Inhibition of *Escherichia coli* DNA polymerase-I by the anti-cancer drug *cis*-diaminedichloroplatinum(II): what roles do polymerases play in *cis*-platin-induced cytotoxicity?

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Abstract Activities of *Escherichia coli* DNA polymerase-I were examined in the presence of the anti-tumor drug *cis*-diaminedichloroplatinum(II) and its inactive geometric isomer *trans*-diaminedichloroplatinum(II). The *trans*-isomer did not inhibit the enzyme activity. The anti-tumor drug, on the other hand, retarded the enzyme in its ability to extend the primer strand of DNA. Two alternative mechanisms of inhibition, covalent binding of *cis*-diaminedichloroplatinum(II) to the polymerase and to the template DNA, were explored. Selective pre-incubations of the platinum drug with the polymerase and DNA reveal that the inhibition is primarily due to covalent binding to the enzyme. The rates of inhibition were found to be first order in enzyme and zeroth order in platinum in the concentration range 0.05–3.0 mM. A mechanism that deals with the formation of an initial platinum–polymerase-I complex with a binding constant $>10^5$ M^{-1} followed by a further reaction to form an inhibitory complex is consistent with the kinetic data. The rate limiting first order rate constant for the formation of the inhibitory complex is comparable to that observed for the thiol coordination of peptides containing cysteine residues. Analyses of known structures and functions of catalytic domains of various polymerases point to the direction that the inhibition is perhaps due to the distortion of the DNA binding domain of the enzyme due to platinum coordination.

Key words: Polymerase-I inhibition; Anti-cancer activity; *cis*-Platin-polymerase-I complex

1. Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is a widely used anti-cancer drug [1,2]. The platinum compound arrests cell synthesis in the G2 phase of the cell cycle by a mechanism commonly known as apoptosis [3–6]. At the molecular level, platinum-DNA adducts are believed to be the lesions responsible for anti-cancer activity [7–9]. However, how the platinated DNA lesions lead to apoptosis is yet to be elucidated. Although both in vitro and in vivo experiments clearly indicate platinum binding to DNA, a significant amount of this metal also binds proteins [10]. Unfortunately, studies related to platinum binding to proteins have mainly addressed issues related to toxicities of the drug [11,12]. The implications of direct covalent binding to replication enzymes which are involved in cell synthesis have not been explored in detail. In order to gain a comprehensive understanding of the anti-cancer mechanism, it is desirable to investigate all molecular events that might lead to apoptosis. Since platinum(II) exhibits a tremendous affinity toward sulfur donor atoms [13–18], such as cysteine and methionine in proteins, over the nitrogen donor sites in DNA, reactions with enzymes rich in cysteine and methionine cannot be ignored. One such enzyme is the DNA polymerase-α that contains many cysteine residues in the DNA binding domain of the enzyme [19]. This key replication enzyme is solely responsible for accurate synthesis of genetic information [20]. Recently, we have shown that *cis*-DDP can directly bind to the enzyme and inhibit its activity [21]. Also, it has been demonstrated that DNA polymerase-β, a repair enzyme, can bypass the platinated DNA lesions in carrying out the chain extension reaction implying that platinated DNA can still be used as a template for DNA synthesis [22]. Furthermore, a very small fraction (∼1%) of the administered *cis*-DDP reaches genomic DNA and the rest of the drug binds proteins, RNA, small thiols and thioether including glutathione, cysteine and methionine [15]. We have undertaken a systematic study to examine the role of replication enzymes toward the anti-cancer mechanism of platinum anti-tumor drugs. Here, we present results related to the inhibition of *Escherichia coli* DNA polymerase-I by *cis*-DDP and compare those with *trans*-DDP, the inactive isomer. Since *E. coli* polymerase-I is both structurally and functionally well-characterized [23–25] and its functions are comparable to those of human DNA polymerases, studies related to the interactions between *cis*-DDP and *E. coli* pol-I might offer insight into the binding sites of the platinum complex that lead to inhibition of the enzyme activities.

