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# Differences in the Inhibition of Translation by Cisplatin, Transplatin, and Certain Related Compounds

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**ABSTRACT.** The non-therapeutic cisplatin congeners transplatin and chloroethylenetriamine platinum (dien) inhibited translation to a similar extent as cisplatin did. The $IC_{50}$ values were: cisplatin 23 μM, transplatin 54 μM, and dien 117 μM. Unlike certain heavy metal inhibitors of translation, the effect of neither cisplatin nor the congeners was reversed by 3′:5′-cyclic adenosine monophosphate (cAMP). This suggests that the effect of these platinum compounds does not occur by the heavy metal mechanism. Polyribosomes and ribosomal subunits formed in transplatin-inhibited reactions differed from those in reactions inhibited by cisplatin. Specifically, large polyribosomes and complete 80S ribosomal subunits accumulated in the presence of transplatin. This indicates that while cisplatin slowed initiation of peptide synthesis, the *trans*-isomer slowed elongation. Substantive differences were not found between cisplatin and the monofunctional compound dien. This congener increased the non-peptidyl disintegrations per minute in the acid precipitates of assays containing [$^{35}$S]methionine. The high background indicated that an interaction between the label and a precipitable component of the system was induced by dien. However, consumption of methionine by this interaction did not appear to be the cause of the inhibition. Although there may be differences in the mechanisms of the effects, the finding that the non-therapeutic congeners inhibit translation at similar concentrations as cisplatin suggests that this inhibition is not responsible for the anticancer effect. On the other hand, the possibility that decreased translation could play an important role in the toxicity of these compounds in certain quiescent cells cannot be ruled out. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;12:1895–1902, 1996.

**KEY WORDS.** cisplatin; transplatin; chloroethylenetriamine platinum; translation inhibition; protein synthesis inhibition; heavy metals

Cisplatin has proven to be highly effective in the treatment of solid tumors of the testes and ovary. This drug inhibits protein, DNA, and RNA syntheses [1–3]. Since the inhibition of DNA synthesis occurs at lower drug concentrations and is the more persistent effect, it had been speculated that the decreases in RNA and protein syntheses are a consequence of impaired DNA functioning. Platination of DNA has been shown to block transcription [4].

Subsequent studies, however, have suggested that cisplatin may decrease protein synthesis by a direct inhibition of translation. In studies of the nephrotoxicity of the drug, Montine and Borch [5, 6] evaluated its effects on protein synthesis in quiescent LLC-$PK_1$ cells. In this model, cisplatin inhibited protein synthesis even when RNA synthesis was suppressed by actinomycin D [5]. This implies that protein formation is blocked at a post-transcriptional step. Previous studies have shown that *in vitro* translation in reticulocyte lysates is inhibited by cisplatin [7–9]. A more recent study has reported that a glutathione-cisplatin reaction product may be critical in producing inhibition [10].

The role that inhibition of protein synthesis plays in the response to cisplatin is uncertain. The mechanism of the anti-tumor action is not well understood; however, it is widely believed that an interaction with DNA is critical [3, 11–14]. While inhibition of protein synthesis is detectable at minimally toxic concentrations of the drug [12], the $IC_{50}$ of *in vitro* translation, 23 μM, is comparatively high. For example, the concentration that decreases the growth rate of exponentially growing L1210 cells by 50% is on the order of 3 μM [15].

The mechanism of cisplatin toxicity in non-proliferating cells is also unclear. Montine and Borch [5, 6] correlated the inhibitions of DNA, RNA, and protein syntheses with the development of toxicity in quiescent LLC-$PK_1$ cells. They found that the amount of inhibition of protein synthesis best correlated with the degree of cytotoxicity and, therefore, hypothesized that this process is a critical target of cisplatin in these cells. They speculated that because DNA synthesis is less important to non-proliferating cells,

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a post-transcriptional process becomes the primary cause of toxicity. Leibbrandt and coworkers [16] have supported this hypothesis, reporting that inhibition of protein synthesis is an early indicator of toxicity of cisplatin and certain active congeners in rat renal proximal tubule cells. The concentration of cisplatin needed to inhibit *in vitro* translation is less than that which reduces viability by 50% in LLC-$PK_1$ cells ($IC_{50} \approx 200$ μM).

To question whether translation plays a role in the actions of cisplatin, the effects of related compounds that lack its therapeutic properties were examined. Transplatin, the stereoisomer, and dien,§ a monochlorinated congener, do not possess anti-tumor activity and are less toxic than cisplatin [3, 17]. If the inhibition of translation leads directly to the unique actions of the drug, the inactive agents should not produce the same effect. Figure 1 shows the structures of these compounds.

In addition to these platinum-containing congeners of the drug, mercuric chloride was also examined. Like several other heavy metals, this compound has been shown to inhibit translation [18, 19] in a manner similar to cisplatin. This experiment tested whether the effects could be distinguished.

