# Inactivation of Tetrahymena rRNA self-splicing by *cis*-platin proceeds through dissociable complexes

Peter V.Danenberg, Luke C.C.Shea, Kathleen D.Danenberg and Tetsuro Horikoshi
Department of Biochemistry and the Kenneth Norris Jr. Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033, USA

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## ABSTRACT

**The anti-cancer drug *cis*-diamminedichloroplatinum (II) (*cis*-DDP) reacted with Tetrahymena self-splicing rRNA ribozyme, causing loss of self-splicing activity and formation of a number of platinated RNA species. The formation of one distinct platinated product, migrating at an apparent size of 2400 nt, was closely associated with ribozyme inactivation. This platinated RNA was resistant to T1 ribonuclease digestion, suggesting the presence of inter-strand Pt cross-links. The reaction rate of *cis*-DDP with the ribozyme followed first order kinetics and showed a saturation effect with increasing *cis*-DDP concentration, characteristic of an affinity-label type of interaction rather than bimolecular collision. The apparent $K_I$ for binding of *cis*-DDP to the ribozyme was 62 μM. Ribozyme treated with urea was not inactivated by *cis*-DDP, indicating that the native structure of the RNA is required for reaction with *cis*-DDP. $Mg^{++}$, which binds to the ribozyme and causes conformational changes in the molecule, protected the ribozyme from inactivation by *cis*-DDP and also prevented the formation of platinated RNA. These results suggest that binding of *cis*-DDP to sites formed by certain secondary or tertiary structural elements of the RNA enhance the rate and the specificity of reaction of the reagent with the ribozyme.**

## INTRODUCTION

*Cis*-DDP is a widely-used and remarkably effective anti-cancer drug (1–3). Because of early studies which showed that treatment of cells in culture or tumor cells *in vivo* with *cis*-DDP results in the inhibition of DNA synthesis and cell division (4,5), the reaction of *cis*-DDP with DNA has been extensively investigated. *cis*-DDP has been shown to form DNA-protein crosslinks, and both DNA interstrand and DNA intrastrand crosslinks, the latter being more closely associated with cytotoxicity (reviewed in 6 and 7). The replication of DNA has been found to be strikingly sensitive to platination. For example, the inhibition of SV-40 DNA replication is observed at an extent of reaction of 4 Pt atoms per $10^4$ bases (8). In contrast to DNA, the reaction of *cis*-DDP with RNA has been little studied. The chemical reactivity of RNA toward *cis*-DDP should be similar to that of DNA, and recent advances in the understanding of RNA catalysis suggested to us that RNA catalytic functions such as splicing could also be quite sensitive to platination. RNA catalysis has been shown to involve many of the same principles as protein enzyme catalysis, and thus conceivably even one inter-strand cross-linking event on a precursor RNA could prevent the conformational changes and hinder the associations with other macromolecules that are required to effect splicing. RNA splicing is essential for generating the final mRNA copy that is to be translated into proteins, so interference with mRNA processing would exert a significant deleterious effect on the viability of cells.

For investigating the potential of *cis*-DDP to interfere with RNA splicing, we used as a model system the Tetrahymena Group I self-splicing rRNA (9). This autocatalytic RNA (or 'ribozyme') contains one intron (IVS) and two exons, and in the presence of GTP and $Mg^{++}$ undergoes self-splicing, as shown in Fig. 1. After ligation of the exons, the excised IVS undergoes a self-cyclization reaction, resulting in the formation of a covalently closed circle (C-IVS). The Tetrahymena ribozyme has binding sites for its substrates, catalytic activity that is dependent on the proper folding of the molecule into a specific tertiary structure and undergoes conformational changes during catalysis (10).

We find that *cis*-DDP inactivates the self-splicing activity of the Tetrahymena rRNA in a time- and concentration-dependent manner. Platinated RNA species are formed probably containing interstrand cross-links. The formation of these products and the inactivation of ribozyme activity requires the native secondary and tertiary structure of the RNA, suggesting that the reaction is mediated by *cis*-DDP-RNA complexes at specific sites prior to covalent reaction.

