

Topology Effect for DNA Structure of Cisplatin: Topological Transformation of Cisplatin-Closed Circular DNA Adducts by DNA Topoisomerase I

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The reaction of *cis*-Pt(NH₃)₂Cl₂ (*cis*-DDP)-closed circular DNA adducts with DNA topoisomerase I (topo I) were studied by electron microscopy. We identified unique topoisomers such as a singly-linked catenane (2₁¹), trefoil (3₁), and dimetric catenane (2₂²), etc., by analysis with electron micrographs. These unique recombination products resulted from *cis*-DDP-intra-twisting looped DNA adducts by DNA topo I, and the products could be explained a new mechanism based on an odd-even number rule. Our results suggest a new model on the working mechanisms for DNA topology of *cis*-DDP which enhances the recombination of DNA. Based on our results, we propose the topological idea that the yields of a mini closed circular DNA and pseudo trefoil DNA, etc., can be expected by reaction of *cis*-DDP-DNA-histone complexes with DNA topo I in the body.

Key words *cis*-diamminedichloroplatinum(II); DNA topology; DNA topoisomerase I; cancer therapy; trefoil; catenane

Recently, knots and links theory has fascinated chemists and biologists as a new method for the analysis of DNA topologies.¹⁾ In particular, the knotting of DNA is important as a pathway for changing DNA topology for transcription and replication in cells. The enzyme reactions in cells are catalyzed by DNA topoisomerase families, which are well known as type I, II, and *Escherichia coli* (*E. coli*) DNA gyrase, etc.²⁾ Further, the knot and link structure of DNA are related to the action of DNA topoisomerase. This caused an extensive basic and clinical interest in the chemotherapy of cancer.

For topoisomerase I (=topo I) targeting drugs, for instance, the inhibition activities of camptothecin derivatives³⁾ for DNA topo I in clinical trial have shown that the drugs are excellent anticancer compounds with potent anticancer activities. DNA topo I alters DNA topology by a single-strand DNA passage mechanism, and the eukaryotic topoisomerase II in the presence of ATP alters the DNA topology by a double-stranded DNA passage mechanism; these enzymes generally convert the supercoiled state of DNA to a relaxed state.²⁾ The inhibitor of DNA topo I, camptothecin, is of special interest as it inhibits the activity of the enzyme which alters the linking number of a DNA circle in steps of passage.

Now, another anticancer drug, *cis*-diamminedichloroplatinum(II) (*cis*-Pt(NH₃)₂Cl₂, *cis*-DDP, or cisplatin) (1), is presented in Fig. 1, and although it has toxic side effects, it is widely used as an effective cancer chemotherapeutic agent. The working mechanism of *cis*-DDP differs from that of camptothecin. Numerous studies have suggested that the cytotoxic action of *cis*-DDP is related to its ability to react with DNA, but the working mechanism of its effects remain uncertain.⁴⁾ We consider that this is mostly due to a lack of evidence of the tertiary structure details of *cis*-DDP-DNA adducts. Information about topological structure is essential in elucidating the mechanism of action and the conversion of types of cross-links⁵⁾ occurring in DNA as a result of formation of the *cis*-DDP-DNA adducts.

In our previous study, we found the production of the topoisomers such as catenane and trefoil by treatment of *cis*-

DDP-pBR322 DNA adducts with DNA topo I.⁶⁾ In the cell cycle, therefore, DNA topoisomerases may play a main role for the metabolization of *cis*-DDP-DNA adducts. The ability of DNA topoisomerases for the interaction with *cis*-DDP-DNA adducts has not been elucidated *in vivo* and *in vitro* at all. Studies on the topological changes of *cis*-DDP-DNA adducts by DNA topo I should offer much more information of the tertiary structures and shapes of the adducts in cells. From this, the working mechanism of *cis*-DDP could be elucidated.

We report here that the binding of *cis*-DDP to closed circular pBR322 DNA molecules transforms the supercoil nodes into plectonemic supercoil nodes, and the formation of mainly three unique topological distinct invariant DNAs, such as dimetric catenane, singly-linked catenane, and trefoiled knot, etc. are caused by reaction with *cis*-DDP-DNA adducts of DNA topo I. The topological mechanism for the formation of the unique knots and links can naturally be explained on the basis of the existence of a twisted looped DNA intermediate. We visually confirmed the existence of the intra-twisted looped circular DNA by electron microscopy. In a topological approach to the topo-reaction with *cis*-DDP-DNA adducts, the formation mechanism of tertiary structure for the cisplatinated trefoils and catenanes, etc. can be analyzed under our diagram (Chart 1). The diagram is a convenient tool to predict the topological shapes of topoisomers. The results may provide a new key to facilitate understanding of the working mechanism of the anticancer activity of *cis*-DDP in cells. Our findings imply that *cis*-DDP is a fac-

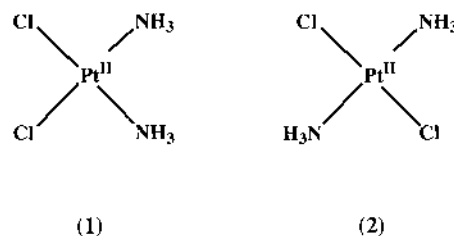


Fig. 1. Structures of *cis*-Pt(NH₃)₂Cl₂ (1) and *trans*-Pt(NH₃)₂Cl₂ (2)

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to enhance the activity of recombination of DNA topo I (or DNA topoisomerases).⁷⁾

Methods and Materials

Drugs and Enzymes Circular supercoiled pBR322 plasmid DNA was isolated by chloramphenicol amplification of *E. coli* cultures. The platinum complex, *cis*-Pt(NH₃)₂Cl₂, was kindly provided by Bristol Myers Squibb Co., (Tokyo, Japan). DNA topoisomerase I from calf thymus and human was purchased from Takara Shuzo Co., (Tokyo, Japan) and TopoGEN, Inc. (Ohio, U.S.A.), respectively.

