Human and bovine serum albumin (38-377 μM) bound approximately 1 mol of platinum per mol of protein when incubated with 300 to 600 μM cis-dichlorodiammineplatinum (II) (cis-DDP) for 6 h at 37 °C. Significantly increased binding was not demonstrated with higher concentrations of cis-DDP or longer incubation periods. Bovine albumin that was carboxamidomethylated retained 0.63-0.06 mol of sulfhydryl group/mol, compared with 0.62 mol/mol of unmodified bovine albumin, and bound 65-80% less platinum when reacted with cis-DDP. Competition experiments were performed in which bovine or human albumin were incubated with cis-DDP and the plasma protease inhibitor, α2-macroglobulin (α2M). The albumins failed to protect α2M from the previously described inactivation by cis-DDP (Gonias, S. L., and Pizzo, S. V. (1981) J. Biol. Chem. 256, 12478-12484), even when present at concentrations 270 times that of the protease inhibitor. Equivalent results were obtained when competition experiments were performed with cis-DDP that was preincubated in a manner that yielded large amounts of the more reactive "aquo" forms of the drug. Platinum-albumin complex was resolved from unreacted drug and incubated with α2M. Partial loss of the protease inhibitor activity was observed. Dialysis experiments showed that the complexes formed between albumin and cis-DDP do not dissociate to a significant extent. It is suggested that the inactivation of α2M by the platinum-albumin complex may involve direct reaction of the protease inhibitor with the complex.

The square planar complex, cis-dichlorodiammineplatinum (II), is a potent antineoplastic agent (1, 2). cis-DDP1 reacts with a variety of small molecules, proteins, and nucleic acids. The mechanism common to all of these reactions is substitution of nucleophilic groups for one or both of the coordinated chlorides (3, 4).

The major antitumor activity of cis-DDP most likely results from DNA modification occurring in a setting of rapid cell division (5). Before this modification occurs, intracellular cis-DDP molecules are converted into more reactive intermediates (aquated forms) in which the chloride-leaving groups have exchanged with water (5-7). Significant quantities of aquation products are not generated in the plasma because of the high extracellular concentration of free chloride (3). Nevertheless, an appreciable fraction of an intravenously administered cis-DDP dose binds to plasma proteins (5, 9). It is unclear whether certain plasma protein-platinum complexes may be therapeutically effective or contribute to the toxicity observed with cis-DDP.

α2-Macroglobulin is a tetrameric plasma protease inhibitor (four identical subunits per molecule) that is inactivated by cis-DDP in vitro (10). Intramolecular cross-linking of α2M subunits by platinum may be demonstrated after reaction of the protease inhibitor with less than 10 μM cis-DDP. The coordination of two sites on the α2M with a central platinum ion constitutes a chelate. This type of complex usually is extremely stable and it is, therefore, not surprising that the reaction of α2M with cis-DDP is difficult to reverse (10). The interaction between cis-DDP and serum albumin is important to understand because of the extremely high concentration of albumin when compared with other plasma proteins. Human (11) and bovine (9) albumin bind cis-DDP nearly quantitatively when present at large molar excess to the drug and incubated for long periods of time in vitro. While these studies suggest that cis-DDP may interact with albumin in the plasma, they do not permit a characterization of the reaction.

In this report albumin is shown to bind cis-DDP with high affinity until reaching an approximate saturation point. The single sulfhydryl group (Cys-34 in most albumin molecules (12-14)) seems to be responsible for 75% of the observed binding. Studies are presented comparing platinum-albumin complexes to the unreacted drug. From these experiments, some conclusions may be drawn concerning the reversibility of the reaction of cis-DDP with albumin and the activity of platinum that is protein bound.

**EXPERIMENTAL PROCEDURES**

Reagents—cis-DDP was purchased from Aldrich. Iodoacetamide and stannous chloride were from Fisher. DTNB, p-nitrophenyl-p'-guanidinobenzoate-·HCI, and N+·benzoyl-dl-arginine-p-nitroanilide·HCl were from Sigma. Cibacron blue F3-88-38-Sepharose was obtained from Bio-Rad Laboratories. Norit A was from Pfanstiehl Laboratories. All the other reagents were of the best commercial grade available.