## MATERIALS AND METHODS

Plasmid pT7-TT1A3 was obtained from Dr. T.R. Cech, University of Colorado. [$^{32}$P]CTP (specific activity 3000 $C_i$/mmol) was purchased from Amersham, Inc., T7 polymerase was obtained from Pharmacia, and *cis*-DDP was purchased from Sigma Chemical Co. All other reagents were molecular biology grade. 5-Fluorouridine-5′-triphosphate (FUTP) was purchased from Sierra Biochemicals, Tucson, AZ. Atomic absorption spectroscopy was carried out by Galbraith Laboratories, Knoxville, TN.

### Synthesis of precursor RNA and IVS

Plasmid pT7-TT1A3 was cleaved with ecoRI and then transcribed with bacteriophage T7 RNA polymerase (11). Isolation of the precursor RNA was based on previously described procedures

(12,13). The transcription reaction mixture consisted of 40 mM Tris chloride (pH 7.5), 12 mM $MgCl_2$, 10 mM dithiothreitol, 4 mM spermidine, ATP, UTP, and GTP all at a concentration of 1 mM, 0.5 mM CTP, 100 $uC_1$ of [$^{32}$P]CTP, 1 μg of pT7-TT1A3 cut with ecoRI, 100 units of T7 polymerase and water to a total volume of 50 μl. The mixture was incubated for 1 h at 30°C, and then 1.5 μl of 500 mM EDTA was added to quench the transcription reaction. The precursor RNA was isolated by electrophoresis of the reaction mixture on a 4% polyacrylamide 8 M urea gel using as the elution buffer 25 mM Tris base, 21 mM boric acid and 0.25 mM EDTA. The RNA was visualized on the gel by UV shadowing. The gel material containing the precursor band was removed, crushed, and soaked overnight at 4°C in 0.5 mM ammonium acetate, 0.1% sodium dodecyl sulfate and 0.1 mM EDTA. The precursor RNA was further purified by chromatography on Sephadex G-50 using 10 mM Tris (pH 7.5), 250 mM sodium chloride, and 1 mM EDTA as the elution buffer. Fractions containing the product were combined and the RNA precipitated with 3 volumes of ethanol. The precipitated RNA was dissolved and stored in 100 μl of buffer containing 10 mM Tris (pH 7.5) and 1 mM EDTA.

### Treatment with *cis*-DDP and splicing of precursor RNA

The conditions for carrying out the splicing reaction were based on previously published procedures (14). The splicing mixture normally consists of 30 mM Tris-Cl, pH 7.5, 200 mM sodium chloride, 10 mM magnesium chloride and 0.2 mM GTP and precursor RNA in a volume of 10 μl. For the *cis*-DDP treatments of the Tetrahymena rRNA, the procedure was slightly modified. The platination reaction mixture consisted of precursor RNA (1.6 pmol), *cis*-DDP at the desired concentration, and 4 mM sodium chloride in 9 μL of 1 mM Tris-Cl, pH 7.5. After the desired reaction time, splicing was initiated by addition of 1 μl of '10X' splicing buffer, consisting of 300 mM Tris-Cl, pH 7.5, 2000 mM sodium chloride, 2.0 mM GTP and 100 mM magnesium chloride. After 20 min incubation, the splicing reaction was terminated by addition of 1.5 μl of 115 mM EDTA. The reaction was then analyzed by gel electrophoresis using the same system described above. After electrophoresis of the splicing reaction mixtures was complete, the gels were dried and visualized by autoradiography. The amounts of $^{32}$P in each band of interest were quantitated by cutting out the spot using the autoradiogram as a guide.

### Thiourea treatment of platinated RNA

The band migrating at an apparent size of 2400 nt was isolated from the electrophoretic gel as described above for isolation of precursor RNA. About 0.06 pmol of this product was incubated with 100 mM thiourea, 0.5 mM dithiothreitol, and 1 mM EDTA in 10 μl of 1 mM Tris buffer, pH 7.5, for various times at 90°C. The solutions were then analyzed by 4% PAGE in 0.4% TBE.

### Ribonuclease T1 digestion

The precursor RNA and the 2400 nt-migrating product (about 1.2 pmol) were treated with T1 ribonuclease in the presence of 2.4 μg of tRNA, 1 mM Tris, pH 7.5, and 1 mM EDTA in a total volume of 9.5 μl for 28 min at 37°C. The digested products were fractionated by 6% PAGE.

