Pt/P (molar ratio of platinum to DNA-phosphorus). In contrast, its isomer, *trans*-$Pt(NH_3)_2Cl_2$ (*trans*-platinum), which shows no antitumor activity [4], does not cause this premelting phenomenon.

Experimental

The platinum compounds prepared in this laboratory were dissolved at 1 m*M* concentration in doubly-distilled water and stored in the dark as described [5]. A clarified stock solution of salmon sperm DNA in the native state having a molar absorption coefficient with respect to DNA-phosphorus (DNA-P) of 6320 M^{-1} cm^{-1} at 260 nm was prepared as described [5]. Reactions of each platinum compound at increasing Pt/P doses were carried out for at least 14 days in the dark at 25 °C with a constant DNA-P concentration of either 54.5 μM or 218 μM in 4 m*M* NaCl. Control DNA samples were also subjected to the conditions of reaction. CD spectra were taken (JASCO Model ORD/UV-5, SS-20 CD modification) at the DNA-P concentration of 218 μM. UV absorbance (260 nm) measurements at DNA-P concentration of 54.5 μM were as described [5]. Renaturation of DNA caused by platinum binding was determined by measuring the change of absorbance (260 nm) after thermal denaturation (10 min at 80 °C followed by rapid cooling on ice and equilibration at 25 °C) with respect to the absorbances before and after thermal denaturation of the control DNA sample, as described [6]. Control DNA showed 25% increase in the absorbance after such thermal denaturation.

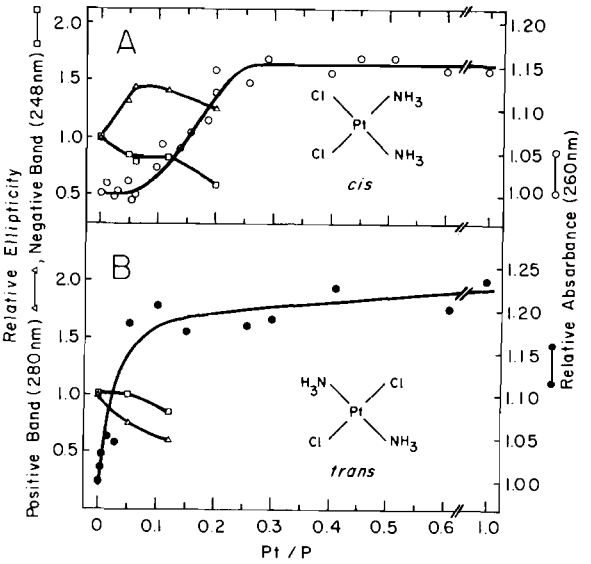

Fig. 1. Effects of increased binding of (A) *cis*-platinum and (B) *trans*-platinum to native salmon sperm DNA in 4 m*M* NaCl observed by CD and UV spectroscopy at 25 °C. Relative ellipticity of either the positive or the negative CD band is the ratio between the ellipticity at the given Pt/P dose and that of the control DNA sample. Relative absorbance is the ratio between the absorbance at the given Pt/P dose and that of the control sample.

Results and Discussion

Increased binding of *cis*-platinum to a native DNA at 25 °C caused premelting and melting of DNA as shown by the sigmoidal absorbance profile (Fig. 1A). In the premelting region with unchanged absorbance, the ellipticity of the positive CD band increased with increasing Pt/P doses up to about 0.06 before decreasing again gradually from its maximum value with the onset of DNA melting at higher Pt/P doses. The

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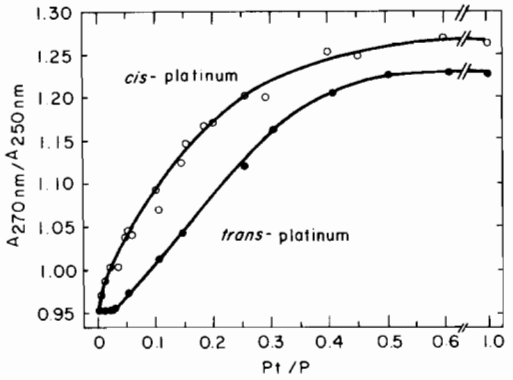

Fig. 2. Platinum binding to DNA bases shown by increases in the UV spectral ratio $A_{270\,nm}/A_{250\,nm}$ with increasing Pt/P doses. Data for DNA treated with *cis*-platinum (○——○) and with *trans*-platinum (●——●) were obtained from the samples described in Fig. 1.

negative CD band on the other hand showed a continuous decrease in ellipticity. These features are remarkably similar to those reported for DNA subjected to increasing temperatures (see Fig. 2 of ref. 2). In contrast, increased binding of *trans*-platinum caused increased melting of DNA secondary structure in that the absorbance profile (Fig. 1B) showed no premelting region even at Pt/P doses near zero. Both the positive and negative CD bands showed a continuous decrease in their ellipticity. Our CD spectral results are essentially consistent with earlier findings [7–10]. However, UV spectral results comparable to the present work at increasing Pt/P doses have not been published previously, although it has been reported that *cis*-platinum or/and *trans*-platinum can cause spectral hyperchromic and bathochromic shifts on binding to DNA [5, 7, 9, 11, 12]. The present finding that low doses of *cis*-platinum cause DNA premelting was not noticed earlier [7–10].

