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Effects of 1,3-chelation induced by *cis*-diamminedichloroplatinum(II) on the stability of DNA duplexes

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Several 1,3-intra-strand cross-linked decadeoxynucleotide duplexes, modified with *cis*-diamminedichloroplatinum(II) (*cis*-DDP), and their base substitution analogues at the complementary site to the intervening base of the coordination sites, were synthesized and measured for UV-melting profiles to determine melting temperature (T_m) values. The results indicated the thermal stability of the oligonucleotide duplexes containing Pt-induced 1,3-intra-strand cross-linking did not depend on the kind of intervening base of the coordination site but rather on its complementary base. These results may explain the mutagenicity of *cis*-DDP from a chemical aspect.

cis-Diamminedichloroplatinum(II); Decanucleotide duplex; 1,3-Intra-strand cross-link; Melting temperature T_m

1. INTRODUCTION

cis-Diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) has potent anti-tumor activity [1] and its DNA adducts are considered responsible for its anti-cancer activity [2,3]. This drug binds preferentially to purine N7 positions, especially guanine N7 positions, in DNA to form 1,2-, 1,3-intra-strand [4,5] and inter-strand adducts [6]. As a result of chelation, DNA duplexes are destabilized and distorted [7–9]. It has been reported that not only 1,2- but also 1,3-intra-strand cross-links inhibit replication [10], and that 1,3-adducts are more mutagenic than 1,2-adducts [11]. Therefore, structural investigations of 1,3-adducts may give further insight into the mechanism of mutagenicity of *cis*-DDP. At present, although several structural investigations for 1,3-intra-strand cross-links have been reported, most of them are single-stranded adducts. Thus, much less information is available on the structure and stability of specific 1,3-intra-strand cross-linked DNA duplexes compared to 1,2-platinated duplexes. In addition, detailed information of the duplex structure is hard to obtain from NMR studies of platinated oligonucleotide duplexes because of the considerable signal broadening [12–14]. Therefore, we synthesized several decadeoxynucleotide duplexes carrying the *cis*-Pt moiety with the binding mode of 1,3-intra-strand cross-linking, and measured the thermal stability. The Pt-chelation was found to probably cause de-stacking of the central base, and the kind of central base at the coordination site was not important but its complementary base was shown to be essential to the duplex stability.

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Abbreviations: cis-DDP, *cis*-diamminechloroplatinum(II); T_m, melting temperature.

2. MATERIALS AND METHODS

2.1. *Materials*

All oligonucleotides were synthesized by a solid-phase β-cyanoethyl-phosphoramidite methods [15]. *cis*-DDP was purchased from Aldrich Chemical Co.

2.2. *Platination*

Platination was performed by a stoichiometric reaction of the upper strand decamers (strand concentration, $4–5 \times 10^{-5}$ M) with *cis*-DDP in unbuffered aqueous solutions, pH 4–5, at 37°C in the dark. The reaction was monitored by reverse-phase HPLC (Shimadzu LC-6A system) on a column (150 × 3.9 mm) of μBondasphere C18-100 Å (Waters Associates) using a linear gradient of CH_3CN in 50 mM ammonium acetate with 254 nm detection. After 10–16 h, NaCl and Tris were added to the reaction mixture (final concentration 0.05 M and 20 mM, respectively), and pH was adjusted to 8.0 with diluted HCl. The solution was applied to a DEAE-cellulose column (165 × 9 mm) (pre-equilibrated with 0.05 M NaCl, 20 mM Tris-HCl, pH 8.0). Elution was performed by a linear gradient of NaCl (0.05–0.4 M) in 20 mM Tris-HCl, pH 8.0. The fractions containing the product were collected and diluted to a salt concentration of 0.05 M by distilled water. The solution was applied to a DEAE-cellulose column (HCO_3^- form). The column was washed with 50 mM triethylammonium bicarbonate buffer, pH 7.5, and then the product was eluted by 1 M triethylammonium bicarbonate. The volatile matter was evaporated and the residue was co-evaporated by distilled water several times. Finally, the platinated decamers were converted to the sodium form by passing them through a column of Dowex 50w (Na^+ form).

2.3. *Atomatic absorption spectroscopy*

Platinum atomic absorption analysis was performed with a Nippon Jarrell-Ash AA-880 spectrophotometer equipped with a FLA-1000 graphite atomizer.

2.4 *Enzymatic digestion*

The platinated decamers (ca. 3 A_{260} U) were digested with 5 μl (1 mg/ml solution) of nuclease P1 (Yamasa Shoyu Co.) at 37°C for 3 h. Aliquots were analyzed by the above HPLC system. Elution was performed by a linear gradient of CH_3CN (0–10%/20 min) in 50 mM potassium phosphate, pH 4.0, with 270 nm detection.

2.5. *UV melting experiments*

Samples were prepared by mixing of equimolar upper and lower strand decamers (duplex concentration was 4.5 μM). The samples, containing 0.5 M NaCl, 10 mM sodium phosphate, pH 7.0, were added into a quartz cuvette (path-length, 1 cm) and the experiments were carried out using a Shimadzu UV-200S spectrophotometer.