## RESULTS AND DISCUSSION

The Tetrahymena self-splicing rRNA was treated with 10 μM *cis*-DDP and the splicing activity was assayed as a function of exposure time to the drug. Electrophoretic gel analysis of the splicing reaction at each time point (Fig. 2) showed that with

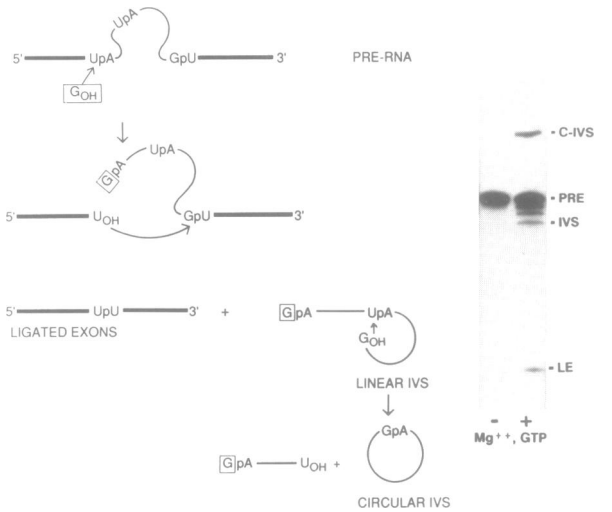


**Figure 1.** Reaction sequence of the Tetrahymena self-splicing RNA. When a guanosine nucleotide ($G_{OH}$) (usually GTP) is added to the precursor RNA (PRE-RNA), cleavage of the ribozyme occurs at the 5′ splice site, and in the process the $G_{OH}$ becomes covalently attached to the 5′ end of the intervening sequence (IVS). The 5′ exon then presumably remains bound to the ribozyme and undergoes a ribozyme-catalyzed reaction with the labile 3′ splice site to give the ligated exons (LE) and the linear IVS. The linear IVS then cyclizes to give the circular IVS with the liberation of a 15-nt fragment. The gel electrophoretogram shows the unspliced precursor RNA (PRE) in the left lane and the spliced mixture of products in the right lane. Note that the circular IVS (C-IVS) migrates much more slowly than the larger linear IVS. The bands located between the IVS and the precursor are splicing intermediates.

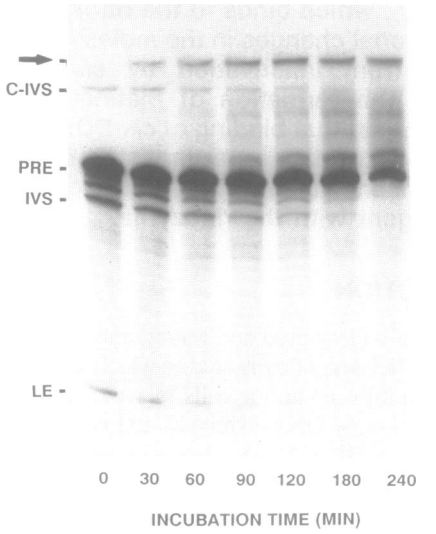


**Figure 2.** Time-dependent loss of self-splicing activity of the Tetrahymena rRNA and formation of platinated products upon treatment with cis-DDP. The ribozyme was incubated with 10 μM cis-DDP for the times indicated and then splicing was initiated as described in MATERIALS AND METHODS. The arrow indicates the platination product that was isolated and subjected to analysis for platinum content. C-IVS is the circularized intervening sequence (intron) of the ribozyme; PRE indicates the unspliced precursor molecule; IVS is the intervening sequence (intron) that is spliced out of the precursor RNA; LE denotes the ligated exons.

increasing reaction time, the formation of the ligated exon product decreased, until after 120 min, no splicing reaction products were detectable. For comparison of the potency of *cis*-DDP inhibition of splicing relative to inhibition of DNA replication, we cite the results of an earlier study that showed about 50% inhibition of SV-40 DNA replication by 10 $\mu$M *cis*-DDP after 24 hr exposure of cultured cells to the drug (8).

