Topological Differences in the Interaction of Human DNA Topoisomerase I with DNA–Histone Complexes Modified by *cis*- and *trans*-DDP

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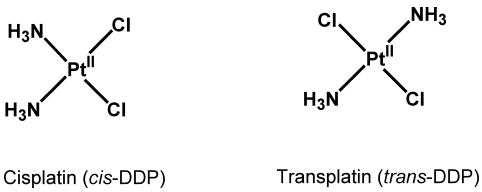
We show that the trefoil, figure-eight knot, and mini circular closed DNA are formed by the reaction of *cis*-DDP-modified ϕX174DNA–histone^{LNCaP} complexes as a new nucleosome model with human DNA topoisomease I. The yields from *cis*-DDP-modified complexes were far higher than that of *trans*-DDP. The topologically-distinct invariant DNA such as the trefoil and figure-eight knot are not produced in the reaction of DNA topo I with ϕX174DNA–histone^{LNCaP} complexes that are not modified by platinum. Therefore, the anti-cancer activity of *cis*-DDP may be related to the production of the trefoil, figure-eight knot, and mini circular closed DNA forms in the living cell. We subsequently demonstrate that the yield mechanism and identification of the topologically-distinct invariant DNA can be explained by the topological method using a Jones polynomial and recombination through the topo I path intra-twisted looped DNA model. These results suggest that the distinguishing of anti-neoplastic activity of *cis*- and *trans*-DDP can be partially explained by the distinct topologies of DNA, trefoil, figure-eight knot, and mini circular closed DNA that they produce.

Key words cisplatin; anti-cancer drug; DNA topology; trefoil; DNA topoisomerase I; electron microscope

cis-Diamminedichloroplatinum(II) (*cis*-$Pt^{II}(NH_3)_2Cl_2$, cisplatin or *cis*-DDP) is an effective chemotherapeutic agent for the treatment of various neoplasms (Fig. 1). The full mechanism of its anti-neoplastic activity has yet to be elucidated, however. Several molecular studies have demonstrated that the bifunctional coordinate bonds between *cis*-DDP and cellular DNA play some role in cell death.[1—3] Although the *trans*-isomer of *cis*-DDP also binds to cellular DNA, it has been shown to be clinically ineffective.[4] The reasons for this difference is unclear.[3,5,6] To explore the differences in anti-neoplastic activity between *cis*- and *trans*-DDP, we have been investigating the topological transformation of DNA structure by the reaction of *cis*-DDP- (*trans*-DDP)-modified DNA with DNA topoisomerase I (topo I). DNA topoisomerases serve critical functions in living cells in the regulation of DNA transcription and replication.[7] DNA topoisomerases alter DNA topology by breaking and rejoining the DNA phosphodiester backbone through a single-strand DNA passage mechanism. While it is clear that *cis*-DDP-modified chromatin interacts differently with DNA topoisomerases, it is unknown how this interaction specifically occurs. In a previous paper, we formed topologically distinct invariant DNAs —trefoil and catenane—by the reaction of *cis*-DDP-modified DNA with DNA topoisomerase.[8] Here, we aim to expand the results of the previous study to a histone-containing chromatin model.

Recently, high-mobility group (HMG) domain proteins that bind specifically to the major *cis*-DDP DNA adducts have been reported.[9] *In vitro*, HMG proteins form more stable platinum DNA-protein complex with *cis*-DDP binding than with *trans*-DDP binding.[9] Yaneva and colleagues have previously reported that the major chromatin protein histone H1 binds preferentially to *cis*-DDP-modified DNA.[10]

In this study, we demonstrate that trefoil, figure-eight knot, fragment DNA, and a mini circular closed knot can be obtained in a yield of approximately 30% by the reaction of DNA topo I with *cis*-DDP-modified ϕX174DNA–histone complexes. The yield of the mini circular closed knot and trefoil from *trans*-DDP-modified ϕX174DNA–histone complexes is only approximately 2%, however. Notably, the production of a mini circular closed knot is not observed in the reaction of *trans*-DDP-modified DNA with DNA topo I. We then demonstrate that *cis*-DDP may be an important factor in controlling the toplological change of DNA by DNA topoisomerase I. The difference in the anti-cancer activity of *cis*-DDP and *trans*-DDP may be related to the generation of the topologically-distinct invariant trefoil DNA and figure-eight DNA or mini circular closed DNA. These results can be explained by the knot theory and topology mathematics.