Preparation of *cis*-DDP-DNA Adducts Method A: A stock solution *cis*-DDP (1.0×10^{-2} M) was prepared by dissolving the complex in a 10 mM Tris-HCl (1×TH) buffer (pH 7.8). In a typical binding experiment, the *cis*-DDP-DNA adducts were prepared by incubation of 0.12 μg of pBR322 DNA with final concentrations of 955.6, 191.1, 95.6, 19.1, 9.56, 0.956, 0.0956, and 0.0 ($\times 10^{-5}$ M) *cis*-DDP in the solution (final 10 μl volume) of 10 mM Tris-HCl (pH 7.8) at 37°C for 2 h and stored at 4°C for 3 d in the dark. After the reaction of the *cis*-DDP-DNA adducts formation, the solutions of the adducts were loaded on a 0.8% agarose gel electrophoresis in TBE (Tris-boric acid-ethylenediaminetetracetic acid) buffer (pH 8.1) at 15 V (1.5 V/cm) constant power for 16 h. The gels were stained with ethidium bromide for 0.5 h, and photographed under UV with a Polaroid camera using Polapan 667 (or 665) films. The mobility and concentration of DNA on the gel were measured by a Pharmacia Image Analyzer (Image Master).

Method B: The *cis*-DDP-DNA adducts were prepared by incubation of 0.08 μg of pBR322 DNA with final concentrations of 101.0, 50.0, 10.1, 5.0, 1.01, 0.101, 0.0101, and 0.0 ($\times 10^{-5}$ M) *cis*-DDP in 10 ml final volume of 10 mM Tris-HCl (pH 7.5) at 37°C for 2 h and stored at 4°C for 3 d in the dark.

Reaction of *cis*-Pt(NH₃)₂Cl₂-DNA Adducts by DNA Topoisomerase I Method C: The topoisomers of the *cis*-DDP-DNA adducts were prepared by incubation of 1.29 μg of pBR322 DNA for 2 h at 37°C at final concentrations of 217.0, 108.0, 21.7, 10.8, 2.17, 1.08, and 0.0 ($\times 10^{-6}$ M) *cis*-DDP (according to Method A). The topoisomers were reacted with 12 units (6 U/μl) of calf thymus DNA topo I in 32 μl of a reaction mixture of 35 mM Tris-HCl (pH 7.8), 72 mM NaCl, 5 mM MgCl₂, 5 mM dithiothreitol (DTT), 5 mM spermidine, and 0.01% bovine serum albumin (BSA) buffer (1×TNM) at 37°C for 1 h. After the topo reaction was terminated by 2 μl of 1% sodium dodecyl sulfate (SDS) solution, 6 μl of the reaction mixture was loaded on an 0.8% agarose gel electrophoresis at 15 V for 15 h, and then analyzed by electron microscope by the Electron microscopy method described below.

Method D: Eight units (4 U/μl) of human DNA topoisomerase I was reacted with the solution of *cis*-DDP-DNA adducts prepared by the method B carried out in the solution of a final volume of 20 μl contained 10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl. The mixtures were incubated at 37°C for 1 h, and analyzed by the above described methods A and C.

Electron Microscopy The preparation of shadowed grids for electron microscopy was performed by essentially the same procedure as that described by Yamagishi.⁸⁾ Photographs were taken at an initial magnification of 20,000× (or 28,000×), as shown in Fig. 7. The contour lengths of circular

DNA molecules, trefoils, catenanes, and *cis*-DDP-DNA complexes were determined by tracing from negatives of the enlarged image and measured with a digitizer (1200 series graphics calculator, Numonics Corp., PA).

Results

Cisplatin-closed Circular pBR322 DNA Adducts The platinum-DNA adducts were prepared by incubation of pBR322 DNA with *cis*-DDP in 20 mM TH buffer (pH=7.8) at 37°C for 2 h and subsequently stored at 4°C for 3 d, and were detected on the bands in 0.8% agarose gel electrophoresis as shown in Fig. 2. Since the changes in electrophoretic mobility for *cis*-DDP-DNA adducts can be readily visualized with agarose gel, the binding effects of *cis*-DDP action on DNA could be easily elucidated. Lanes 1—3 in Fig. 2 (left) show that the mobility of the bands moves upward by the concentration of 95.6×10^{-5} M *cis*-DDP at pH 7.8. In Fig. 2 (right), the electrophoretic mobility of the adducts is given as a function of $-\log[C]$ of *cis*-DDP. Drastic changes in mobility, for instance, are observed on the gel in Fig.2(left) with a binding concentration of 191.1—955.6 $\times 10^{-5}$ M *cis*-DDP, while at the lower concentrations of 95.6—0.0956 $\times 10^{-5}$ M these phenomena are not observed. This decrease in mobility is caused by the unwinding of the negative superhelical DNA.⁹⁾ The shapes of *cis*-DDP-DNA adducts can be recognized based on the curve of mobility vs. the *cis*-DDP concentrations on Fig. 2 (right). The increase in *cis*-DDP molar ratio per DNA causes a reverseal in the increase of electrophoretic mobility of *cis*-DDP-DNA adducts as presented in Fig. 3 (Method B).