2. Materials and methods

2.1. Reagents

cis-DDP was synthesized by the method of Dhara [26]. *E. coli* DNA polymerase-I (pol-I) (EC 2.7.7.7, catalog number D9380) with an activity of 10 U/μl was purchased from Sigma. The highest available purity of all 2′-deoxy nucleoside-5′-triphosphates (dNTPs, 2′-deoxy adenosine-5′-triphosphate, 2′-deoxy cytosine-5′-triphosphate, thymidine-5′-triphosphate and 2′-deoxy guanosine-5′-triphosphate) was also purchased from Sigma. Methyl [^{3}H]thymidine-5′-triphosphate with a specific activity of 15 Ci/mmol was purchased from ICN Pharmaceuticals. Double-stranded calf thymus DNA (type XV) was activated by the method of Aposhian and Kornberg [27] by using deoxyribonuclease-I. A xylene-based scintillation cocktail was purchased from Fischer Scientific. Serum albumin, dithiothreitol (DTT) and L-cysteine (Sigma) were used without further purification. All other common reagents such as $MgCl_2$, KCl and EDTA were used without further purification. Buffers such as phosphate and Tris were prepared from appropriate acids and their conjugate bases.

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2.2. Physical measurements

2.2.1. Liquid scintillation counting. Tritiated material was spotted on a weakly basic anion exchange paper (Fischer Scientific DE-81), dried thoroughly in a microwave oven, then placed in liquid scintillation vials containing 10 ml of scintillene cocktail (Fischer Scientific). Radioactivity was determined in a Beckman LS 6800 liquid scintillation counter with quench detection (^{137}Cs external standard to give the H number) and micro-processed automatic quench correction. The unquenched tritium counting efficiency was 0.61. A quench correction curve was determined from a set of quenched tritium standards (Beckman). Using this quench correction curve and the detected quenching of each sample, we calculated the ^{3}H desintegrations per minute (dpm) directly from the background corrected ^{3}H counts per minute (cpm).

2.2.2. Ultraviolet-visible (UV-vis) measurements. Rates of reactions between *cis*-DDP and DTT were measured on a computer-interfaced UV-Visible spectrophotometer (Perkin Elmer Lambda 600) by following the change in absorbance at 260 nm as a function of the time. The temperature was maintained constant at 37°C and the ionic strength was maintained at 0.5 M by $NaClO_4$. The reactions were carried out by using at least a 10-fold excess of the ligand over the platinum complex.

2.2.3. High performance liquid chromatographic (HPLC) separations. Reactants, intermediates and products of the reaction between *cis*-DDP and DTT were separated on a Gradient HPLC system (Waters 7400) by using a reversed phase C-18 column with a mobile phase, 50 mM Tris buffer (pH = 6.8).

2.2.4. Activity assay of E. coli DNA polymerase-I. The activity of pol-I was determined by the amount of [^{3}H]TMP incorporation into a nicked and gapped calf thymus DNA [28]. The following components were present in a typical assay mixture of a total volume of 100 μl: 400 μg/ml calf thymus DNA, 50 mM Tris buffer (pH = 7.4), 5 mM $MgCl_2$, 20 mM KCl, 5 mM DTT, 50 μg bovine serum albumin, 50 μM each of dATP, dCTP, and dGTP, 50 μM [^{3}H]TTP and 6 U pol-I. The assay mixture was incubated in a thermostat at 37°C for 45 min. The reaction was stopped by adding 50 μg of serum albumin and ice cold 10% trichloroacetic acid containing 100 mM pyrophosphate. The solution was vortexed briefly at a low speed and then placed on a rock salt ice water bath for 45 min to complete the precipitation. The precipitate was collected on an ion exchange paper and washed with 15 ml ice cold trichloroacetic acid containing 50 mM sodium pyrophosphate. The precipitate was further washed with 5 ml water, ethanol and then with acetone. The paper was then soaked in a large Petri dish containing 3 M sodium phosphate to remove any unincorporated dNTPs from the ion exchange paper. Following the removal of unreacted substrates, the paper was placed into a microwave oven for 1 min. Once dried, it was then placed into a 20 ml glass scintillation vial combined with 10 ml scintillation fluid. The radioactivity was then counted on a scintillation counter and ^{3}H cpm were converted to ^{3}H dpm.