Cisplatin (*cis*-DDP) Transplatin (*trans*-DDP)

Fig. 1. Structures of Cisplatin (*cis*-DDP) and Transplatin (*trans*-DDP)

Experimental

Chemical Reagents Cisplatin and transplatin were purchased from Sigma-Aldrich Japan Inc. (Tokyo). Human DNA topoisomerase type I (hDNA topo I; 2005H-1) were obtained from Topogen Inc. (Port Orange, FL, U.S.A.). The ϕX174RF type I DNA (ϕX174 DNA) was purchased from Takara Bio. Inc. (Tokyo).

Cell Culture The LNCaP cell line[11] was obtained from The Rikagaku Institute (Tsukuba, Japan). Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM, ICN Biomedicals Inc., U.S.A.) containing 0.37% sodium bicarbonate, 100 units/ml of penicillin G, and 100 μg/ml streptomycin sulfonate (Gibco Invitrogen Co., U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS, JRH biosciences Inc., U.S.A.), in a water-jacketed 5% CO_2 incubator; the medium was exchanged every 3 d. For all experiments, cells were seeded at a density of 5.0×10^5 cells/ml in tissue culture flasks (25 cm^2/ml) in 10% FBS/DMEM.

Histone Extraction from LNCaP Cells Cultures allowed the cells in the confluent growth phase in 75 $cm^2\times8$ culture flask. LNCaP cells (about 3.2×10^8 cells/total) were harvested and collected by centrifugation (5 min at 1000 rpm) and the pellets were washed twice with cooled PBS(−). Histone (core) extracts were obtained according to the method of Cousens *et al.*,[12,13] with minor modifications. The resulting precipitate was collected by centrifugation and dried in a vacuum for 5 min, dissolved in 500 μl of 10 mM TH buffer, and stored at −20 °C.

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Quantitative Analysis of Histone Proteins The concentration of histone^{LNCaP} proteins extracted was determined using a kit of the modified Lowry method (DC protein assay, BIO-RAD Laboratories, Tokyo).

Preparation of *cis*- and *trans*-DDP Solutions A stock solution of *cis*-DDP (2.5×10^{-2} mol/l) or *trans*-DDP (1.0×10^{-2} mol/l) was prepared by dissolving the complex in a 10 mM Tris–HCl (=TH) buffer (pH 7.5).[14]

Preparation of *cis*- and *trans*-DDP-Modified DNA–Histone^{LNCaP} Complexes The modified DNA–histone^{LNCaP} complexes were prepared by incubation of a final concentration of 4.0×10^{-5} mol/l of *cis*- or *trans*-DDP) with DNA–histone^{LNCaP} complexes in 10 mmol/l TH buffer (pH 7.5) according to methods described previously.[15] The ϕX174RFDNA–histone^{LNCaP} complexes were prepared by incubating 10 mmol/l TH buffer solution (6 μl) of ϕX174RFDNA (0.096 μg) with 2 μl (0.046 μg) of histone^{LNCaP}. These complexes were then modified by incubating with final concentration of 4.0×10^{-5} mol/l of freshly *cis*- or *trans*-DDP (2 μl) at 37 °C for 0, 0.25, 0.5, 1.0, 3.0, 6.0, 8.0, 12.0, 24.0, 30.0, 48.0, and 72.0 h. The reactions were terminated at −20 °C in a dark environment. The resulting complexes were analyzed on 0.8% agarose gel electrophoresis in TBE (90 mmol/l Tris–borate, 2 mmol/l EDTA) buffer (pH 8.1). The voltage was kept below 1.5 V/cm for 16 h.

Reaction of *cis*- or *trans*-DDP-Modified-DNA–Histone Complexes with Human DNA Topoisomerase I A 5 μl solution containing five units of human DNA topoisomerase I was added to a 10ml solution of *cis*- or *trans*-DDP-modified DNA–histone^{LNCaP} complexes. 2 μl of 10X TOPO I buffer (100 mmol/l TH buffer (pH 7.9), 1 mmol/l spermidine, 10 mmol/l EDTA, and 50% glycerol) was added to this mixture. After the mixture was incubated at 37 °C for 1.0 h, the reaction mixture was terminated at −20 °C. The resulting complexes were analyzed by the method described above.

Electrophoresis All complexes were loaded on a 0.8% agarose gel electrophoresis in TBE buffer at 15 V (1.5 V/cm) constant powers for 16 h. The gels were stained with ethidium bromide (0.5 μg/ml) for 1 h in TBE buffer, and photographed under UV with a Polaroid camera using Polapan 667 films.