## MATERIALS AND METHODS

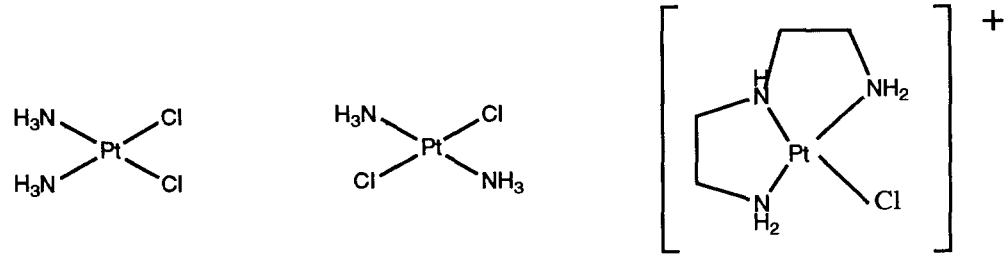
Cisplatin was a gift from the Bristol-Myers Co. (Syracuse, NY). Cisplatin solutions were allowed to stand at least 4 days at 2° prior to testing in translation assays. Transplatin was purchased from the Strem Chemical Co. (Newburyport, MA). Dien was synthesized according to Watt and Cude [20]. The infrared spectrum of the product was consistent with their data. [$^{35}$S]Methionine (> 800 Ci/mmol) was purchased from the Du Pont Co. (Wilmington, DE) and [$^{3}$H]leucine (> 110 Ci/mmol) from ICN Radiochemicals (Costa Mesa, CA). Creatine phosphokinase (type 1 from rabbit muscle), cAMP, and mercuric chloride were purchased from the Sigma Chemical Co. (St. Louis, MO). Hemin hydrochloride was purchased from Kodak (Rochester, NY) and prepared as described [21]. Micrococcal nuclease (nucleate 3′-oligonucleotidohydrolase, EC 3.1.31.1) (from *Staphylococcus aureus*, 9000 U/mg protein) was purchased from Pharmacia P-L Biochemicals, Inc. (Milwaukee, WI). Biosynthesis reaction mixtures (missing either methionine or leucine), consisting of 250 mM HEPES, 400 mM potassium chloride, 100 mM creatine phosphate, and 19 amino acids (500 μM each), were purchased from Gibco BRL (Gaithersburg, MD). Rabbit reticulocyte lysate was purchased from Green Hecters (Oregon, WI). Brome mosaic virus mRNA was purchased from Promega (Madison, WI). All solutions used in translation were either treated with 0.1% diethylpyrocarbonate or prepared with treated water. Glassware was heated to 250°C for 4 hr [21].

### *Translation Assay*

Translation was measured as the incorporation of [$^{35}$S]methionine or [$^{3}$H]leucine. Methionine was diluted 1:10 and leucine 1:80 with unlabeled amino acid and added to the appropriate biosynthesis reaction mixture. Prior to translation, rabbit reticulocyte lysate was made 0.1 mM hemin, 0.5 mM calcium chloride, and 5 U creatine phosphokinase/mL. The inhibitors were dissolved in water. Platinum compounds were added to the lysates and preincubated at 37° for 60 min before translation was started by addition of the biosynthesis reaction mixture. The complete assay mixture contained 1 μCi [$^{35}$S]methionine or 0.012 μCi of [$^{3}$H]leucine and 10 μL of reticulocyte lysate in a volume of 25 μL. Background counts from unincorporated bound label were determined by digesting endogenous mRNA with micrococcal nuclease at 5 μg/mL for 7.5 min at 20°. For translation of exogenous mRNA (from Brome mosaic virus), lysates were digested with micrococcal nuclease as above. The nuclease activity was stopped by addition of EGTA to a concentration of 2 mM.

The translation reactions were incubated at 37° for 60 min unless otherwise noted. Following this incubation 20 μL was removed and treated with 0.5 mL of 1 N sodium hydroxide at 37° for 10 min. This sample was then precipitated by addition of 3 mL of ice-cold 25% TCA containing 2% (w/v) casein hydrolysate. The precipitate was collected by filtration on a Whatman GF/C glass fiber filter. The

§*Abbreviations:* dien, chloroethylenetriamine platinum; cAMP, 3′:5′-cyclic adenosine monophosphate; and TCA, trichloroacetic acid.




*cis*-DDP *trans*-DDP [(dien)PtCl]$^{+}$

**FIG. 1. Structures of cisplatin, transplatin, and dien.**

precipitate was dissolved by placing the filter in 0.5 mL of tissue solubilizer (TS-1, Research Products International Corp., Mount Prospect, IL) for 30 min at 60°. After the filters cooled to room temperature, 10 mL of scintillation fluid (3a20, Research Products International Corp.) was added, the vials were vortexed, and the samples were counted by liquid scintillation spectrometry. Translation activity was determined by disintegrations per minute incorporated into the TCA precipitates minus disintegrations per minute precipitated from reactions using the nuclease digested lysates.

### *Gradient Centrifugation*

Translation reactions analyzed in sucrose gradients had a final volume of 150 μL and were incubated for 10 min at 30°. The reactions were conducted using [$^{35}$S]methionine as described above except that the methionine was not diluted with non-radioactive material and 10 μCi of [$^{35}$S]methionine was used in each assay. A 2-μL aliquot was removed from each assay, hydrolyzed with NaOH, and precipitated with TCA as described above to assess translation activity. Translation was stopped in the remaining lysate by the addition of 600 μL of ice-cold 10 mM Tris–HCl buffer, pH 7.5, containing 10 mM KCl and 1.5 mM $MgCl_2$.