2.2.5. Activity assay in the presence of platinum complexes. The activities of the enzyme in the presence of *cis*- and *trans*-DDP were measured in several different ways. In one approach, platinum compounds were incubated along with the assay mixture for a specified amount of time. These complexes were then deactivated by adding cysteine which reacts fairly rapidly (in minutes) with the platinum(II) center [13–15]. Additions of cysteine did not alter the activity as demonstrated by appropriate controls. In the second method, the platinum compounds were incubated with the enzyme for the desired length of time. The unreacted platinum was then deactivated by adding cysteine. The platinated enzyme was then added to the assay mixture described earlier and the activity was measured. Finally, in the third method, activated DNA was incubated first with platinum compounds for variable times and unreacted platinum was once again removed by cysteine. The platinated DNA was then added to the assay mixture. The same assay protocol was then followed to measure the activity.

2.2.6. Evaluation of rate constants. For a given platinum concentration, the activity of the enzyme was monitored at regular time intervals. The activity-time curves can be described by an exponential decay. These curves were then fitted to the equation:

$$Ac = (Ac_o - Ac_\infty)e^{-kt} + Ac_\infty \quad (1)$$

by a non-linear least-squares method utilizing an iterative computer program. In this equation, Ac_o, Ac and Ac_∞ are the activity at time $t = 0$, at t and at infinite time when no appreciable changes in the activity were observed. The experimental data were found to be in a good fit with the calculated data.

The rate constants for the reaction between *cis*-DDP and DTT were evaluated from the absorbance time curves. These kinetics can be described best by a biphasic profile following a consecutive reaction sequence:

$$\textit{cis}\text{-DDP} + \text{DTT} \xrightarrow{k_1} \text{int} \xrightarrow{k_2} \text{products} \quad (2)$$

where int represents an intermediate in the reaction. Following the above reaction sequence, it can be shown that the absorbance as a function of time can be described by [29]:

$$A = A_o e^{-k_1 t} + [\text{DDP}]_o \frac{k_1 \varepsilon_1}{k_2 - k_1}(e^{-k_1 t} - e^{k_2 t}) +$$

$$\frac{A_\infty}{k_2 - k_1}(k_1 e^{-k_2 t} - k_2 e^{-k_1 t}) + A_\infty \quad (3)$$

A non-linear least-squares fit of the kinetic data yielded values for the two rate constants and the molar absorption of the intermediate. The values of A_o (initial absorbance), A_∞ (absorbance at infinite time) and $[\text{DDP}]_o$ (initial concentration of the platinum complex) were kept invariant. In triplicate measurements, the deviation of the rate constants from their mean values was within 10%.

3. Results

3.1. Inhibition of polymerase activities by cis-DDP

The activity of DNA polymerase-I was measured by monitoring the incorporation of [^{3}H]TMP in a nicked and gapped calf thymus DNA. Fig. 1 shows a typical activity-time curve. The maximum activity was reached after 60 min of incubation of the enzyme with the substrates. Identical experiments were performed in the presence of 1.0 and 3.0 mM *cis*-DDP. These activity-time curves are also displayed in Fig. 1. Although maximum activities were achieved after 30 min, these activities were substantially lower than those observed in the absence of *cis*-DDP. The inhibition thus observed appears to be irreversible, since no enhancement of the activity was observed even with a prolonged incubation. This irreversible inhibition may be due to covalent binding of *cis*-DDP with either DNA or the polymerase. In order to address the origin of this irreversible inhibition, *cis*-DDP was incubated selectively with pol-I and DNA and then, the activities were measured. Furthermore, identical experiments were also carried out with the inactive *trans*-isomer.

3.2. DNA polymerase-I activity from cis-DDP bound enzyme

cis-DDP was incubated with pol-I for various times. Following incubation, the activity of the enzyme was assayed by

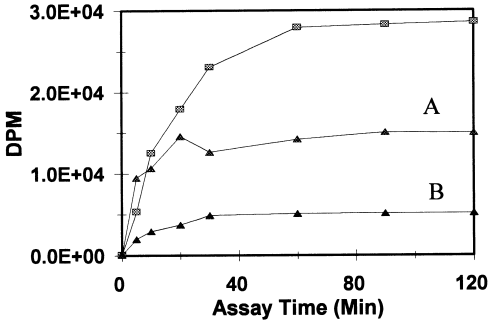

Fig. 1. Activity-time profiles for *E. coli* polymerase-I in the absence (■) and presence (▲) of 1.0 (A) and 3.0 (B) mM *cis*-DDP.