It may be indicated that the *cis*-DDP binding to pBR322 DNA causes the structural change to the relaxed and nicked circular formed DNAs. However, electron microscopic studies of *cis*-DDP-pBR322 DNA adducts gave the explanation for the cause of the changes of electrophoretic mobility for the binding concentration of *cis*-DDP in the DNA. By the addition of *cis*-DDP, the superhelical density of pBR322 DNA was reduced, and thus the DNA shape was relaxed. In addition, intra-twisted looped DNA (see Fig. 6) was also obtained. Furthermore, we previously found by using electron microscopic studies that the positive increase of writhing numbers($\langle Wn \rangle$) in the *cis*-DDP-DNA adducts causes reversal increased mobility, and the sign is positive.¹⁰⁾

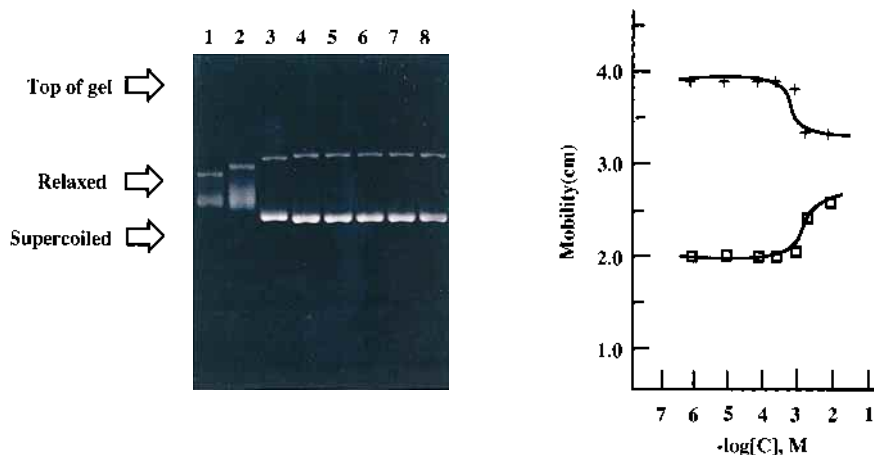


Fig. 2. (Left) Agarose Gel Electrophoresis of *cis*-Pt(NH₃)₂Cl₂-pBR322DNA Adducts Produced by Reaction of pBR322DNA (0.12 μg) with *cis*-Pt(NH₃)₂Cl₂ at Concentrations of *cis*-Pt(NH₃)₂Cl₂ 955.6, 191.1, 95.6, 19.1, 9.56, 0.956, 0.0956, and 0.0 $\times 10^{-5}$ M, Respectively (Lanes 1—8) (Method A). (Right) The Relationship between Concentration ($-\log[C]$, M) of *cis*-DDP and Its Mobility (cm)

Symbols + and □ represent the mobility of *cis*-DDP-supercoiled DNA adducts and *cis*-DDP-relaxed DNA adducts, respectively.

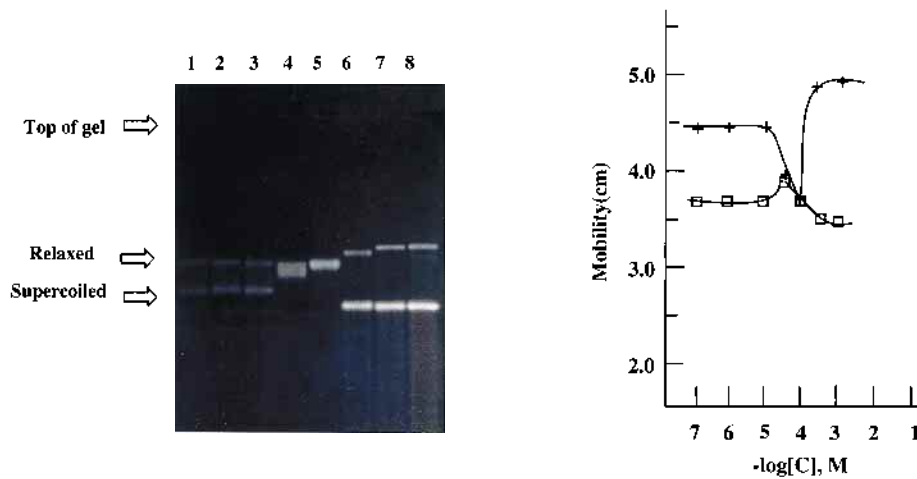


Fig. 3. (Left) Agarose Gel Electrophoresis of *cis*-Pt(NH₃)₂Cl₂-pBR322DNA Adducts Produced by Reaction of pBR322DNA (0.08 μg) with *cis*-Pt(NH₃)₂Cl₂ at Concentrations of *cis*-Pt(NH₃)₂Cl₂ 101.0, 50.0, 10.1, 5.0, 1.01, 0.101, 0.0101, and 0.0×10⁻⁵ M, Respectively (Lanes 1—8) (Method B). (Right) The Relationship between Concentration (-log[C], M) of *cis*-DDP and Its Mobility (cm)

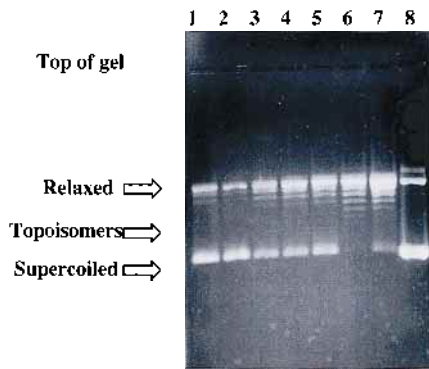


Fig. 4. Agarose Gel Electrophoresis of Topological Isomers Produced by Reaction of *cis*-Pt(NH₃)₂Cl₂-pBR322DNA Adducts with DNA Topoisomerase I (Method C)

Lane 8, DNA only (control); lanes 1—7, the concentrations of *cis*-Pt(NH₃)₂Cl₂ are 216.8, 108.4, 21.68, 10.84, 2.168, 1.084, and 0.0×10⁻⁶ M, respectively. Reactions of *cis*-Pt(NH₃)₂Cl₂-DNA adducts with calf thymus DNA topo I were performed for 60 min at 37.0 °C.