Upon exposure of the Tetrahymena rRNA to *cis*-DDP, new bands appeared on the gel electrophoretogram in a time-dependent manner (Fig. 2), all of which migrated more slowly on the gel than did the precursor RNA. Several of the reaction products appeared as diffuse bands just above the precursor RNA on the gel superimposed onto a light smear of radioactivity which became darker as the reaction progressed. However, one of the new RNA species was a clearly defined band (shown by the arrow in Fig. 2) well separated from the other products and migrating just above the C-IVS at a position indicating an apparent MW of about 2400 nt (compared to the 510 nt of the pre-spliced ribozyme). This product was isolated from the gel and subjected to analysis for bound platinum. Atomic absorption spectroscopy indicated $20 \pm 1$ atoms of Pt bound per ribozyme molecule after 90 min of treatment with *cis*-DDP. To confirm the platination, the slowly migrating band was treated with dithiothreitol and thiourea at 90°C, conditions that are known to reverse Pt-DNA bonds (7). As shown in Fig. 3, thiourea treatment caused the original precursor RNA to be regenerated from the new product. These results convincingly demonstrate that the band migrating at 2400 nt consists of platinated RNA.

By analogy to its reaction with DNA (7), *cis*-DDP presumably could react with RNA to form intrastrand cross-links between adjacent bases (usually G-G in DNA) in a single strand of RNA and cross-links between complementary double-stranded regions (analogous to interstrand cross-links in DNA). Possibly cross-links between two or more RNA molecules could be formed as well, and indeed the slower migration rates of the platinated RNA compared to the unreacted precursor RNA could be interpreted higher molecular weight aggregates between several RNA molecules. However, for several reasons, we believe that most of the platinated products see on the gel are single molecules of RNA that are internally cross-linked by *cis*-DDP. The formation of oligomers of RNA molecules linked to each other by Pt would require a number of successive bimolecular reactions between mono-functional adducts of RNA molecules. Thus, a higher order oligomers would be expected to form at much slower rates than dimers, yet the product migrating at 2400 nt, which according to its apparent molecular weight consists of 4 molecules of precursor, was formed the most rapidly. Interstrand cross-linking of double-stranded hairpin regions can also account for the slow migration rate of the resulting platinated products. Cross-linking between strands would lead to the formation of covalently closed loop structures ($\alpha$-shaped molecules). Circular RNA is known to migrate much more slowly than linear RNA on electrophoretic gels (15); an example of this phenomenon is the Tetrahymena C-IVS, which, as shown in Fig. 1, migrates with an apparent size of 1200 nt but actually only has 400 nt. The formation of numerous platinated products migrating more slowly than the precursor can be accounted for by internal cross-link formation at different locations on the RNA molecule, giving closed circles of various sizes. Some of the products contained within the smear just above the precursor may contain intrastrand cross-links, which have a moderate retarding effect on the migration of small model DNA strands (16).

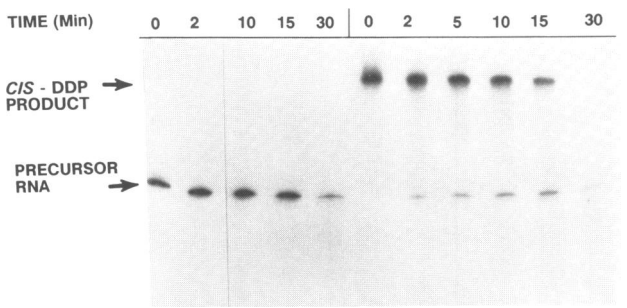


**Figure 3.** Regeneration of precursor RNA from the platinated product. The band migrating at an apparent size of 2400 nt (shown by the arrow in Fig. 2) that was formed by cis-DDP treatment of the Tetrahymena rRNA was isolated from the electrophoretic gel and incubated with 100 mM thiourea for the times indicated as described in MATERIALS AND METHODS. As a control, the precursor RNA was also incubated with thiourea for the same time periods. Some loss of RNA material occurred at the longer reaction times, as shown by the lower intensity of the bands at 30 min exposure time.