Electron Microscopy The treatment of samples used for electron microscopy was essentially the same as that described by Yamagishi.[16] The grids were inserted into a JEOL JEM-SCAN100CX II electron microscope (JEOL Ltd., Tokyo), so that the shadowed surface, followed by rotary-shadowcasting with Au/Pd (3 : 2), of the grid faced toward the emulsion side of the sheet film. Photographs were taken at an initial magnification of 20000× (or 28000×).

Contour Length of ϕX174RFDNA Used in This Study The length of ϕX174RFDNA (control) used in this study was measured with an electron microscope in following the previous method.[14] Results are shown in Fig. 2. The mean length is 1.70±0.26 mm (mean±S.E.M.).

Analysis and DNA Topology The counter lengths and shape of the DNA topoisomers were determined by measuring tracings of the negatives of the enlarged images with a MicroAnalyzer version 1.1 d (Nihon Poladigital, Inc., Tokyo). Figure 3 presents the standard link and knot in DNA topology used in this study. The ϕX174RFDNA used in our study is negative supercoiled closed circular DNA. The relaxed form and the negative and positive sign conventions for form crossings are illustrated in (**1**), (**2**) and (**3**) as the topological representation, respectively. Intra-twisted looped forms (**4**, **5**) and trefoils (**7**, **8**) are simple knots with negative or positive signs, respectively. However, forms **4** (or **5**) and **7** (or **8**) are different knots. The form (**6**) is a catenane. To distinguish the topology quantitatively, we used the Jones polynomial.[17]

The Jones polynomials $V_K(t)$ for knot are expressed with the Eqs. 1 and 2.

$$L_K(A)=(-A^3)^{-w(K)}\langle K\rangle \quad (1)$$

$$V_K(t)=L_K(t^{-1/4}) \quad (2)$$

Here, $L_K(A)$ is the normalized bracket polynomial for oriented knot (K), A is a commutative variable, and $w(K)$ (=Wr) is the invariant as a writhing number.[17] The Wr can be measured using electron microscopy.[14]

Results and Discussion

Structure of *cis*- and *trans*-DDP-Modified DNA–Histone Complexes Figure 4A shows that the elctrophoretic mobility in gel lane 6 is drastically changed in comparison with the lanes 2—5. The electron microscopy visualization shows that the ϕX174RFDNA–histone^{LNCaP} has a mean length of 1.39 μm and is shorter than that of ϕX174RFDNA (control=1.70 μm).[15] The dissociation of ϕX174RFDNA from ϕX174RFDNA–histone^{LNCaP} complexes is accelerated by the reaction of *cis*-DDP.

The geometrical isomer *trans*-DDP is unfavorable to the dissociation of ϕX174RFDNA from ϕX174RFDNA–histone^{LNCaP} complexes, however. Figure 4B shows that the electrophoretic mobility of the *trans*-DDP modified ϕX174RFDNA–histone^{LNCaP} complexes change little during 0.0—72 h incubation.

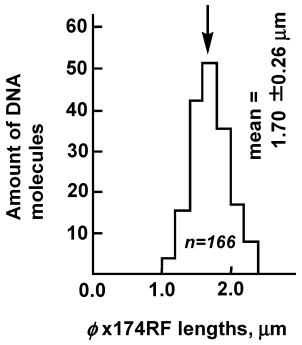

Fig. 2. Contour Length of ϕX174DNA

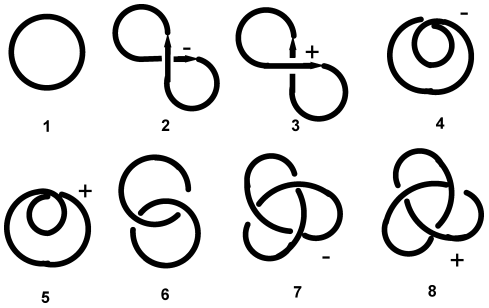

Fig. 3. Topological Structure of DNA

1; trivial knot, **2**; knot having negative crossing point, **3**; knot having positive crossing point, **4**; negative intra-twisted loop, **5**; positive intra-twisted loop, **6**; catenane, **7**; negative trefoil, and **8**; positive trefoil.