Linear sucrose gradients (11.2 mL) at densities described in the figure legends were prepared by dilution of a stock solution of 60% (w/v) sucrose in the above Tris–HCl buffer. The gradients were formed using a Hoefer gradient maker (Hoefer Scientific Instruments, San Francisco, CA). The translation assays were layered over the gradients and centrifuged at 197,000 *g* in an SW-41 rotor at 4° as described in the figure legends.

Following centrifugation, the bottom of each tube was pierced, and 0.45-mL fractions were recovered by displacing the gradients from the bottom with 60% sucrose at 0.8 mL/min using a Beckman Fraction Recovery System (Beckman Instruments, Palo Alto, CA). For analysis of polysomes, fractions were precipitated with 1 mL of 8% (w/v) TCA containing 0.5% (w/v) casein acid hydrolysate and were filtered on glass fiber filters; then the filters were washed with 15 mL of 8% TCA. To analyze the formation of ribosomal subunits, fractions were collected into 1 mL of 0.25 M sodium acetate, pH 5.1, containing 2% cetyltrimethylammonium bromide, followed by the addition of 1 mL of 0.25 M sodium acetate, pH 5.1, containing 500 μg of unfractionated yeast RNA. Samples were vortexed and filtered on glass fiber filters. The filters were washed with 10 mL of 5 mM sodium acetate containing yeast RNA and 10 mL of water. The precipitates were counted as described above.

## RESULTS

When the non-therapeutic cisplatin congeners were added directly to translation assays both compounds were found to inhibit. Therefore, further studies were undertaken to determine if the characteristics of their inhibitions were the same as those of cisplatin.

Several toxic heavy metals, of which mercuric chloride may be prototypical, inhibit translation in hemin-supplemented reticulocyte lysates [18, 19]. As is the case with cisplatin, inhibition has been shown to be due to a decrease in the initiation of polypeptide chains. Because of this similarity, the possibility that the inhibition by cisplatin or these non-therapeutic congeners was this same heavy metal effect was considered. Addition of 10 mM cAMP to lysates inhibited by mercuric chloride restores translation activity [18]. When addition of cAMP was tested on inhibition by the platinum compounds, inhibition was increased rather than being reversed (Fig. 2). The ability of cAMP to reverse inhibition by mercuric chloride is also illustrated. This response to cAMP suggested that cisplatin and the congeners do not act by the same mechanism as the heavy metals.

The effects of transplatin and dien on translation activity were compared with that of cisplatin. Both of the non-antitumor compounds decreased the rate of incorporation of amino acids (Fig. 3). Cisplatin inhibited at slightly lower concentrations. The concentrations that inhibited by 50%, estimated from the intercepts of the concentration versus inhibition graphs were: cisplatin 23 μM, transplatin 54 μM, and dien 117 μM (Fig. 4). This difference, however, did not seem large enough to account for the major differences in their biological actions.

To distinguish the effect of the *trans*-isomer from that of cisplatin, we compared the polyribosomes and initiation complexes formed in inhibited reactions. [$^{35}$S]Methionine was incorporated into the forming initiation complexes of reactions containing a highly inhibitory concentration of either cis- or transplatin (200 μM). As shown in Fig. 4, the concentration versus inhibition curves of cis- and transplatin converge at high concentrations. At 110 μM concentrations, both compounds yielded essentially their maximum inhibition (90%). The concentration selected for this experiment yielded very similar amounts of inhibition with both agents. These reactions were then fractionated in linear sucrose density gradients. It was found that large polysomes were present in transplatin-inhibited reactions (Fig. 5). In contrast, cisplatin caused polysomes to disaggregate. In Fig. 6, analysis of the ribosomal subunits formed in transplatin-containing reactions showed an accumulation of complete 80S initiation complexes. Cisplatin-inhibited reactions, on the other hand, were depleted of complete initiation complexes. While cisplatin inhibited the formation of the initiation complexes, transplatin appeared to slow the rate at which initiated peptide chains were elongated.

When dien was combined with reticulocyte lysates containing [$^{35}$S]methionine, the amounts of unincorporated label in the acid precipitates were very high. In the absence of dien, background dpm was 1628 ± 27. At 1 mM dien, 7485 ± 223 dpm of $^{35}$S were precipitated. In contrast with [$^{3}$H]leucine, background dpm were 1952 ± 218 and 1986 ±