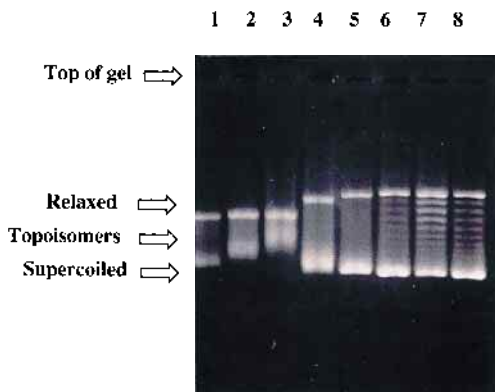


Fig. 5. Agarose Gel Electrophoresis of Topological Isomers Produced by Reaction of *cis*-Pt(NH₃)₂Cl₂-pBR322DNA Adducts with Human DNA Topoisomerase I (Method D)

Lane 8, DNA only (control); lanes 1—7, the concentrations of *cis*-Pt(NH₃)₂Cl₂ are 101.0, 50.0, 10.1, 5.0, 1.01, 0.101, 0.010, and 0.0×10⁻⁵ M, respectively. Reactions of *cis*-Pt(NH₃)₂Cl₂-DNA adducts with human DNA topo I were for 60 min at 37.0 °C.

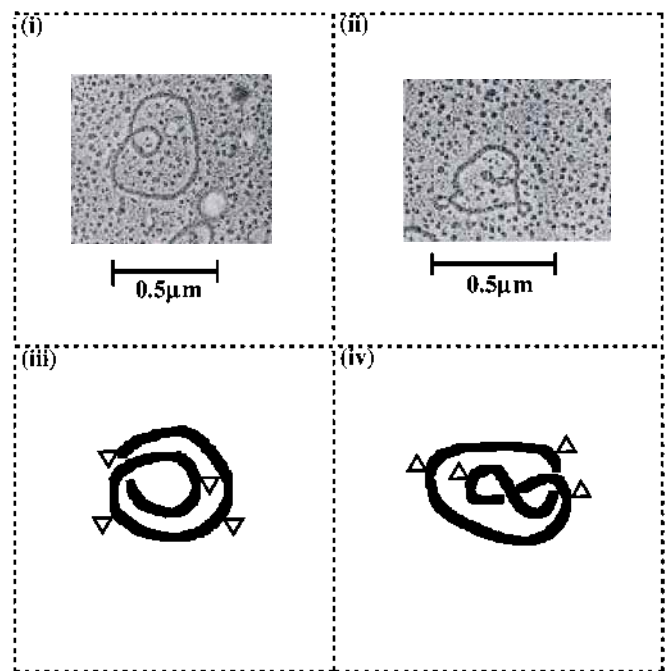


Fig. 6. Electron Micrographs of Intra-Twisting Looped DNAs ((i) and (ii)) Produced by Reaction (condition of (B) in Method C) of *cis*-Pt(NH₃)₂Cl₂-pBR322DNA Adducts with DNA Topoisomerase I

Its DNA is expected to act on the intermediate in the DNA topological transformation. Illustrated (iii) and (iv) are depicted by tracing of electron micrographs (i) and (ii), respectively. Symbol (Δ) represents *cis*-DDP.

Topoisomerase I reaction and Micrographs In order to investigate the effect of *cis*-DDP for relaxation and supercoiling of *cis*-DDP-DNA adducts, the *cis*-DDP-DNA adducts were reacted with DNA topo I. Figure 4 shows the ladders on 0.8% agarose gel electrophoresis for topoisomers of *cis*-Pt(NH₃)₂Cl₂-pBR322 DNA adducts reacted with calf thymus DNA topo I. Unplatinated control DNA in lane 7 appears as a negative topoisomer. *cis*-DDP does not act as a DNA topo I inhibitor. As shown in lane 6 in Fig. 4, the reaction with DNA topo I of *cis*-DDP-DNA adducts which were prepared at a low concentration, 0.108×10⁻⁵ M *cis*-DDP, causes a negative relaxation upwards on the gel. In the presence of the