To obtain further evidence for the formation of internal Pt cross-links, the product migrating at 2400 nt was subjected to digestion by ribonuclease T1. This enzyme cleaves single strands of RNA after G residues, and has been used to analyze RNA for UV-induced interstrand cross-links because the RNA near the site of the cross-link is resistant to digestion (17). As shown in Fig. 4, the platinated product was digested substantially more slowly by the T1 nuclease than was the unreacted precursor DNA, as would be expected if the presence of interstrand cross-links made the molecule more rigid and its single-stranded regions less accessible to the nuclease. Intrastrand cross-links between G-G residues within the same strand would probably also prevent enzymatic hydrolysis at the point of the cross-link, but unless the RNA were very highly platinated, these lesions should not have a drastic effect on the initial rate of digestion of the molecule. In fact, the total number of platinated sites resistant to nuclease appears to be quite low, since more complete digestion of the RNA with 5 units of T1 nuclease (two right lanes of Fig. 4) showed only 3 or 4 unique undigested fragments (indicated by arrows).

Among the various platinated products that were formed, the Pt-cross-linked RNA migrating at 2400 nt appears to be the most closely linked with loss of ribozyme activity. The appearance of this product paralleled the inactivation of the ribozyme whereas the other products did not. During the initial velocity period of ribozyme inactivation by *cis*-DDP, the 2400 nt species was the major product, accounting for about 85% of the loss of RNA from the ligated exons and other splicing products. Furthermore, the amount of RNA in the 2400 nt band did not increase any further after the ribozyme was inactivated, while the more rapidly migrating bands kept increasing even after the ribozyme was inactivated. After 120 min of reaction time with *cis*-DDP when ribozyme activity was inhibited by 86%, formation of the 2400 nt product was 80% of its maximal, but the other platinated RNAs migrating between the pre-mRNA and the C-IVS had reached only 40% of their maximal amounts at this point.

We interpret these results in terms of the hypothesis that Tetrahymena rRNA exists in both active and inactive conformations that do not readily equilibrate among each other (18). It has been found that under normal splicing conditions, a substantial proportion of precursor RNA is unreactive (18) and

in our experience also a typical yield of splicing products is often only around 15−20%. For example, the extent of splicing in the untreated control lane of Fig. 1 is at about 90% of its maximum and thus most of the precursor molecules in this reaction will remain unspliced. However, the splicing yield can be increased by raising the pH or placing the RNA in mildly denaturing conditions such as 4 M formamide (18), which presumably facilitates the transitions from inactive to active conformations. Thus, we suggest that the 2400 nt species would appear concomitantly with inactivation if it were generated by reaction of *cis*-DDP specifically with the subpopulation of catalytically active ribozyme, whereas those products still increasing after loss of ribozyme activity are formed as a result of the slower reaction of *cis*-DDP with catalytically inactive forms of the ribozyme. Indeed, it can be seen in Fig. 2 that the latter bands were formed at the expense of the unreactive pre-rRNA band.

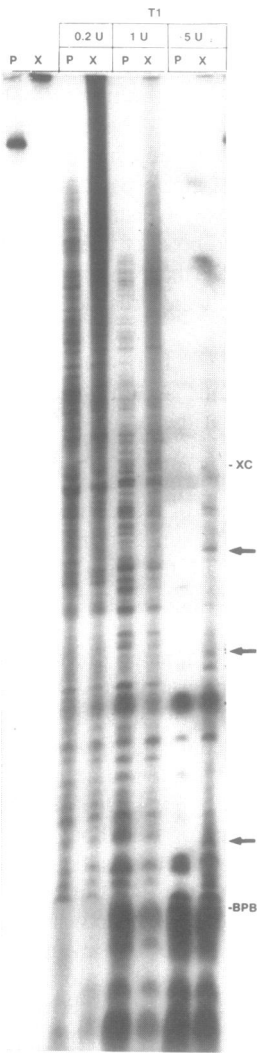


**Figure 4.** Ribonuclease T1 digestion of the Tetrahymena rRNA precursor (P) and the cis-DDP product migrating at 2400 nt (X). The precursor RNA or the platinated product were digested with the indicated number of units of T1 ribonuclease. BPB and XC indicate the migration of the marker dyes bromophenol blue and xylene cyanol, respectively. The arrows show fragments that appear to be resistant to digestion.