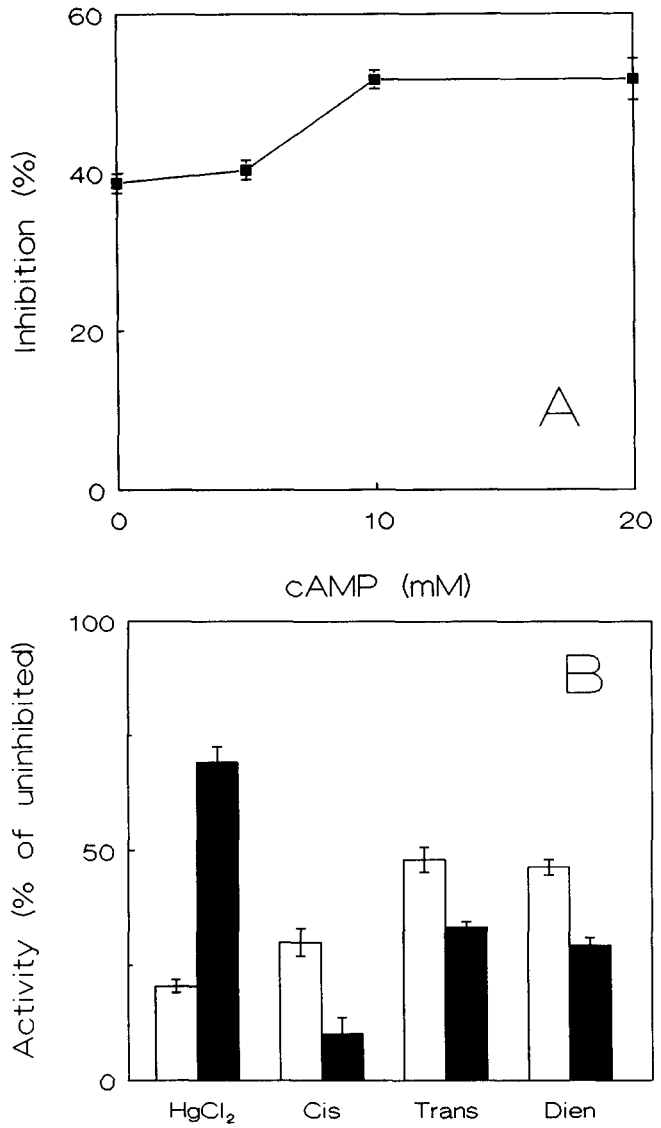



FIG. 2. Inability of cAMP to restore activity to reactions inhibited by the platinum compounds. (A) Lysates were preincubated with cisplatin (90 μM) and with increasing concentrations of cAMP for 30 min at 22°. Each point represents six individual assays. Error bars represent ± 1 SEM. cAMP at 10 and 20 mM significantly increased inhibition, $P < 0.05$. In the uninhibited reactions, activity increased with increasing concentrations of cAMP from $1.25 \pm 0.03 \times 10^5$ dpm in the absence of cAMP to $2.50 \pm 0.04 \times 10^5$ dpm in the presence of 20 mM cAMP. (B) Inhibition by cisplatin (80 μM), transplatin (90 μM), and dien (90 μM) along with mercuric chloride (25 μM) was tested in the absence (open columns) or presence (closed columns) of 10 mM cAMP. Activities are presented as compared with an uninhibited reaction, that is, the reaction in the absence or presence of cAMP but not containing the inhibitor. [$^3$H]Leucine incorporation was measured with dien and [$^{35}$S]methionine with the other inhibitors. Each bar represents six individual assays. Error bars represent ± 1 SEM. In each case, there was a significant difference in the presence of cAMP, $P < 0.05$. In the uninhibited reactions with mercuric chloride, cis- and transplatin activity was $1.87 \pm 0.02 \times 10^5$ dpm in the absence of cAMP and $3.74 \pm 0.03 \times 10^5$ dpm in the presence of cAMP. The activity of the uninhibited control paired with dien was measured using [$^3$H]leucine. Uninhibited activity in the absence of cAMP was $5.5 \pm 0.2 \times 10^4$ dpm and with cAMP was $8.9 \pm 0.5 \times 10^4$ dpm.