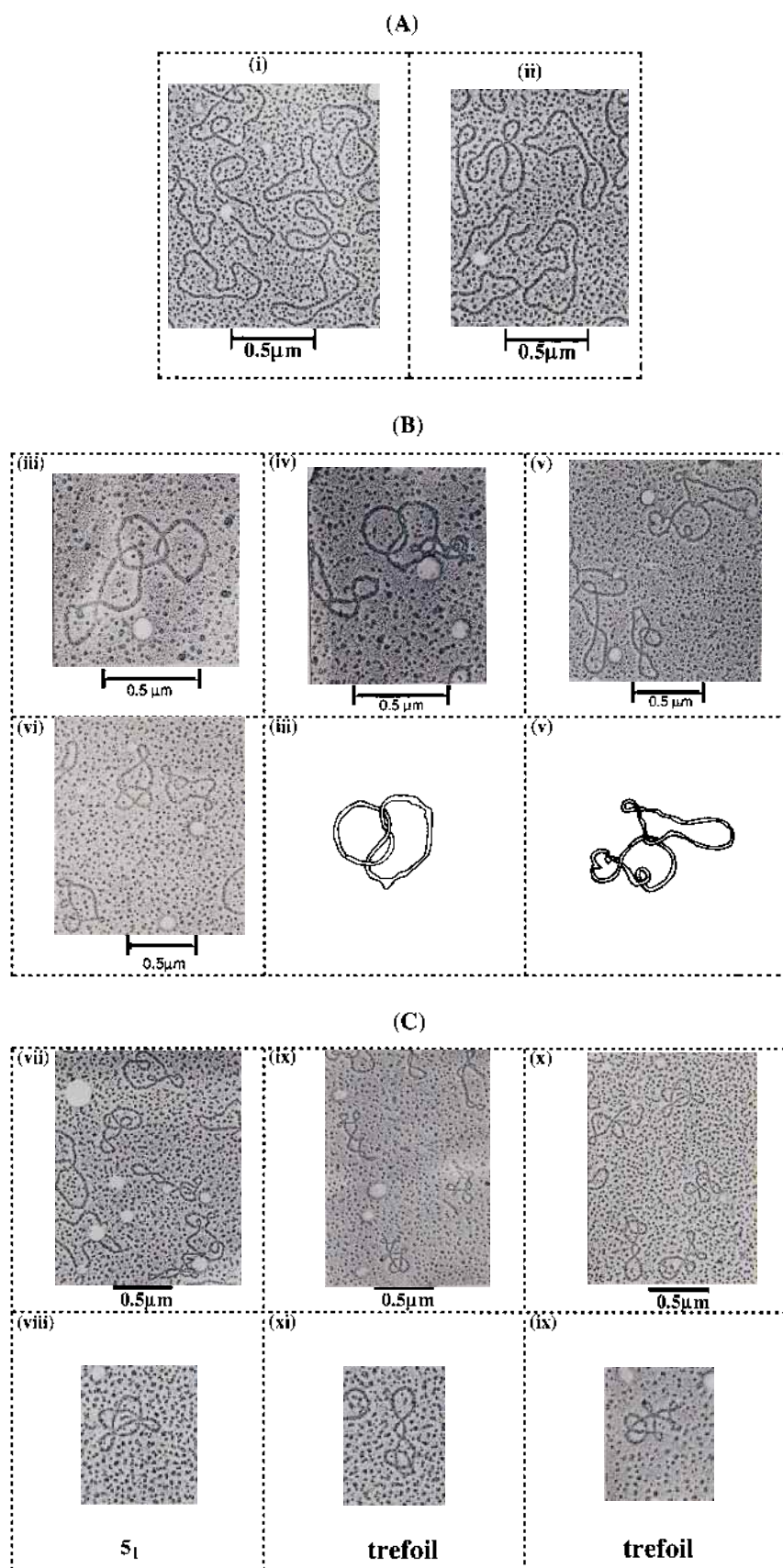


Fig. 7. Electron Micrographs of Supercoiled, Trefoil Knot, and Singly-Linked Catenane of *cis*-Pt(NH₃)₂Cl₂ Binding Produced by Reaction of *cis*-Pt(NH₃)₂Cl₂-pBR322DNA Adducts with DNA Topoisomerase I

The concentration of *cis*-Pt(NH₃)₂Cl₂: (A) 0.0, (B) 1.084×10^{-6} M, and (C) 1.084×10^{-4} M. The representations of DNA isomers are (i) and (ii) relaxed form, (iii) singly-linked catenane, (iv) trefoil and singly-linked catenane, (v) dimetric catenane, (vi) trefoil, (vii) trefoil and supercoil, (viii) 5_1 knot, (ix) trefoil and supercoil, (x), (xi) trefoil, 5_1 knot, and supercoil, respectively.

Table 1. Correlation between the Number of Observed Knots and Links and Concentration of Cisplatin in Reaction of *cis*-DDP–DNA Adducts with DNA Topoisomerase I

Cisplatin (M)	Number of DNAs observed	Number of observed nodes in intra-twisted loop ^{a)}			Observed linking DNAs	Observed knotting DNAs
		1	2	3		
0	821	174 (21.2) ^{b)}	7 (0.9)	1 (0.1)	9 (1.1)	2 (0.2)
1.08×10^{-6}	592	143 (24.2)	5 (0.8)	1 (0.2)	66 (11.1)	24 (4.1)
1.08×10^{-4}	866	61 (7.0)	16 (1.8)	1 (0.1)	36 (4.2)	15 (1.7)

a, b) Number in parenthesis means %, for instance, $174/821 \times 100 = 21.2\%$.

cis-DDP–DNA adducts bound at high concentrations, 217.0, 108.0, 21.7, 10.8×10^{-6} M *cis*-DDP, respectively, the positively supercoiled topoisomers appear downwards on the gel (lanes 1–4 in Fig. 4). Figure 5 shows that similar results are obtained with human DNA topo I in 10 mM TH buffer. We could observe the *cis*-DDP–DNA topoisomers by electron microscopy, and the results for the reaction of DNA topo I (Method C) with *cis*-DDP–DNA adducts are summarized in Table 1. We paid attention to the number of nodes in intra-twisted loop formed by binding of *cis*-DDP as shown in Fig. 6. The results show that the intra-twisted looped DNAs are a key intermediate for the topological transformation of DNAs by DNA topo I.

When the number of *cis*-DDP binding increases with concentration, the number of linking DNAs, such as catenane, increased from 1.1% to 11.1%. The number of knotting DNAs also increased from 0.2% to 4.1%. We found that the results are caused by the production of unique topological DNAs. In presence of *cis*-DDP, the amount of the linking and knotting DNAs increases. Figure 7 shows the electron microscopy visualization of trefoil, catenane, and singly-linked catenane, *etc.* Especially, the catenane and singly-linked catenane are formed at the lower concentration of 0.108×10^{-5} M *cis*-DDP (Fig. 7(B)), and this means that the shapes of the link and knot's nodes are related to the binding amount of *cis*-DDP to DNA. Figures 7(B) (iv), (vi) and (v) show a trefoil knot and a dimetric catenane, respectively. Below the tracings (iii–iv) are a redrawing of the trefoil and dimetric catenane. Figures 7(B) (iii) and (iv) shows a singly-linked catenane which was observed in the same manner in the presence of 0.108×10^{-5} M *cis*-DDP. Its catenane is composed of two circular DNAs of 0.77 and 0.79 μ m length.