Several studies [4, 13, 14] suggest that DNA is the primary target for the antitumor activity of *cis*-platinum which binds irreversibly to the DNA bases [11, 15] in the order guanine > adenine > cytosine and predominantly to guanines at low Pt/P doses [12]. Significance of the *cis*-geometry in the antitumoral action of this drug, as it appears from our present findings, lies in its ability to reach and bind to guanine bases at extremely low Pt/P doses. Such binding produces irreversible premelting conformational changes in the DNA molecule without causing melting as indicated by the unchanged absorbance (Fig. 1A). The binding of *cis*-platinum to DNA bases even at Pt/P doses approaching zero is shown (Fig. 2) by the increased ratio of absorbances at 270 nm and 250 nm ($A_{270\,nm}/A_{250\,nm}$). This ratio gives a direct measure of the spectral batochromic shift [5] and is correlated with platinum binding to DNA bases [5, 7, 12]. In contrast, *trans*-platinum at low Pt/P doses below 0.05 showed no binding to DNA bases (Fig. 2). However, it was evidently bound to other sites of DNA causing DNA melting as shown by the increased absorbance (Fig. 1B). Involvement of phosphate groups of DNA with *trans*-platinum has been suggested by others (9) and may explain the effects at extremely low doses. However, at higher doses it does bind to DNA bases [15] although consistently to a lesser extent than *cis*-platinum (Fig. 2). It appears from these results that *trans*-platinum cannot easily approach and bind to DNA bases without causing DNA melting, possibly due to the steric hindrance caused by the NH_3 groups.

Existence of interstrand cross-links induced by *cis*-platinum in the DNA secondary structure is well-known [5, 6, 7, 8, 14]. Consistent with earlier results [5], we found again that at Pt/P doses corresponding to DNA melting, the observed partial melting was irreversible and the remaining portion of DNA could be renatured after denaturation. Thus, the plateau of the melting curve with 15% increase in the absorbance (Fig. 1A) indicated that nearly half of the DNA molecule was irreversibly melted by *cis*-platinum. With *trans*-platinum (Fig. 1B) the observed denaturation of DNA was too high for any meaningful renaturation study and only below Pt/P dose of 0.05 we observed partial renaturation. Understanding of DNA premelting caused by *cis*-platinum may help elucidate the molecular mechanism of antitumor action of this drug. Several proposals in the literature [4–6, 8–10, 14–17] about how this drug acts on DNA may be reexamined in the light of our present findings.

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References

1 P. K. Ganguli and T. Theophanides, *Abstracts, Second Chemical Congress of the North American Continent, San Francisco*, California, August 24–29, 1980; No. BIOL 208.
2 E. Palecek, *Progr. Nucl. Acid Res. Mol. Biol., 18*, 151 (1976).
3 B. Rosenberg, L. VanCamp, J. E. Trosko, V. H. Mansour, *Nature (London), 222*, 385 (1969); B. Rosenberg, *Platinum Metals Rev., 15*, 42 (1971); J. L. Marx, *Science, 192*, 774 (1976); C. J. Williams and J. M. A. Whitehouse, *Brit. Med. J., 1*, 1689 (1979).
4 B. Rosenberg, *Naturwissenschaften, 60*, 399 (1973); B. Rosenberg, *J. Clin. Hematol. Oncol., 7*, 817 (1977).

5 P. K. Ganguli and T. Theophanides, *Eur. J. Biochem., 101,* 377 (1979).
6 H. C. Harder, *Chem-Biol. Interact., 10,* 27 (1975).
7 P. Horacek and J. Drobnik, *Biochim. Biophys. Acta, 254,* 341 (1971).
8 A. D. Kelman, H. J. Peresie, P. J. Stone, *J. Clin. Hematol. Oncol., 7,* 440 (1977).
9 J. P. Macquet and J. L. Butour, *Biochimie, 60,* 901 (1978).
10 R. C. Srivastava, J. Froehlich, G. L. Eichhorn, In *'Platinum Coordination Compounds in Cancer Chemotherapy'*, T. A. Connors and J. J. Roberts, Eds., Springer, Heidelberg (1974) 48, p. 75; R. C. Srivastava, J. Froehlich, G. L. Eichhorn, *Biochimie, 60,* 879 (1978); J. P. Macquet and J. L. Butour, *Eur. J. Biochem., 83,* 375 (1978).
11 J. P. Macquet and T. Theophanides, *Inorg. Chim. Acta, 18,* 189 (1976).
12 L. L. Munchausen and R. O. Rahn, *Biochim. Biophys. Acta, 414,* 242 (1975).
13 H. C. Harder and B. Rosenberg, *Int. J. Cancer, 6,* 207 (1970); J. A. Howle and G. R. Gale, *Biochem. Pharmacol., 19,* 2757 (1970); P. Lecointe, J. P. Macquet, J. L. Butour, C. Paoletti, *Mutat. Res., 48,* 139 (1977).
14 J. J. Roberts and A. J. Thomson, *Progr. Nucl. Acid Res. Mol. Biol., 22,* 71 (1979).
15 J. P. Macquet and T. Theophanides, *Bioinorg. Chem., 5,* 59 (1975). *Idem, Biopolymers, 14,* 781 (1975).
16 D. Hermann, C. Houssier, W. Guschlbauer, *Biochim. Biophys. Acta, 564,* 456 (1979).
17 M. M. Millard, J. P. Macquet. T. Theophanides, *Biochim. Biophys. Acta, 402,* 166 (1975).