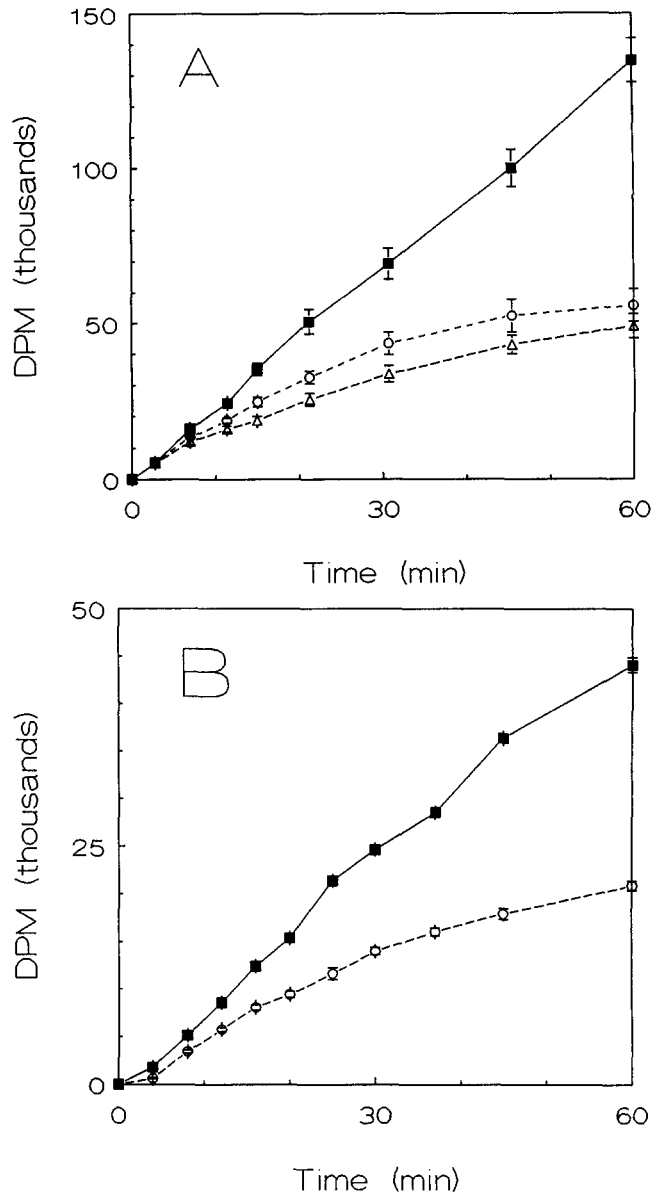



FIG. 3. Rates of amino acid incorporation in cisplatin-, transplatin-, and dien-inhibited reactions. In part A, 50 μM cisplatin (○), 100 μM transplatin (△), or solvent (■) and in part B, 110 μM dien (○) or solvent (■) was incubated with reticulocyte lysates for 60 min at room temperature. Lysates were then allowed to translate endogenous mRNA in the presence of 10 mM cAMP. Aliquots were removed at the indicated times and precipitated to determine the incorporation of [$^{35}$S]methionine (panel A) or [$^3$H]leucine (panel B). Each point represents the average of four individual assays. Error bars represent ± 1 SEM. In both graphs, all time points in the presence of the platinum compound were significantly less than the solvent control after 10 min, $P < 0.05$.

262, respectively. The [$^{35}$S]methionine background dpm increased, reaching a maximum at a dien concentration of 6 mM, which was about 10,000 times the methionine concentration in the assay. While this high background demonstrated that an interaction between $^{35}$S, in methionine or its metabolite, and the assay mixture occurred, it is un-

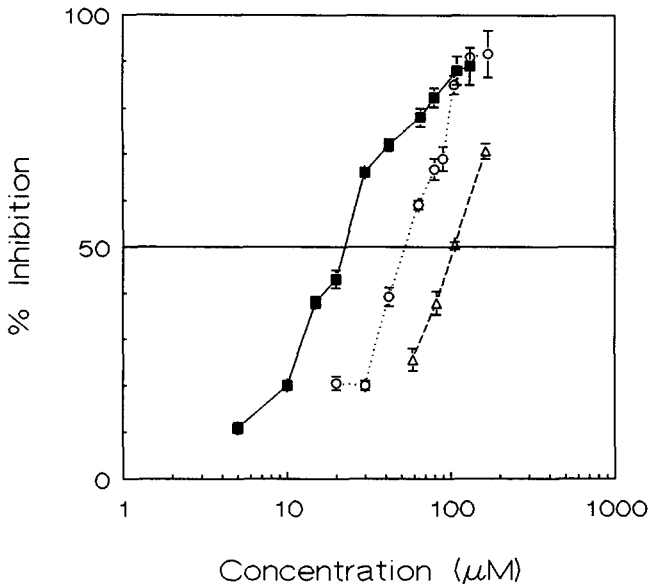



FIG. 4. Cisplatin, transplatin, and dien concentration versus inhibition of translation activity relationships. Endogenous mRNA was translated after preincubation with increasing concentrations of cisplatin (■), transplatin (○), or dien (△) as described in Materials and Methods. Incorporation of [$^{35}$S]methionine was determined with cis- and transplatin, and [$^{3}$H]leucine was measured with dien. Inhibition of translation was determined by comparison with assays preincubated in the absence of a test compound. Each point represents six individual assays. Error bars represent ± 1 SEM. In the uninhibited reactions paired with cis- and transplatin, activity was 3.16 ± 0.12 × $10^5$ dpm. Incorporation of [$^{3}$H]leucine in the uninhibited reaction paired with dien was 8.9 ± 0.5 × $10^4$ dpm.

likely that consumption of methionine was the cause of the inhibition. Only a small amount (≈1%) of the $^{35}$S contained in the reaction precipitated, and addition of excess unlabeled methionine (3–2000 μM) to reactions inhibited by dien (130 μM) did not lessen the inhibition significantly. Despite the fact that methionine has been shown to interact with both cis- and transplatin [22], neither affected the background counts and inhibition was the same when measured with either [$^{3}$H]leucine or [$^{35}$S]methionine. This high background precluded evaluation of the dien-inhibited reaction by density gradient centrifugation of the components that associated with methionine as was done with cis- and transplatin.