On the other hand, the topo-reaction of *cis*-DDP–DNA adducts at a concentration of 10.8×10^{-5} M *cis*-DDP yielded a trefoil knot and trimetric catenane in Fig. 7(C) (vii–x). The bottom in Fig. 7(C) shows the magnification of two types of trefoils and 5_1 DNA¹¹⁾ by electron microscopy. Thus, we conclude the following: first, DNA knots and catenanes are not generated from DNA without *cis*-DDP binding by DNA topo I (see Fig. 7(A)); second, supercoiled DNA increases with increasing concentrations of *cis*-Pt(NH₃)₂Cl₂ in the reaction with DNA topo I. The curved supercoils are completely different from the molecular shape of the native supercoiled pBR322 DNA; third, a unique singly-linked catenane (2_1^2)¹¹⁾ is formed together with a dimetric catenane (2_1^2) and a trefoiled knot (3_1) from *cis*-DDP–DNA adducts.

Lengths of Topoisomers We measured the precise lengths of the topoisomers which obtained from *cis*-DDP bound or non-bound to pBR322 DNA by catalytic DNA topo

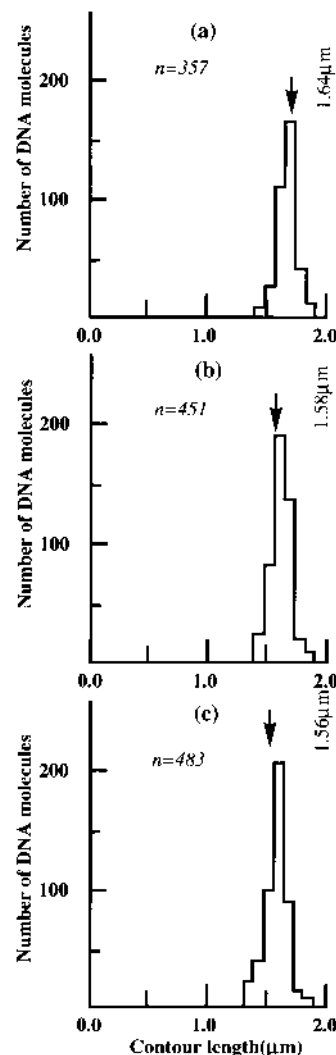


Fig. 8. Distribution of Contour Lengths of Topoisomers Produced by Reaction of *cis*-Pt(NH₃)₂Cl₂–pBR322DNA Adducts with DNA Topoisomerase I

The concentration of *cis*-Pt(NH₃)₂Cl₂: (a) 0.0, (b) 1.08×10^{-6} , and (c) 1.084×10^{-4} M. DNA exists as a cluster of molecules with a mean contour length of (a) 1.64 μ m ($N=357$ molecules measured), (b) 1.58 μ m ($N=451$), and (c) 1.59 μ m ($N=483$), respectively.

I. Statistically, the length of the *cis*-DDP–DNA adducts treated with topo I is shorter than that of the native DNA treated with it in the same manner. The results are summarized in Fig. 8. The DNA lengths for reaction with topo I of non-platinated pBR322 DNA are shown in Fig. 8(a). The arrow represents the mean value of contour length, and the value was 1.64 μ m. However, the length of topoisomers by topo-reaction of the platinated DNA is shorter (1.58–

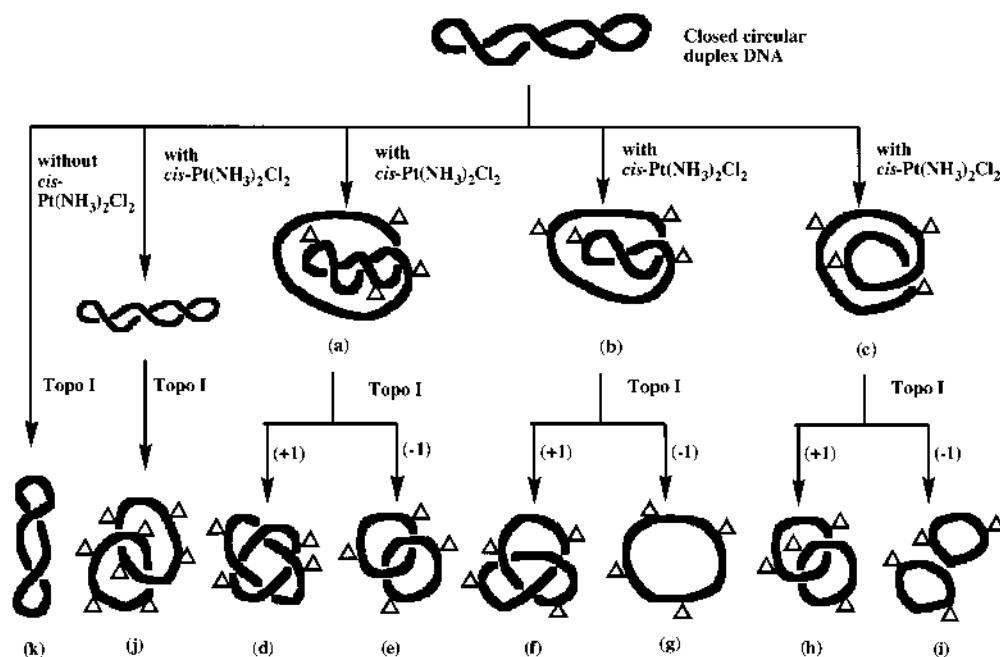


Chart 1. Possible Topological Mechanisms on Recombination of *cis*-Pt(NH₃)₂Cl₂-DNA Adducts by calf Thymus DNA Topoisomerase I

(a) Writhing number (Wn) of intra-twisted loop is 3; (b) (Wn) is 2; (c) (Wn) is 1; (d) redrawing of catenane in drawing, 4₂; (e) singly linked catenane, 2₂; (f) trefoil knot, 3₁; (g) trivial knot, 0₁; (h) singly linked catenane, 2₂; (i) trivial knot, 0₁; (j) dimetric catenane, 2₂; and (k) supercoiled DNA.