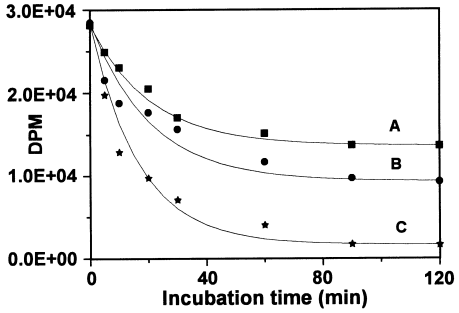

Fig. 2. Inhibitions of *E. coli* polymerase-I activity due to *cis*-DDP pre-incubations with the enzyme. Concentrations of *cis*-DDP were A, 0.050 mM, B, 0.4 mM and C, 3.0 mM. Solid lines are simulated profiles using Eq. 1 with the rate constants listed in Table 1.

[^{3}H]TMP incorporation. The activity-time curves at several platinum concentrations are shown in Fig. 2. The activity at time $t=0$ min corresponds to the activity of the enzyme in controls. As can be seen from the diagram, at a given concentration, the activity first decreases with an increasing incubation time and then finally levels off at longer times. In particular after 120 min, insignificant changes in the activity were observed for the platinum concentrations employed here, 0.05–3.0 mM. These data clearly indicate that *cis*-DDP is an inhibitor of pol-I.

The activity-time curves can be described by a first order rate profile. The rate constants obtained from the non-linear least-squares fit of the first order equation are listed in Table 1. The data indicate that there is no significant variation of the rate constant with [*cis*-DDP], indicating that the inhibition is zeroth order with respect to platinum under the experimental conditions. The kinetic data can be explained by a mechanism:

$$\textit{cis}\text{-DDP} + \text{pol-I} \overset{K}{\leftrightarrow} \textit{cis}\text{-DDP, pol-I} \tag{4}$$

$$\textit{cis}\text{-DDP, pol-I} \xrightarrow{k} \textit{cis}\text{-DDP}-\text{pol-I} \tag{5}$$

$$\textit{cis}\text{-DDP}-\text{pol-I} + \text{DNA} + \text{NTPs} \rightarrow \text{no reaction} \tag{6}$$

According to this mechanism, the observed rate constant k_0 can be related to:

$$k_0 = \frac{kK[\textit{cis}\text{-DDP}][\text{pol-I}]}{1 + K[\textit{cis}\text{-DDP}]} \tag{7}$$

Since the observed rate constant was invariant in platinum concentrations, $K[\textit{cis}\text{-DDP}] > 1$, the lower limit of K can be set to 10^5 M^{-1}.

cis-DDP also undergoes a parallel reaction with DTT. As can be seen from Table 1, at lower platinum concentrations (< 500 μM), complete retardation was not observed primarily due to the depletion of platinum complex by a parallel reaction with DTT. In order to estimate the amount of platinum available for the enzymatic reaction, kinetics of the *cis*-DDP–DTT reaction were examined under identical conditions. The reaction between *cis*-DDP and DTT follows consecutive kinetics with the formation and decay of a *cis*-DDP–DTT intermediate. The formation of the intermediate follows the well-established two-term rate law with the rate constants for the aquation (k_o), $(1.2 \pm 0.1) \times 10^{-4}$ s^{-1} and for the direct reaction (k), $(1.0 \pm 0.1) \times 10^{-2}$ M^{-1} s^{-1}. The formation of the product was found to be a first order process with respect to the intermediate with a rate constant of $(2.3 \pm 0.2) \times 10^{-4}$ s^{-1}. The sequence of reactions is shown by Eqs. 8–10:

$$\textit{cis}\text{-Pt}(NH_3)_2(Cl)_2 + H_2O \xrightarrow{k_0} \textit{cis}\text{-Pt}(NH_3)_2(Cl)(H_2O)^+ + Cl^- \tag{8}$$

$$\textit{cis}\text{-Pt}(NH_3)_2(Cl)_2 + \text{DDT} \xrightarrow{k} \textit{cis}\text{-Pt}(NH_3)_2(Cl)-\text{DTT(int)} + Cl^- \tag{9}$$

$$\textit{cis}\text{-Pt}(NH_3)_2(Cl)-\text{DTT(int)} \xrightarrow{k_2} \textit{cis}\text{-Pt}(NH_3)_2(\text{DTT}) + Cl^- \tag{10}$$

In the intermediate, one of the two available thiol groups of DTT is bound to the platinum center. However, we have detected two products in the HPLC chromatograms. The products are most likely to be a bis-(DDT) complex in which two molecules of DTT are bound to the platinum center in a monodentate fashion and a DTT chelate in which both thiol groups of the molecule are coordinated. The first order kinetics and magnitude of k_2 support that the formation of the products is mainly limited by the rate of aquation [30–32]. Since our objective was to evaluate the fraction of platinum bound to this reducing agent, we did not attempt to fully characterize these DTT complexes. The concentration profiles for the *cis*-DDP, the intermediate and products for a typical reaction are shown in Fig. 3.