The above interpretation of the data requires that *cis*-DDP does not react by indiscriminate bimolecular collision with the RNA but with enough selectivity to show a preference for some conformational states over others. Two mechanisms can be envisaged by which selectivity of reaction might be achieved: 1) a certain conformation of the ribozyme possesses an unusually nucleophilic site that reacts more rapidly with the reagent; or 2) *cis*-DDP forms a complex with the ribozyme prior to covalent reaction and the active conformations of the ribozyme have a binding site for *cis*-DDP with stronger affinity than sites on the inactive conformations. Upon binding, the reactive groups of the *cis*-DDP would then be placed in proximity to nucleophilic groups on the ribozyme and react with them in a first order manner:

$$\textit{cis}\text{-DDP} + \text{ribozyme} \underset{}{\overset{K_I}{\rightleftharpoons}} \textit{cis}\text{-DDP}\cdot\text{ribozyme} \xrightarrow{k_{inact}} \text{Pt-ribozyme}$$

In the above scheme, $K_I$ represents the binding constant of the *cis*-DDP-ribozyme complex and $k_{inact}$ is the rate constant for inactivation. Since this mechanism is analogous to the mode of action of affinity labels of protein enzymes, it would similarly be expected to 1) display first order kinetics, rather than second order kinetics expected from a bimolecular reaction and 2) show saturation kinetics (19). Accordingly, to determine if the *cis*-DDP-ribozyme interaction possessed these characteristics, we measured the time dependent loss of ribozyme activity at various fixed levels of *cis*-DDP. A semilog plot of these data was linear, as expected for a pseudo-first order reaction involving complex formation (Fig. 5). To look for rate saturation effects, we measured the formation of the Pt cross-linked product over a broad range of *cis*-DDP concentrations. For this experiment, in addition to the natural uracil (U)-rRNA, we also used ribozyme substituted with 5-fluorouracil (FU) (20), which appears to have somewhat different conformations than the (U) RNA due to the lower pK

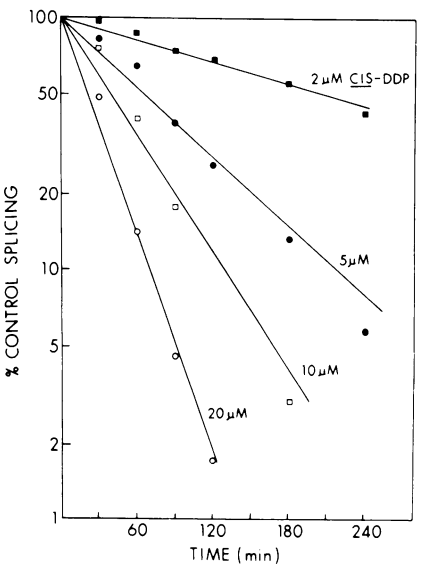


**Figure 5.** Loss of splicing activity as a function of time at different concentrations of cis-DDP. The precursor RNA was treated with cis-DDP for the times indicated, before addition of splicing buffer. Splicing was allowed to proceed for 30 min at 30°C. After electrophoresis of the reaction mixtures on 4% polyacrylamide gels, the bands corresponding to the ligated exons were cut from the dried gels and were quantitated by liquid scintillation counting.

of the FU base (21) and thus may react differently with *cis*-DDP. Fig. 6A shows that the curves for rates of product formation from both RNAs were initially linear with *cis*-DDP concentration, but then reached a plateau phase, consistent with the inactivation of the ribozyme by a complexed inactivator. Each point of Fig. 6A represents the slope of a linear pseudo-first order plot of the formation of the 2400 nt product as a function of time (not shown). From the horizontal intercept of the linear double reciprocal plots of these data (Fig. 6B) apparent $K_I$ values were determined to be 62.5 $\mu$M and 32.0 $\mu$M for the binding of *cis*-DDP to the (U)- and the (FU)-ribozymes, respectively. The initial velocity of the inactivation of (FU)-ribozyme by *cis*-DDP at a non-saturating concentration (10 $\mu$M) was about 2-fold faster than that of the (U)-ribozyme (data not shown), which is precisely accounted for by the 2-fold lower $K_I$ value of *cis*-DDP with the (FU)-ribozyme. This result further supports the formation of a Michaelis-Menten type of complex between *cis*-DDP and the ribozyme because it shows that the rate difference is associated with enhanced binding of the reagent to (FU)-RNA rather than greater chemical reactivity ($k_{inact}$) of the RNA. The latter, as indicated by the vertical intercepts of the double reciprocal plots in Fig. 5, is quite similar for both (U)- and (FU)-RNAs.