Due to this problem with the background, translation activities of reactions containing dien were measured by [$^{3}$H]leucine incorporation. What differences were found between the inhibitions of dien and cisplatin might be explained by their different reactivities. For example, inhibition occurred more quickly with dien. Transplatin also in-

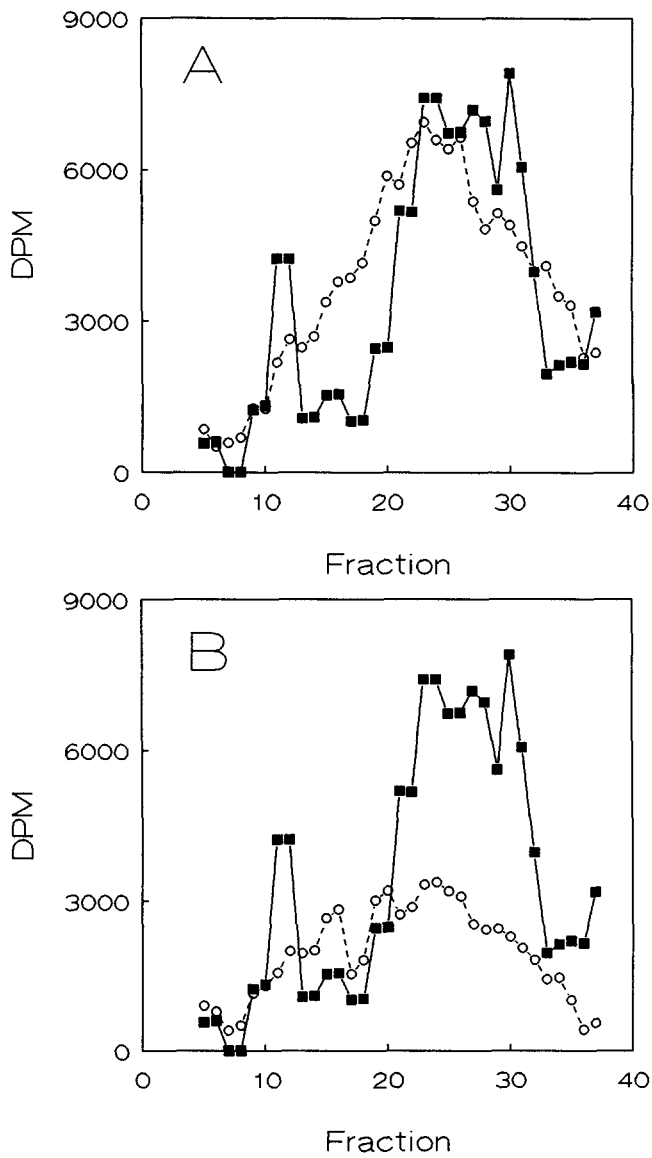



FIG. 5. Polyribosome formation in transplatin-inhibited reactions. Reaction mixtures (150 μL), supplemented with 10 mM cAMP, were allowed to translate endogenous mRNA for 10 min at 30°. Translation was carried out in the presence of 200 μM transplatin (■) and, in panel A compared with the absence of inhibitor (○) or, in panel B, with 200 μM cisplatin (○). Samples were analyzed by centrifugation for 80 min in sucrose density gradients (15–35%, w/v, linear sucrose gradients). TCA-precipitable [$^{35}$S]methionine was determined in the gradient fractions. Sedimentation is from left to right. The total number of disintegrations per minute in all fractions of the uninhibited reactions varied somewhat from day to day when the experiments were repeated. Therefore, the results from each trial were standardized so that replicates could be compared. An uninhibited control was run with each assay. The average total dpm in all fractions of these controls was approximately 125,000 dpm. The total dpm of each control was multiplied by a factor to yield 125,000. The counts in each fraction of the control and the paired inhibited assay were then multiplied by this same factor. For example, the uninhibited control in one trial had 100,000 dpm that were precipitated in all its fractions. Therefore, the dpm in each fraction of that control and of the paired inhibited reaction were multiplied by 125,000/100,000. Each point represents the average of fifteen control, twelve cisplatin or three transplatin assays. Fractions 11, 12, 21–32 and 35–37 of the transplatin assay were significantly higher than the cisplatin-inhibited assay at $P < 0.05$.