1.59 μm). This may be due to an increase in small DNA knot nodes and a change in the DNA twist angle by platination to DNA.

Mechanisms of Topo I-Reaction for *cis*-CDDP-DNA Adducts and DNA Topology Analysis

We used the topological method in order to elucidate the mechanism for the topological pathway to the resulting topoisomers of singly-linked catenane, trefoil, and dimetric catenane, as shown in Fig. 7 (see (B) and (C)). How is linked and knotted DNA generated by a Topo I-reaction mechanism? It was found that the *cis*-DDP-DNA adducts are transformed to the trefoil knot (f), singly-linked catenane (e and h), dimetric catenane (j), etc., by recombination of DNA topo I. In this topo-reaction mechanism, the catenanes must be present in an even number for writhing of intra-twisted loop (Wn) (a–c), and knotting must occur an odd number of times. We propose a mechanism to construct the catenanes and knots by reaction of *cis*-DDP-DNA adducts with DNA topo I (Chart 1). In Chart 1(b), for instance, if the sign¹² is (1+), the DNA topo I acts positively on the platinated DNA, and the knotting DNA (trefoil, f) is generated because the (Wn) is 3 ($=2+1$). Nevertheless, if the sign is (1–), the recombined trivial knot (g) is generated because the (Wn) is 1 ($=2-1$). Then, the singly-linked catenane is generated from *cis*-DDP-DNA adducts (a) or (c) in Chart 1.

We propose a tree diagram, as shown in Fig. 9, in order to summarize the pathway of the topological mechanisms, based on the experimental findings as shown in Chart 1. The bold type numbers represent patterns and (Wn) of intra-twisted loop,¹³ and the numbers in parentheses represent manipulation of recombination. For instance, the tree diagram (Fig. 9) predicts that the reaction of *cis*-DDP-2 adducts ((b) in Fig. 9) with DNA topo I generates both trefoil DNA 3 ((f) in Chart 1) and trivial knot DNA 1 ((g) in Chart 1). *cis*-DDP-1 adducts ((c) in Scheme 1 or (a) in Fig. 9) generate

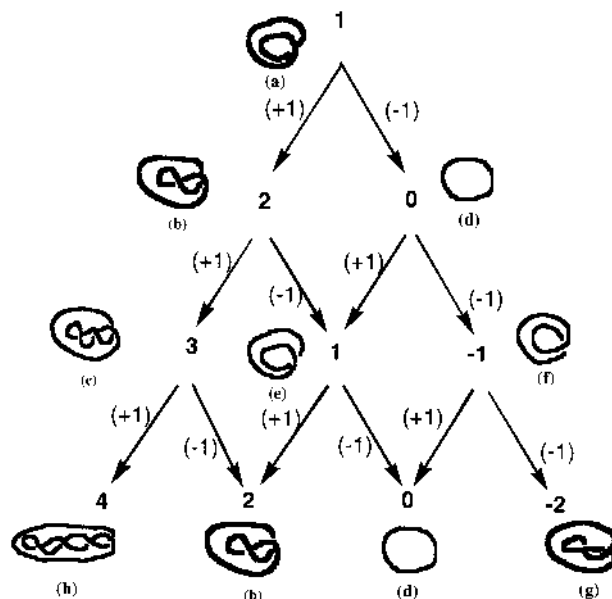


Fig. 9. Tree Diagram for the Reaction of *cis*-Pt(NH₃)₂Cl₂-DNA Complex with DNA Topoisomerase I

The transformation between both intra-twisting looped DNA decides what shape of distinct invariant DNA is constructed. The sign means that the manipulation of +1 winds DNA to negative and -1 unwinds DNA to positive.

singly linked catenane 2 ((h) in Chart 1) and mini trivial knot DNA 2 ((i) in Chart 1). Here, the manipulation (+1) and/or (-1) winds or unwinds double-stranded DNA. The sign (+) represents positive manipulation, and the sign (-) negative manipulation. In types for intra-twisted loop, we found at least four types of topology; 0, 1, 2, and 3, e.g. 0=(i), 1=(g), 2=(e) and (h), and 3=(f) as shown in Chart 1, by electron microscope. Thus, based on the rule of an odd and even number, we could explain the construction of the topoisom-

mers in the topological pathway of knot and catenane. It indicates that *cis*-DDP plays an important role to obtain the intra-twisted looped DNA.

Discussion

We propose a new mechanism for the action to the *cis*-DDP-DNA adducts of DNA topo I. Although the tertiary structural and topological characters of the *cis*-DDP-DNA adducts have not been reported yet, if *cis*-DDP interacts with DNA in cells, the *cis*-DDP-DNA adducts are expected to be affected by DNA topo I (and topo II). In this respect, the present study showed a new idea to elucidate a topological role for interaction between *cis*-DDP and DNA. As a result, we found that platinum ion generates the intra-twisted looped DNA by the reaction with DNA, and when the platinum ion-DNA complexes are reacted with DNA topo I, unique DNA topoisomers such as trefoil and catenane DNA are easily obtained. Our data imply that cisplatin enhances the recombination catalyzed by DNA topo I, because the general mechanism for relaxation of negative supercoils can not cover the mechanism to generate a trefoil, singly-linked catenane, or dimetric catenane (see topological method). Therefore, we discussed the mechanisms for topological pathway of *cis*-DDP-DNA adducts based on DNA topology, as illustrated in Chart 1.

The unique intra-twisted looped DNA adducts containing platinum (in Chart 1(a), (b), and (c)) were observed by electron microscope, and the *cis*-DDP-DNA adducts can be interwound as plectonemic supercoils. In fact, the intra-twisted looped DNAs resulted from cisplatin (Fig. 6). The difference of the shapes is clear in comparison with the supercoils and relaxed DNA, and the intra-twisted looped DNAs can be identified from the mobility of the bands on agarose gel electrophoresis.