Another criterion for an affinity type of interaction is whether or not the inactivation is subject to competition by another molecule (19). For affinity labels of protein enzymes, this competing molecule is usually the normal substrate. In the case of the Tetrahymena rRNA, we thought that the magnesium ion ($Mg^{++}$) might be a possible candidate for a competitor with *cis*-DDP. $Mg^{++}$ has a distinct binding site on the ribozyme, with a dissociation constant of about 2 mM (23). The binding of $Mg^{++}$ causes the 3′ splice site to become exceedingly reactive presumably by altering the conformation of the RNA precisely in such a way as to place abnormal strain on the phosphodiester bond at the splice site (24). When *cis*-DDP is placed in aqueous solution, the chloride ions are displaced by water (7). The resulting hydrated Pt atom may have either one or two positive charges and thus may resemble $Mg^{++}$ to some extent. In order to test for competition between these ligands, we measured the inactivation of the ribozyme by 20 $\mu$M *cis*-DDP in the presence of increasing levels of $Mg^{++}$. Fig. 7 shows that as $Mg^{++}$ levels were raised the ribozyme was increasingly protected until there was no detectable inactivation after 30 min of exposure to *cis*-DDP at 5 mM $Mg^{++}$. However, high levels of $Mg^{++}$ prevented the formation of all of the platinated products instead of just one specific platinated species that might be formed by reaction at the $Mg^{++}$ site. Thus, $Mg^{++}$ exerts an overall

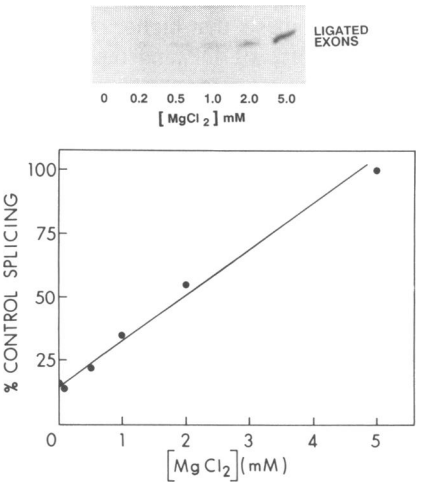


**Figure 7.** The effect of increasing $Mg^{++}$ concentration on the inactivation of ribozyme activity by cis-DDP. Treatment of the ribozyme with 20 $\mu$M cis-DDP was carried out as described in MATERIALS AND METHODS, except that $MgCl_2$ was present during incubation at the indicated concentrations. Splicing was initiated by addition of GTP. Ligated exon formation was assayed by cutting the bands from the dried 4% polyacrylamide gel. The inset at the top of the figure shows the ligated exon formation increasing as the $MgCl_2$ concentration is raised.

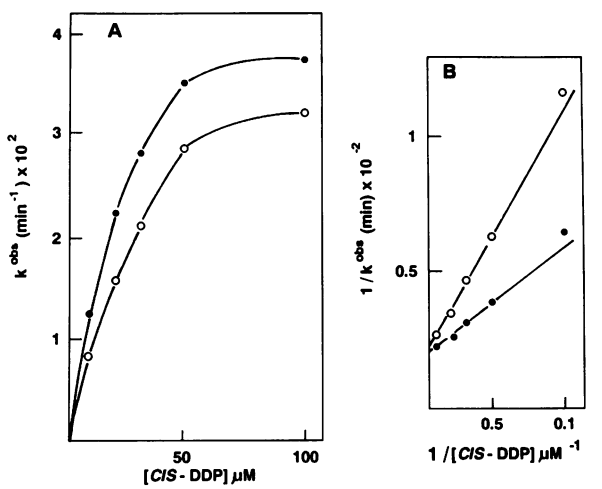


**Figure 6.** A. Rates of formation of platinated RNA migrating at 2400 nt as a function of cis-DDP concentration from treatment of both uracil (U)-containing (O) ribozyme and 5-fluorouracil (FU)-containing ribozyme (●). The (FU) RNA was synthesized as described in MATERIALS AND METHODS, except that FUTP was used instead of UTP in the transcription mixture. B. Double reciprocal plot of the data in A.