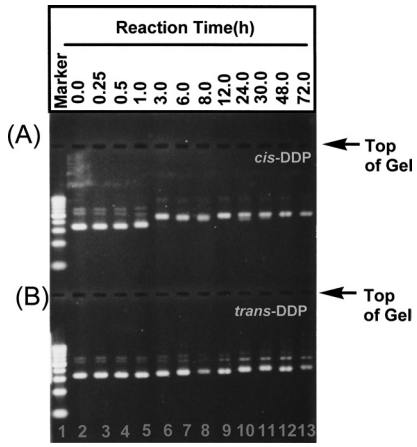

Fig. 4. Changes in Electrophoretic Mobility of ϕX174DNA–Histone Complexes Modified by (A) *cis*-DDP and (B) *trans*-DDP

Lanes 2—13 contain the ϕX174RFDNA–histone^{LNCaP} complexes. The complexes were incubated in the presence of final concentration of *cis*- or *trans*-DDP: 4.0×10^{-5} mol/l for 0.0, 0.25, 0.5, 1.0, 3.0, 6.0, 8.0, 12.0, 24.0, 30.0, 48.0, and 72.0 h at 37 °C. The reactions were terminated by freezing reaction at −20 °C and analyzed by electrophoresis and electron microscopy.

These results indicate that the dissociation of the wound DNA from ϕX174DNA–histone^{LNCaP} complexes by *cis*-DDP is stronger than that by *trans*-DDP.[15] Therefore, the following equilibrium equations may occur:

$$\textit{cis}\text{-DDP} + \phi\text{X174RFDNA–histone}^{LNCaP} \rightleftarrows \textit{cis}\text{-DDP–}\phi\text{X174RFDNA} + \text{histone}^{LNCaP} \quad (3)$$

$$\textit{trans}\text{-DDP} + \phi\text{X174RFDNA–histone}^{LNCaP} \rightleftarrows \textit{trans}\text{-DDP–}\phi\text{X174RFDNA–histone}^{LNCaP} \quad (4)$$

Our model indicates that DNA partially dissociates from chromatin (nucleosome) by *cis*-DDP binding in the nucleus and this may be actually generated in the living cells.

Reaction of *cis*- and *trans*-DDP-Modified DNA–Histone Complexes with Human DNA Topoisomerase I Gel electrophoresis of the topo-reaction is shown in Figs. 5A and B for *cis*- and *trans*-DDP, respectively. Clearly, both *cis*- and *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes react with hDNA topo I in the Topo I reaction buffer.

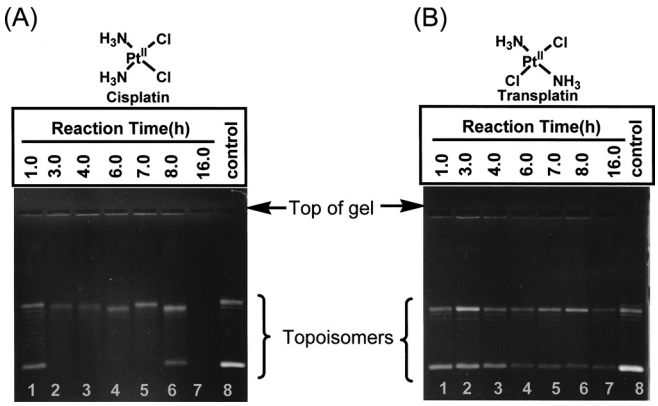

Fig. 5. Agarose Gel Electrophoresis of the Distinct *cis* and *trans* Geometrical Effects of Cisplatin and Transplatin

ϕX174RF–histone^{LNCaP} complexes modified by (A) *cis*-DDP and (B) *trans*-DDP were incubated with 5 U of hDNA Topo I in Topo I reaction buffer for 1.0 h at 37 °C, respectively. Reaction time represents, respectively, the *cis*-DDP- and *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes incubated for 1.0, 3.0, 4.0, 6.0, 7.0, 8.0, and 16.0 h at 37 °C in the reaction buffer (pH 7.5).

The gel mobility of *cis*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes with hDNA topo I differ from that of the gel mobility of *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes. This is likely due to the observation that the number of nodes of the negative super coiled DNA in the complexes decreases with an increasing reaction time of *cis*-DDP with ϕX174RFDNA–histone^{LNCaP} complexes. The DNA changes toward positive supercoiled DNA (in lanes 2—5 in Fig. 5A). On the other hand, negative supercoiled DNA remains in the reaction mixture by reaction of the hDNA topo I with *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes (in lanes 1—7 in Fig. 5B). This means that *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes do not easily react with DNA topo I.