As indicated earlier, zeroth order kinetics of pol-I inhibition with respect to platinum requires a binding constant, $> 10^5$ M^{-1}, for an inhibitory enzyme-platinum complex. An independent estimate of the binding constant can also be obtained from the activity data with the consideration that the measured activities at time > 90 min, i.e. when no further changes were observed, were due to establishment of an equilibrium.

Table 1
First order rate constants for *E. coli* polymerase-I inhibition as a function of [*cis*-DDP] at 37°C and at pH 7.0

[*cis*-DDP]$_o$, M	[*cis*-DDP]$_{eq}$, M	k_o, s^{-1}	Activity$_{eq}$, dpm
5.0×10^{-5}	1.4×10^{-5}	1.0×10^{-3}	1.36×10^{4}
1.0×10^{-4}	2.7×10^{-5}	8.9×10^{-4}	1.25×10^{4}
4.0×10^{-4}	1.1×10^{-4}	8.0×10^{-4}	9.29×10^{3}
3.0×10^{-3}	8.2×10^{-4}	1.1×10^{-3}	1.64×10^{3}

zThe equilibrium concentrations of *cis*-DDP and the polymerase activity in dpm are also listed

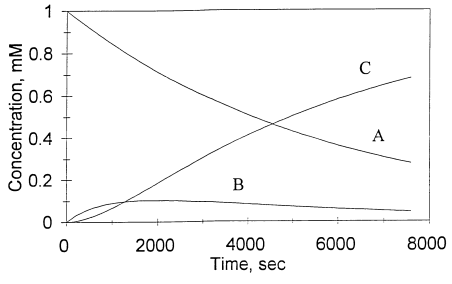

Fig. 3. Concentration profiles of *cis*-DDP (A), *cis*-DDP–DTT intermediate (B) and products (C) for the reaction of *cis*-DDP (1.0 mm) and DTT (5.0 mM) at pH 7.0, determined based on the kinetic scheme I by utilizing the rate constants indicated in the text.

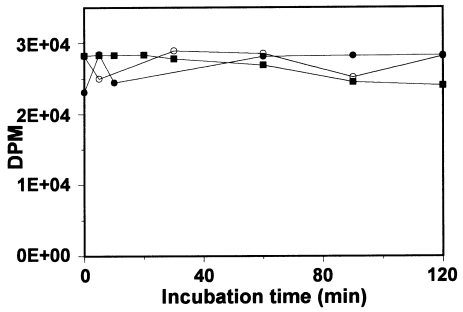

Fig. 4. Activity-time plots for *E. coli* polymerase-I due to pre-incubation of *cis*-DDP with DNA (■) and *trans*-DDP with DNA (○) and the polymerase (●). Note that none of these pre-incubations results in significant inhibitions.

Table 1 also lists the activity of the enzyme as a function of *cis*-DDP concentrations at 120 min which can be used to evaluate the binding constant as described below. The binding constant, K, for the reaction:

$$\textit{cis}\text{-DDP} + \text{pol-I} \overset{K}{\leftrightarrow} \textit{cis}\text{-DDP}-\text{pol-I} \tag{11}$$

can be expressed as:

$$K = \frac{[\mathrm{E}]_o - [\mathrm{E}]}{[\mathrm{E}]([\mathrm{Pt}]_o - [\mathrm{E}]_o + [\mathrm{E}] - [\mathrm{Pt}-\mathrm{DTT}])} \tag{12}$$

where $[\mathrm{Pt}]_o$ and $[\mathrm{E}]_o$ are the initial concentrations of the platinum complex and enzyme and [E] and [Pt–DTT] are the concentrations of the enzyme and platinum–DTT complexes at equilibrium. Since both $[\mathrm{E}]_o$ and [E] are several orders of magnitude smaller than $[\mathrm{Pt}]_o$ and the activity of enzyme is proportional to its concentration, the binding constant can be expressed as:

$$K = \frac{(Ac)_o - (Ac)}{(Ac)([\mathrm{Pt}]_o - [\mathrm{Pt}-\mathrm{DTT}])} \tag{13}$$

where $(Ac)_o$ and (Ac) correspond to the activities of the enzyme of the control experiment (without platinum) and in the presence of platinum when equilibrium was achieved. A plot of $[\mathrm{Pt}]_{ef}([\mathrm{Pt}]_o - [\mathrm{Pt–DTT}])$ versus $(Ac)^{-1}$ yielded a binding constant of $(5 \pm 2) \times 10^4$ M^{-1}. This value of the binding constant must be a lower limit since at physiological pH of *cis*-DDP is also known to form hydroxo-bridged dimers and trimers [33,34] and other complexes through parallel reactions and accurate estimates of concentrations of these species are difficult to achieve.

3.3. DNA polymerase-I activity in the presence of trans-DDP

The polymerase activities were also determined in the presence of *trans*-DDP, the inactive isomer. The same experimental protocol as described for the *cis*-DDP counterpart was followed, i.e. the polymerase activities were measured following selective pre-incubation of *trans*-DDP with the polymerase. Fig. 4 shows the activity time data for the highest platinum(II) concentration, 3.0 mM. As can be seen from the plot, even at this high platinum concentration, there is no significant inhibition of the enzyme. There is, however, considerable scattering of the activity data compared to those obtained for the *cis*-DDP reactions. This scattering appears not to be entirely related to experimental uncertainty or artifacts because of its reproducibility.

3.4. Inhibition of DNA polymerase-I activity due to cis- and trans-platination to DNA

In order to understand the role of platinated DNA toward polymerase inhibition, *cis*- and *trans*-DDP were individually incubated with the nicked and gapped DNA. The enzyme activities were measured by deactivating the unreacted platinum complexes with cysteine. Fig. 4 shows the activity-time curves for *cis*- and *trans*-DDP reactions. Unlike the *cis*-DDP–polymerase pre-incubation experiments, at times up to 30 min, the incubation of *cis*-DDP with DNA did not result in any significant inhibition. In fact, activity-time curves obtained by pre-incubations with DNA appeared to be indistinguishable from those obtained for the control experiments. However, at longer times, some inhibition was apparent for the DNA pre-incubations. After 120 min, less than 10% inhibition was observed. Likewise, *trans*-DDP–DNA incubations did not result in any significant inhibition of the enzyme.

4. Discussion

Results presented in the previous section unequivocally establish that *cis*-DDP but not the *trans*-isomer inhibits the activity of DNA polymerase-I as observed by the nick and gap translation assay. Furthermore, the lowest *cis*-DDP concentration used in this study is comparable to doses administered to patients [1]. The inhibition mechanism appears to be irreversible. Comparative experiments dealing with DNA versus polymerase pre-incubations clearly point primarily to enzyme binding events responsible for the inhibition. The rate of inhibition should correspond to the rate of substitutions onto platinum(II) if the irreversible complex formation between DNA or polymerase was the event responsible for the lack of enzyme activity. The postulated mechanism requires the formation of an initial complex for which only a binding constant $> 10^5$ M^{-1} can be estimated but not the rate constant. This initial complex then undergoes further reaction (rearrangements) to form a product, presumably an inhibitory complex by a first order process. However, the first order rate constant, $(1.0 \pm 0.1) \times 10^{-3}$ s^{-1}, is comparable to what we measured for a chelation step for a peptide with two cysteine residues. Furthermore, this rate constant is about two orders of magnitude larger than that of aquation of *cis*-DDP, the process that limits DNA coordination.