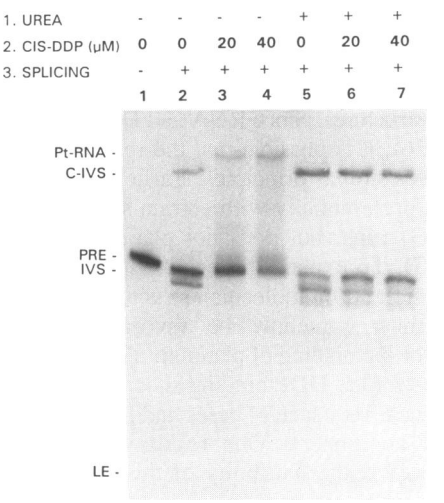


**Figure 8.** The effect of urea on platination and inactivation of the self-splicing activity of the Tetrahymena rRNA. Lane 1: Unspliced precursor RNA. Lane 2: Ribozyme spliced for 20 min. Lane 3 and 4: Precursor RNA treated with 20 $\mu$M and 40 $\mu$M cis-DDP, respectively, for 90 min as described in MATERIALS AND METHODS. Lane 4: Splicing reaction with 4 M urea added to the splicing buffer. Lanes 6 and 7: Precursor RNA treated with 20 $\mu$M and 40 $\mu$M cis-DDP, respectively, for 90 min in the presence of 4 M urea and then spliced for 20 min.

protective effect on the whole RNA molecule, most likely by inducing conformational changes which eliminate or alter all of the sites at which *cis*-DDP would react. A similar phenomenon was noted by Latham and Cech (25), who showed that $Mg^{++}$ protects many regions of the ribozyme against the RNA cleavage reagent Fe(II) EDTA, presumably by causing the helices of the RNA to be packed more closely together. Although inconclusive as to whether *cis*-DDP acts as an affinity label for the $Mg^{++}$ binding site, these results do show that the reactivity of the RNA toward *cis*-DDP is sensitive to the conformation of the RNA and thus are consistent with binding sites for *cis*-DDP that are formed by specific secondary and tertiary structures.

To confirm the hypothesis that higher order RNA structures promote the binding of *cis*-DDP and therefore accelerate the platination reaction, we examined the effects of a denaturant on the reactivity of *cis*-DDP with the ribozyme. The rationale behind this experiment was that mildly denaturing conditions may affect the conformation of the ribozyme in such a way that any binding sites for *cis*-DDP are altered or destroyed, thereby decreasing the interaction of the reagent with the RNA. On the other hand, if the platination of the RNA occurred through bimolecular reaction of *cis*-DDP with single bases, the partially denatured molecule should react just as well as the native one. Accordingly, we treated the ribozyme with 20 $\mu$M *cis*-DDP for 120 min in the presence of 4 M urea before adding the splicing buffer. After exposure to *cis*-DDP under these conditions, the ribozyme still spliced normally and no visible platinated products were formed, whereas ribozyme treated identically but without urea was completely inactivated (Fig. 8). The reactivity of the *cis*-DDP itself is not affected by urea. We have observed that the hepatitis delta virus RNA, a smaller ribozyme that carries out an auto-catalyzed cleavage-ligation reaction (22), is platinated and inactivated by *cis*-DDP despite the presence of 4 M urea (P.V. Danenberg and L.C.C. Shea, unpublished results).

## CONCLUSIONS

In this study, we have shown that *cis*-DDP causes inactivation of ribozyme catalysis and have presented evidence that the reagent does not appreciably react with RNA by bimolecular collision, but largely through the prior binding to sites formed by higher order RNA structures. Since RNA and DNA can form analogous structures (26), it is possible that the reaction of *cis*-DDP with DNA involves similar principles. Earlier work showed that *cis*-DDP reacts preferentially with certain sequences in DNA such as oligo (dG) runs, but does not platinate randomly all such sequences (7). To explain this selectivity, it was proposed that 'the electronic and/or molecular structures of the surrounding sequences must somehow be favorable relative to other sequences, for the binding of platinum' (7). That is, the structural requirements for *cis*-DDP binding sites in DNA consist of more than just a linear sequence of bases and probably include a three-dimensional component. Our results with the Tetrahymena ribozyme support the feasibility of this concept.

## ACKNOWLEDGEMENTS

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