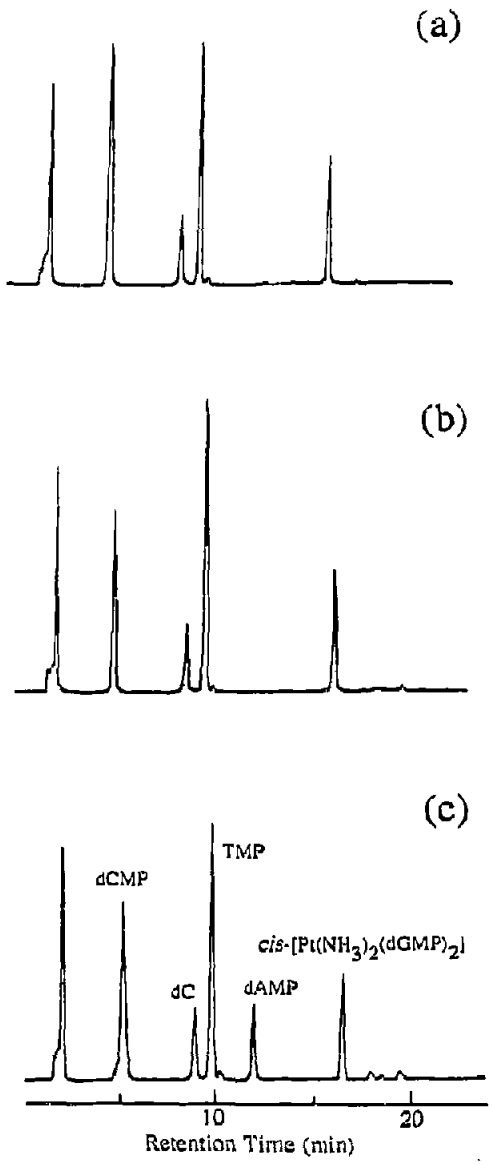

Fig. 1. HPLC analysis of the enzymatic digestion with nuclease P1 of the platinated upper strand decanucleotides (a) X = C, (b) X = T and (c) X = A. Elution was performed with a linear gradient of CH_3CN (0–10%) over 20 min in potassium phosphate (pH 4.0) with UV detection at 270 nm.

3. RESULTS AND DISCUSSION

The sequence of the decanucleotides used in this study was designed by the placement of the coordination core at the center of the upper strand decanucleotides, and by following with oligopyrimidine sequences on both 5′- and 3′-flanking sequences in order to avoid undesirable side reactions upon the platination reaction. A typical platination condition for the preparation of *cis*-Pt[NH_3)$_2$]{d(CTCTGXGCTC)-N7-G(5), N7-G(7)}] was 5×10^{-5} M upper strand oligomer concentration in unbuffered aqueous solution, within a pH range of 4–5 at 37°C with a stoichiometric amount of *cis*-DDP. The platination reactions proceeded correctly, and afforded a single major product, except for the upper strand decamer which had A as an X base: the platination of this decamer yielded a significant amount of by-products as a result of undesired 1,2- (AG and GA) chelation.

5′-d(CTCT*G*XGCTC)
(GAGACYCGAG)d-5′

X
C; Duplex 1
T; Duplex 2
A; Duplex 3

The crude products were purified by DEAE-cellulose column chromatography. The obtained decanucleotides and their Pt complexes were more than 95% pure with reverse-phase HPLC.

Pt contents of the all platinated decanucleotides were measured by carbon-rod atomic absorption spectroscopy which indicated the coordination of 1 Pt atom per strand. Their platination sites were determined with nuclease P1 digestion. It is known that the digestion of a 1,3-adduct with this enzyme afford corresponding dNMP (N is a central base of the adducts) and *cis*-Pt(NH_3)$_2$(dGMP)$_2$ [4,5]. Indeed, the platinated decanucleotide (X = A) affords dAMP and *cis*-

Table I

Melting temperatures of unplatinated decanucleotide duplexes, d(CTCTGXGCTC)·d(GAGCYCAGAG)[a]

X	Y			
	G	A	C	T
C	54.6	35.7	29.1	34.4
T	37.8	50.6	32.7	36.4
A	33.8	35.1	32.2	48.4

[a] T_m values were determined by measuring changes in absorbance at 274 nm as a function of temperature at a duplex concentration of 4.5 μM in 10 mM sodium phosphate buffer (pH 7.0) containing 0.5 M NaCl.