**Proteins**—BSA was purchased from Sigma. α2M was not a detectable contaminant of this preparation. Carboxamidomethylated BSA was prepared by two methods. BSA (650 μM) was reacted with a 1.5-fold molar excess of iodoacetamide in 50 mM Tris-HCl, pH 7.3, for 2 h (CmAlb-1) or with a 6-fold molar excess of iodoacetamide in the same buffer for 6 h (CmAlb-2). Both solutions were dialyzed exhaustively to remove unreacted iodoacetamide. HSA was purified with...
immobilized Cibacron blue F3-GA as described by Travis et al. (15) and delipidated by the method of Chen et al. CmAlb-2 was purified from human plasma as described by Kurecki et al. (17) and modified by Imber and Pizzo (18). Trypsin was purchased from Worthington Biochemicals. This preparation was 70% active as determined by active site titration (19). Soybean trypsin inhibitor was from Sigma.

**Protein Concentrations**—The concentrations of a2M and albumin preparations were determined by absorbance, utilizing the following constants: BSA, CmAlb-1, CmAlb-2, A\textsubscript{\text{LAM}} = 6.67, M = 66,200 (20); HSA, A\textsubscript{\text{LAM}} = 5.26, M = 66,200 (21); a2M, A\textsubscript{\text{LAM}} = 8.93, M = 718,000 (22).

**DTNB Assays**—A modification of the method described by Ellman (23) was used to determine the sulphydryl group content of albumin preparations. BSA, CmAlb-1, and CmAlb-2 were dissolved in 0.1 M Tris-HCl, 0.01 M EDTA, 6 mM guanidine HCl, pH 8.0 (0.04 mM of protein in 2.5 ml of total volume). The background absorbance of each tube was measured at λ = 412 nm and compared to a blank that contained no protein. Freshly dissolved DTNB was then added to each tube (100 μl of a 10 mM solution in 50 mM NaF, pH 7.0) and the increase in absorbance was monitored relative to the blank. Maximum color development was observed within 10 min. The molar absorption coefficient for the 3-carboxylate-4-nitrophenolate ion at λ = 412 nm is 13,600 M\textsuperscript{-1} cm\textsuperscript{-1} (24).

**cis-DDP Solutions**—Crystalline cis-DDP was freshly dissolved in 20 mM Tris-HCl, 80 mM NaCl, pH 7.3, at 37°C for most experiments. Reactions were conducted in the same buffer. A second method for preparing cis-DDP involved dissolving the drug in water and incubating the solution at 37°C for 4 h. This method was designed to convert a large percentage (greater than 50%) of the drug into aquo and hydroxo forms before addition to a solution containing protein (5, 25). The protein-drug reaction buffer remained 20 mM Tris-HCl, 80 mM NaCl, pH 7.3, in these experiments.

**a2M Activity Assay**—The a2M activity assay is based on the reaction of a2M with trypsin to form a complex that has amidolytic activity toward a small substrate but does not react with soybean trypsin inhibitor (26). A detailed description of the assay is presented elsewhere (27). a2M was allowed to react with different concentrations of cis-DDP in the presence and absence of albumin at 37°C and then compared by assay to untreated a2M. The results of the a2M activity assay are not altered by unbound cis-DDP nor does the drug inactivate trypsin or soybean trypsin inhibitor under the assay conditions (10, 28). Control experiments were performed in which a2M activity was assayed in the presence of up to 380 μM albumin (HSA or BSA). No significant difference was observed between these samples and others that contained only a2M. In separate control experiments, HSA and BSA were assayed alone and did not contain any inherent amidolytic activity.

**Platinum Assays**—The assays presented are modifications of the procedures described by Ayres and Meyer (29). Stannous chloride (SnCl\textsubscript{2}·2H\textsubscript{2}O) was dissolved in concentrated HCl (2.2 g in 3 ml) approximately 8 h before the reagent was required. An additional 7 ml of water was added when the solution had become clear.