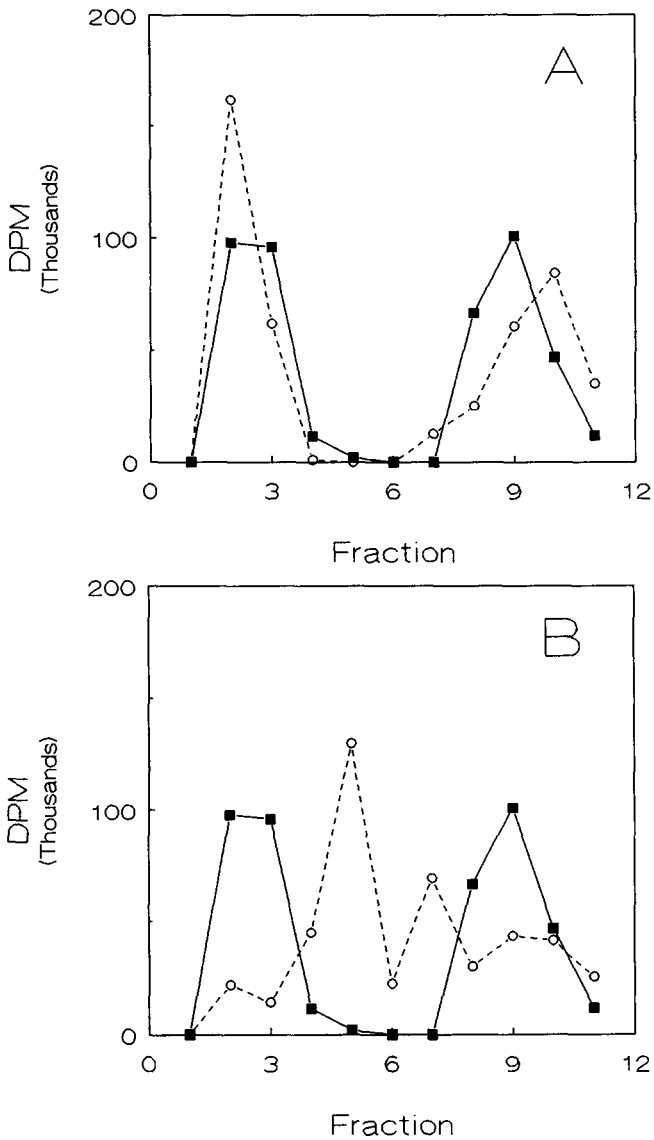



**FIG. 6. Ribosomal subunits in transplatin-inhibited lysates. Endogenous mRNA was translated for 10 min at 30° as in Fig. 5. Translation was carried out in the presence of 200 μM transplatin (■). The gradient profile was compared with reactions in the absence of inhibitors (○) (panel A) or in the presence of 200 μM cisplatin (○) (panel B). Samples were analyzed by centrifugation (180 min) in sucrose density gradients (15–35%, w/v, linear sucrose gradients). Cetyltrimethylammonium bromide-precipitable [$^{35}$S]methionine was determined in each fraction. Sedimentation is from left to right with 43S sedimenting mainly in fraction 2, 48S at fraction 5, 75S at fraction 7, and 80S at fraction 10. Each point represents fifteen individual control assays, sixteen cisplatin assays, or three transplatin reactions. The results from each treatment were standardized as described in Fig. 5 so that results of different trials could be compared. Fractions 2, 3, 5, 7, and 9 were significantly different between the cisplatin and transplatin gradients at $P < 0.05$.**

hibited more quickly than cisplatin (Fig. 7). Dithiobiuret prevented inhibition by dien; however, addition of DNA to dien had no significant effect.

Finally, in order to locate the target of dien inhibition, mRNA and lysate were treated individually with the compound. Message in lysates was removed by digestion with micrococcal nuclease. Digestion was stopped and dien was then incubated with either these RNase-treated lysates or mRNA. The dien-message or -lysate mixtures were then combined with their dien-free counterpart and translation begun. Dien inhibited more efficiently when it was incubated first with mRNA. This suggested that an interaction with message was important in the inhibition. We had shown previously that mRNA is also more sensitive than lysate to cisplatin [7]. However, mRNA was more susceptible to inhibition by dien in the absence of lysate. Cisplatin inhibited about equally when added to the message or to combined message and lysate. In contrast, incubation of mRNA with the concentration of dien needed to inhibit 50%, when added to combined message and lysate, resulted in over 90% inhibition with message alone (Fig. 8).

## DISCUSSION

The cisplatin-like compounds, dien and transplatin, have been used to identify elements of the drug's structure that are essential for its selective toxicity to cancer cells. All three of these compounds react with nucleophiles such as guanosine in DNA and RNA and thiols [17, 22]. Because components of the translation system contain enumerable such sites, it is not surprising that inhibition occurred.

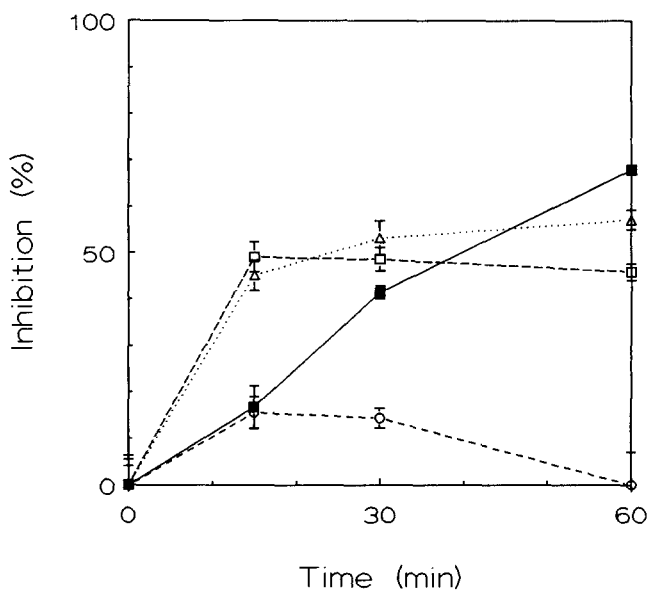



**FIG. 7. Increase in inhibition with time of exposure to platinum compounds. Lysates were preincubated with the platinum compound at 37° for the times indicated. Translation was then started by the addition of the reaction mixture and the activity was determined. The concentrations were transplatin (160 μM) (■), dien (90 μM) (△) and cisplatin (80 μM) (■). Control reactions (○) not containing an inhibitor are also shown. Each point represents four individual assays. Error bars represent ± 1 SEM. All reactions were measured by incorporation of [$^{3}$H]leucine. In the uninhibited reaction, translation activity was 1.3 ± 0.03 × $10^{4}$ dpm.**