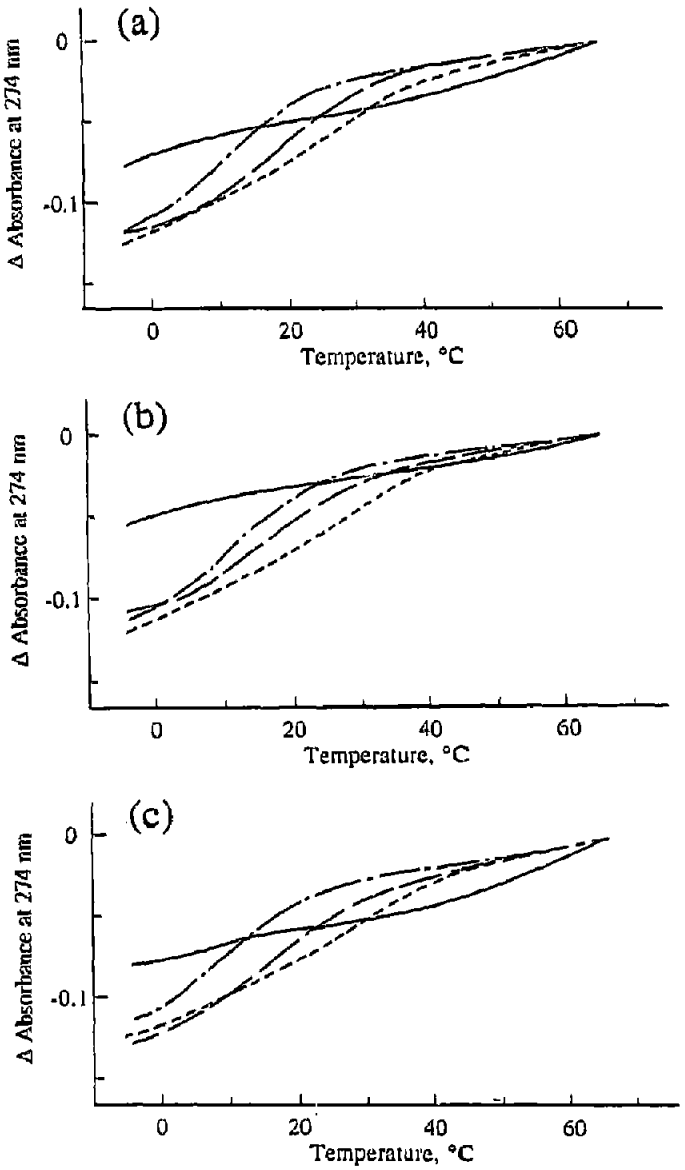

Fig. 2. UV melting profiles of the *cis*-platinated duplexes. (a) Duplex 1 (X = C), (b) Duplex 2 (X = T) and (c) Duplex 3 (X = A), at 274 nm with 4.5 μM of duplex in 10 mM sodium phosphate buffer (pH 7) containing 0.5 M NaCl: Y = G (——), Y = T (– – – –), Y = A (——) and Y = C (— – —).

$Pt(NH_3)_2(dGMP)_2$ corresponding to its platination core sequence, in addition to dCMP and TMP (Fig. 1c). Similarly, the remaining platinated decamers (X = C and T) also afford *cis*-$Pt(NH_3)_2(dGMP)_2$ and additional dCMP and TMP, respectively (Fig. 1a and b). Thus, the chemical structure of the platinated decanucleotides was confirmed.

The thermal stability of DNA duplexes reflects the relative strength of hydrogen bonding and base–base stacking interactions between the strands. The stability of the uncoordinated duplexes decreased with base substitution at the Y site. As shown in Table I, the effects of base mismatch at the Y residue on duplex stability are in the order of G > T > A > C (X = C), A > G > T > C (X = T) and T > G > A > C (X = A). These effects of the base-pair mismatch were well explained by Tinoco Jr. and co-workers [16].

The stability of the platinated duplexes, on the other hand, remarkably decreased (> 30°C), even when the base-pair mismatch was not contained. Although it was, therefore, difficult to estimate the reliable T_m values, the stability differences of the duplexes is clearly distinguishable from their melting profiles (Fig. 2). The UV melting curves of the Y = G duplexes show relatively sharp transitions at high temperature. The lower strand alone, which has a G as the Y base, showed a similar melting profile, although this strand showed standard melting profiles when it was mixed with the unplatinated upper strands. Thus, the unusual melting of the platinated duplexes containing a G as the Y base should reflect the lack of or much weaker interaction of this lower strand with the platinated upper strands. The order of stability of platinated Duplex 1 is T > A > C > G with substitution of the Y base. The effects of the base-pair mismatch of Duplex 2 and 3 are also the same as those of Duplex 1. In other words, the thermal stability of oligonucleotide duplexes carrying a *cis*-Pt-induced 1,3-adduct is independent of the central base of the platination core sequence. This residue would be stacked-out from the strand, as suggested by NMR studies. The duplexes carrying a 1,3-*cis*-Pt adduct are always the most stable when the complementary base is a T, although 1,2-*cis*-platination does not alter the selectivity for the base-pairing of guanines in oligonucleotides [9].

Since synthesis of an 1,3-*cis*-Pt-oligonucleotide complex, which has a G at the internal position of the coordination core sequence, presents many difficulties, such a complex was not studied in this work. Because the X base hardly participates in the duplex stability, as described above, the platinated duplex containing a G as the X base seems to afford the same results. The present results may explain the mutagenicity of *cis*-DDP from a chemical aspect.

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