Although the bands disappeared on the gel in the reaction of *cis*- or *trans*-DDP with ϕX174RFDNA–histone^{LNCaP} complexes (Figs. 4A, B), the bands of the aggregated DNA were observed in Figs. 5A and B. Aggregation between the complexes was probably strengthened by the DNA topo-reaction. The *cis*- or *trans*-DDP was cut down with DNA topo I from the modified ϕX174RFDNA–histone^{LNCaP} complexes, and the resulting complexes were insoluble due to their relative hydrophobicity. Moreover, we are interested in the bands of lower electrophoretic mobility more than the bands of the relaxed forms.

Structure of DNA and Electron Micrographs To understand the reaction of hDNA topo I with *cis*-DDP modified DNA–histone complexes, the reaction products were analyzed by an electron microscope. We first observed the production of topologically-distinct invariant isomers by incubation of hDNA topo I with *cis*-DDP modified ϕX174RFDNA–histone^{LNCaP} complexes as a *cis*-DDP modified nucleosome model. Figures 6 and 7 show the visualization of trefoil, figure-eight knot, and mini circular closed knot (mini cc) by electron microscopy. We classified the length, shape, DNA conformation, and topology to characterize the products.

The amount of the trefoil DNA (**1a**—**1d** in Fig. 6A) and

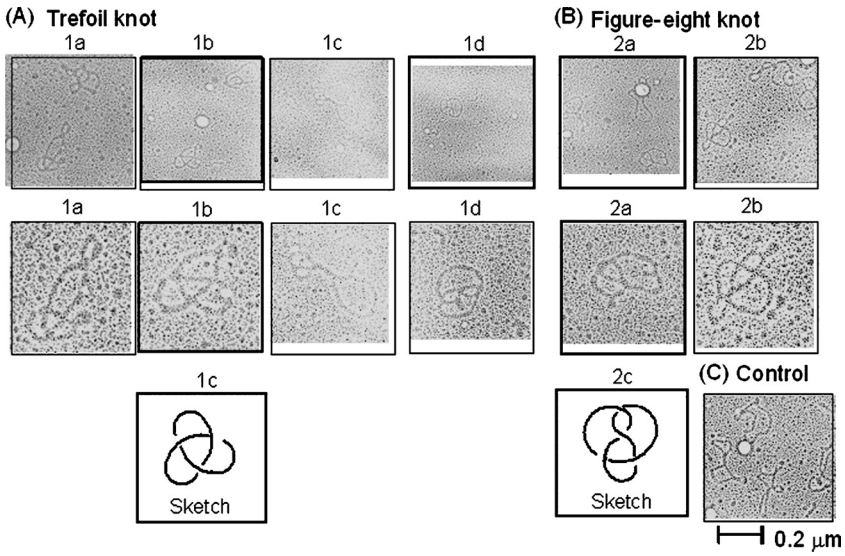

Fig. 6. Visualization of Topologically-Distinct Invariant Trefoil Knot (A) and Figure-Eight Knot (B) by Electron Microscopy

Results by reaction of hDNA Topo I with modified ϕX174RF–histone^{LNCaP} complexes produced by incubation with *cis*-DDP for 4 h (see lane 3 in Fig. 4). (A) and (B): the numbers **1a**—**d**, **2a**—**b**, and **1c**, **2c**, represent trefoils and figure-eight knots. (C) Control: results of the reaction of hDNA topo I with ϕX174RF–histone complexes. trefoil and figure-eight knot are not visualized.

mini circular closed DNA (**a**, **b**, and **c** in Fig. 7B) increases with increasing reaction time of *cis*-DDP with the ϕX174RFDNA–histone^{LNCaP} complexes. The structure of trefoil DNA (see Figs. 6A, 7A) clearly differs from the mini circular closed DNA (see Fig. 6B) and has a knot. The length of trefoil DNA has a mean length of approximately 1.70 μm. We surmise, therefore, that the trefoil DNA yielded from ϕX174RFDNA through a process of topological reaction since the mean length of the control is 1.70±0.26. Furthermore, it is characterized by the formation of mini ccDNA by reaction of the *cis*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes with hDNA topo I. The prominent products are mini circular DNA of short length. For example, the length of mini ccDNA **a**, **b**, and **c** is 0.74, 0.48, and 0.24 μm, respectively, in Fig. 6B, and they are clearly shorter than the trefoil DNA and the control. The results of the length distribution and shape are summarized in Table 1.

The amount of *cis*-DDP bound to the ϕX174RFDNA–histone^{LNCaP} complexes increases with time, and trefoil (3_1) and mini cc DNA (0_1^2) are major products in a yield of 13—27% and 15%, respectively. A figure-eight knot (4_1) and catenane are also produced as minor products (Figs. 6B, 7C). The hDNA topo I reaction of *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes produced the trefoil and mini ccDNA in low yield, respectively, of <2% and <1% under the same conditions. These results are listed in Table 2.