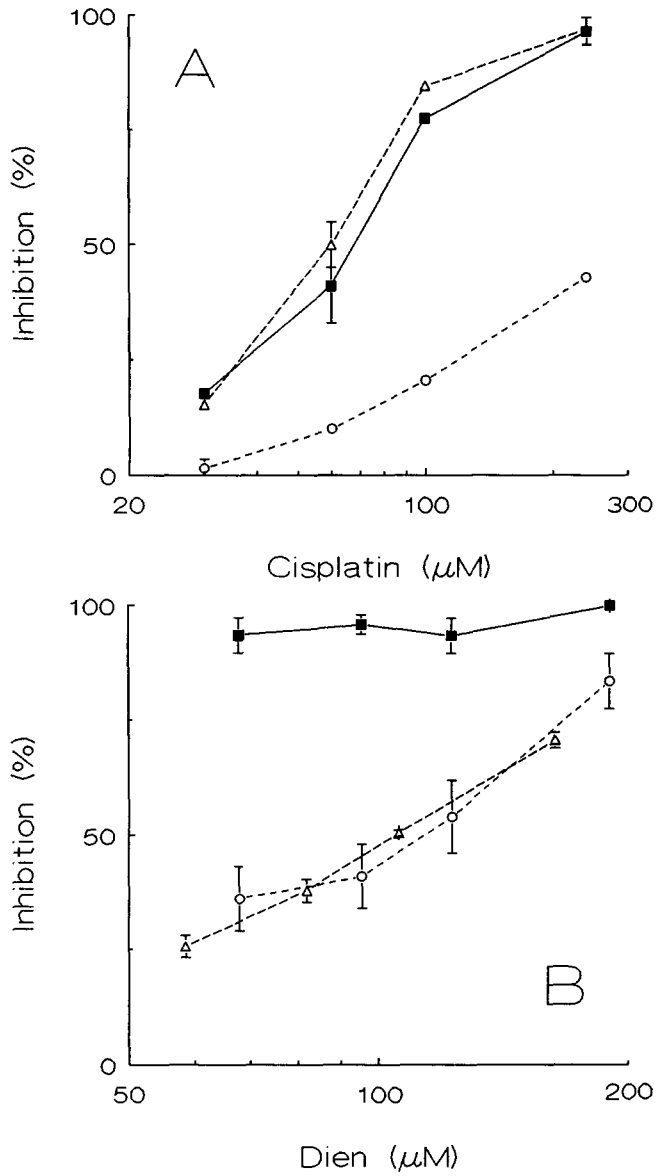



**FIG. 8. Sensitivity of message to dien. Increasing concentrations of cisplatin (panel A) or dien (panel B) were incubated with either Brom mosaic virus (BMV) mRNA (0.5 μg in 10.75 μL) (■), lysate (10.75 μL) that had been digested with nuclease (○), or a mixture of the two (△) for 60 min at 37°. Nuclease-digested lysate was then added to the platinum–mRNA mixture or mRNA to the platinum–lysate mixture, and translation was started immediately by addition of the reaction mixture. Activity was compared with that in assays preincubated without cisplatin or dien. Each point represents the average of four individual assays. Error bars are ± 1 SEM. In panel A, activity was measured by incorporation of [$^{35}$S]methionine. Activity of the uninhibited control reaction was 4.2 ± 0.1 × $10^4$ dpm. In panel B, activity was measured by incorporation of [$^3$H]leucine. Activity of the uninhibited control reaction was 4.5 ± 0.1 × $10^4$ dpm.**

Besides the differences in the number and orientation of the reactive positions of these molecules, there are differences in their reactivity. For example, of the three, cisplatin reacts most slowly with salmon sperm DNA; the $T_{1/2}$ for their reactions are cisplatin 3.9 hr, transplatin 2.5 hr, and dien 0.65 hr [17]. The rate of reactivity may be reflected in the rate of development of inhibition with the three compounds. Inhibition developed more quickly with transplatin and dien than with cisplatin. Despite the fact that the non-therapeutic congeners produced inhibition more quickly, they were not more potent inhibitors.

Lysate appeared to protect mRNA from dien but not cisplatin. Dien incubated with message alone inhibited far more than when added to combined message and lysate. This was not true for cisplatin; incubation of message alone or of message in the presence of lysate led to equal amounts of inhibition. If it is true that an interaction with message causes the inhibition, it may be that cisplatin has a preference for reaction with the message that dien does not.

While neither transplatin nor dien has measurable antitumor activity, both inhibited translation at concentrations only slightly higher, 2.3- and 5.1-fold respectively, than cisplatin. In contrast, in L1210 leukemia cells the concentrations of transplatin and dien that reduce the growth rate by 50% are, respectively, approximately 30 and 100 times higher than that of cisplatin [17]. Therefore, these findings argue against inhibition of translation being key to the antitumor action of the drug. However, because the characteristics of the inhibition by transplatin and the intracellular disposition of these compounds differ, it is conceivable that compounds with similar effects on *in vitro* translation would lead to different outcomes in cells.

There is less difference between the toxicities of cis- and transplatin in the quiescent LLC-$PK_1$ cell nephrotoxicity model. The concentration of cisplatin that decreases cell viability by 50% is about one-fifth that of the *trans*-isomer [5]. Hence, the inhibitory effect of this compound was less inconsistent with the hypothesis that decreased translation is a factor in cisplatin toxicity in certain non-dividing cells.

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