Electron microscopy of the topoisomers formed by reac-

tion with *cis*-DDP-DNA adducts of topo I showed the generation of topologically distinct *cis*-DDP-DNA adducts, singly-linked catenane, trefoil, dimetric catenane, and positive supercoiled *cis*-DDP-DNA. It is interesting to note that the recombination by topo I at the twisted looping position introduces the exchange of topologically distinct invariant. We found the rule of the generation of these unique *cis*platin-topoisomers, as shown in Chart 1 and summarized in Table 2. For instance, in the illustrated DNA of negative supercoil in Chart 1, if the writhing number is 3 (a), the resulting links generate the topoisomers of nodes 4_1^2 (d) and singly-linked catenane 2_1^2 (e). However, a knotting DNA would not be obtained. On the other hand, if $\langle Wn \rangle$ is 2 (b), the topoisomers of knot 3_1 (f) and the recombined knot 0_1 (g) are generated. If $\langle Wn \rangle$ is 1 (c), the singly-linked catenane 2_1^2 (h) and the small trivial DNA (i) are generated. The topoisomers of (f), (e), and (h) are formed by cisplatin mediated recombination between two DNA strands. In fact, the yield of catenane increases from <1.0% to 11.1% at a concentration of cisplatin 1.08×10^{-6} M, as summarized in Table 1. This means that cisplatin enhances the recombination by DNA topo I, as previously described.

As shown in the model of recombination in Chart 2, we conclude that the knots (d), (e) in Chart 2 are produced from an intra-twisted looped DNA-like structure with different handling. The mechanism indicates that the *cis*platinated intra-twisted looped DNA ($\langle Wn \rangle$ is 2) (b) is converted by winding (+ sign, d') into trefoil (d) and unwinding (-sign, e') into trivial knot (e). Our results suggest that the intra-twisted looped DNAs produced by treatment of *cis*-DDP with DNA are an important intermediate in the products of unique topological DNA. We showed an intra-twisted looped DNA in topological transformation.

Based on our suggested model, we discuss the topological transformation of DNA-histone complex, chromatin. *Cis*-

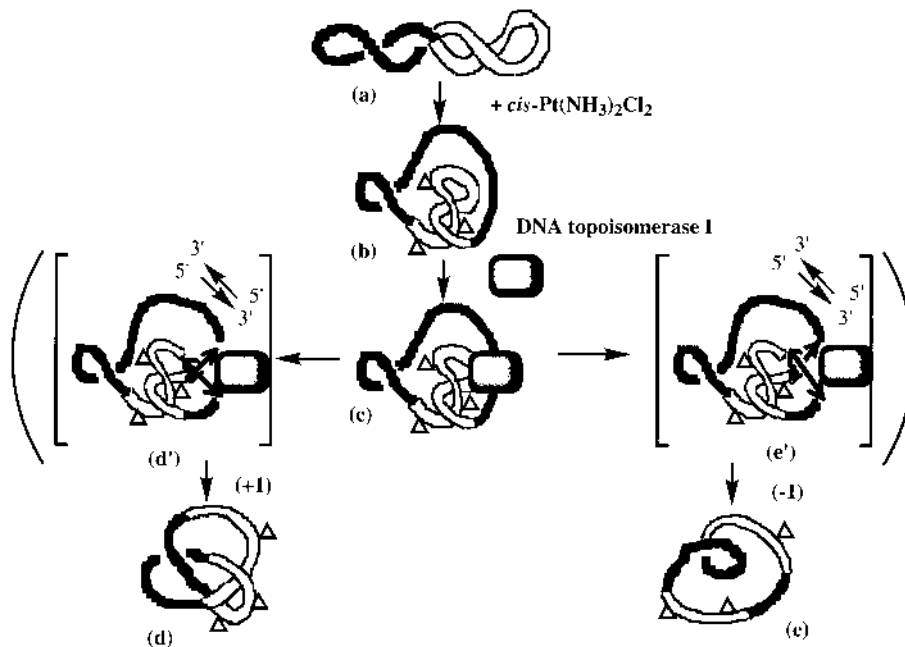


Chart 2. A Possible Recombination Model for the Productive Mechanism of DNAs of Different Topological Distinct Invariants

The DNAs, (d) and (e), are produced from recombination of DNA topo I which binds with twisted looped DNA. According to our model, the recombinations are able to proceed by two routes of (d') of sign(+1) and (e') of sign(-1). Trefoil DNA (d) yields from recombination of more winded loop. DNA (e) yields from recombination of unwinded loop.

Table 2. Odd and Even Number Rule between Writhing Number and Products on Recombination of *cis*-DDP-DNA Adducts by DNA Topoisomerase I

Writhing number	Number of node in product					
	0 (even)	1 (odd)	2 (even)	3 (odd)	4 (even)	5 (odd)
1 (odd)	Catenane	—	Catenane	—	—	—
2 (even)	—	Knot	—	Knot	—	—
3 (odd)	—	—	Catenane	—	Catenane	—
4 (even)	—	—	—	Knot	—	Knot

DDP binding to DNA-histone produces an intra-twisted loop. The yields of a mini circular closed DNA and a pseudo trefoil DNA are expected from reaction of *cis*-DDP-DNA-histone complexes with DNA topoisomerase I. This indicates that the *cis*-platinated linear DNAs also are transformed by reaction with DNA topo I. This result can be applied to the synthesis of unique invariant DNA and to the analysis of the transformation in the early state of replication. It was presumed that *cis*-DDP has a role to control the topology of DNA in the reaction with *cis*-DDP-DNA adducts by DNA topo I. *cis*-DDP enhances the recombination by the topological reaction with DNA by DNA topoisomerase I. This suggestion proposes a new model for understanding the action mechanisms of *cis*-DDP in the body.

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