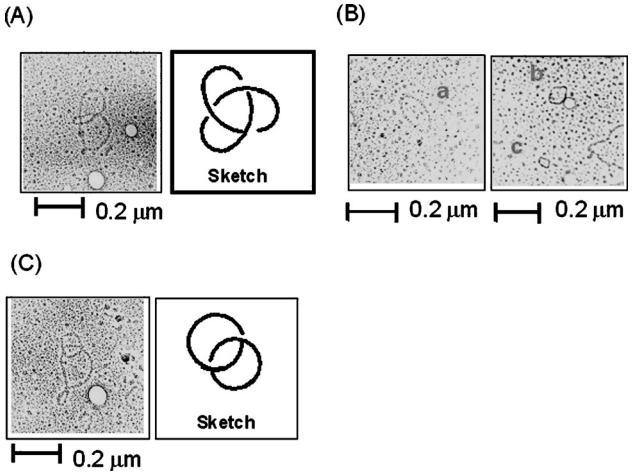

Fig. 7. Visualization of Topologically-Distinct Invariant Trefoil Knot, Catenane, and Mini Closed Circular DNA by Electron Microscopy

Results of the reaction of DNA Topo I with modified ϕX174RF–histone^{LNCaP} complexes obtained by incubation with *cis*-DDP for 6 h (see lane 4 in Fig. 4A). (A); trefoil, (B); mini ccDNA (length, **a**; 0.74 μm (=2350 bps), **b**; 0.48 μm (=1520 bps), and **c**; 0.24 μm (=760 bps)), and (C); singly-linked catenane.

The topologically-distinct invariant isomers was not formed from ϕX174RFDNA–histone^{LNCaP} complexes without *cis*-DDP binding by reaction with hDNA topo I (Fig. 5C). Additionally, the mini ccDNA and fragment DNA were not produced by the reaction of hDNA topo I with *cis*-DDP-modified ϕX174RFDNA. The difference for the production of the topoisomers shows that there are distinct topological pathways for the reaction of platinum ion-modified DNA with hDNA topo I by binding of *cis*-DDP and *trans*-DDP.

A New Model of DNA Topology in Cellular Nucleus The formation of trefoil (major), figure-eight knot (minor), mini closed circular DNA, and fragment DNA in our nucleus model in living cells shows that their knots may be produced in the nucleus by stimulation of *cis*-DDP with chromatin. By comparing the reactions of DNA topo I with *cis*- and *trans*-DDP-modified DNA–histone complexes, we have shown that the key actions of *cis*-DDP and *trans*-DDP are related to whether they easily dissociate DNA from DNA–histone complexes.

The formation of trefoil, figure-eight knot, mini ccDNA, and fragment DNA are generated through the reaction of hDNA topo I with *cis*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes. The Rolfsen numbers of trefoil, figure-eight knot, and mini ccDNA are 3_1, 4_1, and 0_1^2, respectively.[18] Topological invariants of these three DNA are different. Here, we present a method to explain the formation mechanism of unique trefoil, figure-eight knot, mini ccDNA, and fragment DNA in Fig. 8.

The bracket polynomial of product trefoil DNA **8** (Fig. 6A) is $L_{\text{Trefoil}}(A)=(-A^3)^{-3}\langle K\rangle=-A^{-9}(-A^5-A^{-3}+A^{-7})=A^{-4}+A^{-12}-A^{-16}$. The Jones polynomial $V_K(t)$ is obtained $t+t^3-t^4$. Next, the $V_K(t)$ of figure-eight knot **9** and mini ccDNA **10** is $t^2+t^{-2}-t-t^{-1}+1$ and 1, respectively. The structure of the DNA was quantitatively classified not only by the shape but also by the Jones polynomial. Therefore, we have shown that the knot and link DNAs obtained by the reaction of hDNA topo I with *cis*-DDP modified ϕX174RFDNA–histone^{LNCaP} complexes can be quantified with the topologically-distinct invariant using the Jones polynomial.