In order to inhibit polymerase-I activities, *cis*-DDP can either directly bind to the active sites of the enzyme [38–42], modify the substrates which are not recognized by the enzyme, or cause structural changes elsewhere in the enzyme such that it does not recognize substrates or template DNA. The possibility that the modified substrates, due to direct platinum binding to dNTPs, are the source of inhibition can be ruled out. The reactions of dNTPs and dNMPs with the platinum(II) complex are much slower compared to those observed here [35–37]. Furthermore, if modified substrates are the source of inhibition, similar activities were expected for the enzyme regardless of the polymerase or DNA pre-incubations. The inhibition was observed when pol-I was pre-incubated with *cis*-DDP or both DNA and pol-I are co-incubated with the platinum complex. Moreover, we did not observe the inhibition for the *trans*-DDP system, yet the re-

actions of the *trans*-isomer are generally faster than that of the *cis*-counterpart. Platinum(II) binding at the polymerization active site and dNTP binding sites can also be argued against. The absence of potential coordination sites in these amino acids except histidine makes it difficult to bind *cis*-DDP. Although histidine has a potential nitrogen donor site, its reaction with the platinum complex is comparable to that of N7 donor sites of purines but slower than that observed here.

Since reactions between platinum(II) and sulfur donor sites, especially thiols and thioethers, are shown to be much faster, it is conceivable that platinations at these residues might also have led to major structural distortions. In fact, X-ray structural data reveal that the Klenow fragment of the enzyme contains a large cleft between the two major helices which contain several cysteine, methionine and histidine residues. The template DNA is believed to fit snugly in the cleft [23]. Therefore, it is highly likely that structural changes due to covalent binding to *cis*-DDP might lead to a lack of DNA recognition. The structural distortions can also be supported from our earlier observation that *cis*-DDP readily replaces Zn(II) from the DNA binding domain of PA3 polymerase-α [28]. Furthermore, we have reported the facile formation of *cis*-DDP–peptide complexes containing nine and 31 amino acids from this DNA binding domain of polymerase-α [43] which undergo severe structural perturbations due to platination [44,45].

The polymerase inhibition results should be evaluated in the context of an anti-cancer mechanism of *cis*-DDP. The anti-cancer mechanism is commonly believed to be due to the formation of a variety of platinum–DNA adducts including DNA–protein cross links. To date, roles of polymerases have been confined to their inabilities to utilize platinum–DNA adducts as the template toward DNA synthesis [22,46–48]. However, Harder et al. [46] reported significant inhibition of pol-α and -β isolated from RPMI cells with 500 μM *cis*-DDP, but at lower platinum concentrations, marginal inhibitions were observed. It is not clear whether the lack of substantial inhibitions was due to limited exposure of the drug to the enzyme, a substantial loss of *cis*-DDP through dimerization and rapid reaction of pre-equilibrated *cis*-DDP with DTT, the nature of the enzymes or difficulty to isolate the enzymes in their pure forms. Furthermore, Hoffman and co-workers [22] reported that polymerase-β recognized the platinated DNA template in contrast to observations reported by Harder [46]. Moreover, we have reported [21] polymerase-α inhibitions by both *cis*-DDP and dichloro(ethylenediamine)platinum(II). Certainly, more studies are needed to address the issues pertaining to the diversities of polymerase activities isolated from a variety of cells and organisms and comparison of the rates of inhibitions due to DNA versus polymerase binding to platinum drugs.

Although platinum-adducts are believed to be the primary lesions responsible for the anti-cancer mechanism, other mechanisms including protein-platinum bindings have yet to be ruled out. Roles of a variety of repair enzymes and architectural proteins have been examined in relation to acquired drug resistance and differential behaviors of *cis*- and *trans*-DDP toward cytotoxicity [49–52]. For example, a number of structure specific recognition proteins block the repair of *cis*-DDP-mediated DNA damage caused mainly by an intra-strand 1,2-binding mode to adjacent G or G-A, while *trans*-platin-DNA adducts are repaired by the same proteins. These bifunctional *cis*-DDP–DNA adducts cause severe structural distortions to DNA [53–56]. Since anti-tumor platinum complexes with one binding site [57] cannot cause such distortions, a different mechanism must be invoked. In search for a common mechanism, we are currently examining the polymerase inhibition by a variety of platinum complexes that offer both one and two coordination sites [58]. Finally, although the data presented here did not unambiguously establish a new anti-cancer mechanism since the fate of *cis*-DDP in the cellular milieu cannot be defined to a specific molecular event, they provided impetus for thorough and systematic investigations for the roles of platinum-protein adducts toward the cytotoxicity of platinum drugs. It is interesting to note that polymerase inhibition has led to apoptosis by an antibiotic, aphidicolin, and the onset of DNA degradation by both *cis*-DDP and the antibiotic appears to be the same [3–5].

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