Mechanism The differences of the action of *cis*-DDP and *trans*-DDP might be explained by the knot and link method. Our previous paper demonstrated that the major role of *cis*-DDP is to dissociate DNA from DNA–histone complexes through interacting with DNA.[15] The power of this interaction is far stronger than that of *trans*-DDP. Following dissociation, the now-modified DNA–histone complexes forms intra-twisted loop DNA (**12** or **13**) as shown in Fig. 8, and hDNA topo I-catalyzed recombination takes place on the

Table 1. Distribution of Trefoil, Figure-Eight Knot, and Mini ccDNAProduced by Reaction of hDNA Topo I with *cis*-DDP-Modified ϕX174DNA–Histone Complexes

Method	Reaction time (h)	Distribution (%)					
		Trefoil and figure-eight knot	Mini CC[c]	Topo isomers	Relax	Others[d]	Total (Amount)[e]
cis-DDP modified ϕX174–histone/topo I[a]	3[b]	27.4	*ca.* 0.0	44.9	10.7	17.0	100 (354)
cis-DDP modified ϕX174–histone/topo I[a]	4[b]	13.3	14.9	44.9	8.5	18.4	100 (316)

a) Histones extracted from LNCaP cells. *b*) Used *cis*-DDP-modified ϕX174–histone^{LNCaP} complexes produced by incubation for 3 and 4 h at 37 °C, respectively. *c*) Mini cc: mini closed circular DNA. *d*) Fragment DNA, *etc*. *e*) Counted number.

cis-DDP-modified DNA–histone–Topo I complexes. We have shown that the topological changing of the DNA structure occurs by the formation of intra-twisted loop in the DNA partially dissociated by *cis*-DDP binding. The topological DNA structure changes depending upon the odd or even writhing number for winding numbers of intra-twisted loops.

In Fig. 8, the topological pathway in **11** predicts that the recombined trivial knot (**1**) Jones polynomial $V_K(t)=1$ is produced. This prediction coincides with the experimental results (see Fig. 6C). The sign (+1) indicates that the recombination progresses in the direction in which the writhing number increases by 1. Inversely, the sign (−1) means that the recombination progresses in the direction which the writhing number decrease by 1. When the intra-twisted looped DNA with the odd numbers of Wr reacts with hTopo I, Wr is reduced one, as shown in the small circle of Fig. 8. In **12** (writhing number (Wr)=−1), the DNA **12** transforms mini circular closed DNA ($V_K(t)=1$); total Wr is (−1)+(+1)=0. However, when the Wr increases by 1, total Wr is (−1)+(−1)=−2, and singly linked catenane **6** is produced by the topo I recombination. In the actual reaction using the *cis*-DDP modified ϕX174RFDNA–histone^{LNCaP} complexes, we obtained mini circular closed DNA (Fig. 7B) and singly linked catenane (Fig. 7C). The $V_K(t)$ of the catenane is $+t^{-1/2}-t^{-5/2}$. When the Wr number is increased by one (**13** in Fig. 8), total Wr is (−2)+(−1)=−3, the trefoil **7** or **8** is produced by the topo I recombination (Figs. 6A, 7A), and the Jones polynomial ($V_K(t)$) is $t^{-1}+t^{-3}-t^{-4}$ or $t+t^3-t^4$. When the Wr is reduced by one (Fig. 8), total Wr of the DNA is (−2)+(+1)=−1. The trivial knot **1** ($V_K(t)=1$) should be produced by the recombination.

In contrast, *trans*-DDP-modified ϕX174RFDNA–histone^{LNCaP} complexes are quite different from those modified by *cis*-DDP. The total yield of the topological isomers, trefoil, catenane, and mini ccDNA using *trans*-DDP is only about 2% that of *cis*-DDP (Table 2).

Our model on the mechanism of trefoil, mini cc DNA, and figure-eight formation consists of the recombination of DNA by topo I based on an even-odd number rule. We can derive the following rules.

Rule 1: Wr number of intra-twisted looped (itl) DNA; Wr^{itl}+sign (at recombination point)
Wr^{itl}+sign
Odd+(−1)=mini circular closed DNA
Odd+(+1)=singly linked catenane
Where the number of Wr^{itl} is 1 in the maximum.

Rule 2: Wr number of intra-twisted looped (itl) DNA; Wr^{itl}+sign (at recombination point)
Wr^{itl}+sign
Even+(−1)=trivial knot
Even+(+1)=trefoil
Where the number of Wr^{itl} is 2 in the maximum.

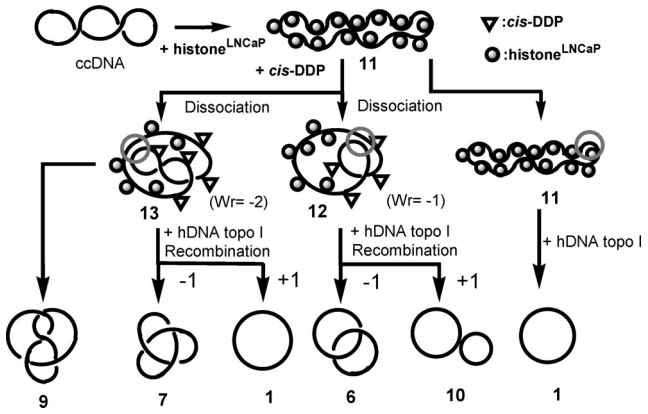

Fig. 8. Possible Mechanism for the Production of Topologically-Distinct Invariant DNA Through the Reaction of *cis*-DDP-Modified ϕX174RF DNA–Histone Complexes with hDNA Topoisomerase I

Trefoil **7** (and **8**) and mini closed circular DNA**10** *etc.* are yielded by recombination of DNA topo I *via cis*-DDP (or *trans*-DDP) modified intra-twisted looped DNA–histone complexes. The platinum ion modified complexes **13** and **12** have writhing number (Wr) of sign −2 and −1, respectively. When *cis*-DDP adducts with DNA–histone complex **11**, the chemical equilibrium moves to the right-hand side (Eq. 3) in order to form *cis*-DDP-modified intra-twisted looped DNA–histone complexes, **12** and **13**. When *trans*-DDP adducts with DNA–histone complex **11**, however, the chemical equilibrium moves to left-hand side (Eq. 4). The formation of *trans*-DDP-modified intra-twisted looped DNA–histone complexes decreases (see Tables 1, 2).

Conclusion

Our data suggest that trefoil, mini ccDNA, and fragment DNA are produced by a reaction of hDNA topo I with *cis*- or *trans*-DDP-modified chromatin in the nucleus of cancer cells. Three topological processes, the local dissociation of DNA, the formation of intra-twisted looped DNA, and the recombination by hDNA topo I, have achieved an important action in the nucleus (see Fig. 9). Therefore, our model predicts that trefoil and mini ccDNA also are formed in the nucleus of living cells. The yield of trefoil and mini ccDNA by DNA topo I recombination was higher *cis*-DDP-modified DNA–histone complexes than *trans*-DDP-modified DNA–histone complexes. Therefore, the differences of topological distinct invariant DNA formation may be related to the level of anti-cancer activity. These results suggest that a major reason why the *trans*-DDP isomers clinically ineffective is that it produces a topologically-distinct invariant DNA. If this is the case, the production of topologically-distinct invariant DNA will be an important measure in developing platinum

Table 2. Distribution of Trefoil, Figure-Eight Knot, and Mini ccDNA Produced by Reaction of Topo I with *trans*-DDP-Modified ϕX174–Histone Complexes

Method	Reaction time (h)	Distribution (%)					
		Trefoil and figure-eight knot	Mini cc[c]	Topo isomers	Relax	Others[d]	Total (amount)[e]
trans-DDP modified ϕX174–histone/topo I[a]	3[b]	1.4	0.7	59.5	9.2	29.2	100 (284)
trans-DDP modified ϕX174–histone/topo I[a]	4[b]	1.9	0.9	58.6	11.4	27.2	100 (210)

a) Histones extracted from LNCaP cells. *b*) Used TDDP–ϕX174–histone^{LNCaP} complexes produced by incubation for 3 and 4 h at 37 °C, respectively. *c*) Mini cc: mini closed circular DNA. *d*) Fragment DNA, *etc*. *e*) Counted number.

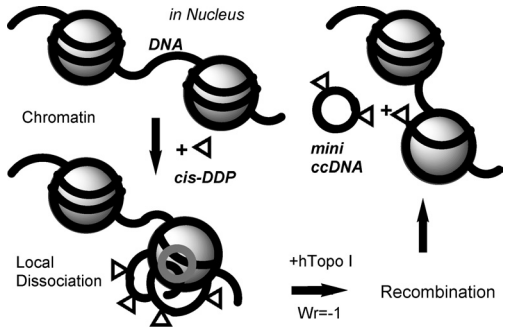

Fig. 9. Model of Proposed Mechanism of Mini ccDNA Production through the Reaction of *cis*-DDP-Modified DNA–Histone Complexes with HDNA Topoisomerase I in the Nucleus

Local dissociation of DNA from chromatin by *cis*-DDP binding accelerates the form of *cis*-DDP modified intra-twisted looped DNA. Mini ccDNA is then produced by strands recombination of DNA topoisomerase I in the circle.

(Pt^{2+}) ion related anti-cancer drugs.

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