BSA, HSA, CmAlb-1, and CmAlb-2 were incubated with cis-DDP at 37°C in a final volume of 40 μl. The solutions were cooled to 4°C, and then 0.16 ml of 6.3% (w/v) cold trichloroacetic acid was added. The samples were mixed and incubated at 4°C for approximately 5 min before centrifugation for 30 min in an Eppendorf microcentrifuge.

The supernatants were separated from the proteinaceous precipitates and transferred to Pyrex test tubes. Volumes were brought to 450 μl with water and the following reagents were added in sequence with mixing: concentrated HCl (0.1 ml), 20% (w/v) NH\textsubscript{4}Cl (0.25 ml), SnCl\textsubscript{2}·2H\textsubscript{2}O (0.2 ml). Maximum color development within 2 h. Absorbances were measured at λ = 403 nm. A standard curve was generated with standard solutions of cis-DDP and no protein and was linear within the range of 3 to 40 ng of platinum. When low concentrations of cis-DDP were studied, the initial incubation volume and the volume of trichloroacetic acid added were doubled without changing the concentrations of any of the solution components.

Control reactions were performed in which cis-DDP was added to the different albumin solutions at 4°C. The protein was then immediately precipitated with acid as described above. Recovery of platinum in the supernatant was quantitative. Any acid-soluble peptides that may have been associated with the albumin solutions had no effect on the assay results.

A final modification of the platinum assay was designed to measure total platinum (protein bound and free) in chromatography fractions. Solutions containing 0.45 ml of protein and/or platinum were acidified with 100 μl of HCl and then heated at 110°C for 10 h. Standard samples containing variable amounts of cis-DDP and the same concentrations of albumin as in the unknowns were treated equivalently. After cooling, NH\textsubscript{4}Cl and SnCl\textsubscript{2}·2H\textsubscript{2}O were added as described above. A slight turbidity which occasionally developed after cooling was readily removed with a short period of low speed centrifugation. All reported platinum assay results represent the averages of at least three experiments.

**Chromatography Experiments**—BSA and CmAlb-1 (440–500 μg of protein) each were reacted with a 1.6-fold molar excess of cis-DDP for 6 h. The samples were then chromatographed on Sephadex G-25 columns (26 × 0.3 cm) equilibrated in 20 mM Tris-HCl, 80 mM NaCl, pH 7.4. A constant flow rate of 10 ml/h was maintained with a peristaltic pump. Elution fractions (approximately 0.4 ml) were measured for protein by absorbance and by the method of Lowry et al. (30). Fractions were also assayed for ability to inactivate a2M by incubating 55 μl of each with 5 μl of an a2M solution (6 ng/ml for 6 h. a2M activity was then measured as described above. The platinum content of selected fractions was also measured as described above.

**Polyacrylamide Gel Electrophoresis**—Electrophoresis of denatured proteins was conducted on 5% gel slabs in an ammediol-buffered gel system containing sodium dodecyl sulfate in the sample buffer and upper reservoir, as described by Wyckoff et al. (31). Protein solutions were not reduced during denaturation.

**Platinum-Albumin Dissociation Experiments**—BSA was reacted for 6 h with a 1.6-fold molar excess of cis-DDP and then chromatographed on Sephadex G-25 to resolve the protein from unbound drug. The recovered albumin was placed in one side of an equilibrium dialysis cell containing two 1-ml chambers separated by a dialysis membrane with a surface area of 2.8 cm\textsuperscript{2}. A solution of 0.8 μg of a2M in the equivalent buffer was placed in the second chamber. The dialysis cell was rotated slowly at 37°C. Aliquots of 16 μl were removed periodically from the a2M chamber and frozen immediately. Upon completion of the experiment, samples were thawed, denatured, and compared by electrophoresis. In control experiments, freshly dissolved cis-DDP was substituted for the reacted albumin. The amount of cis-DDP in the presence and absence of albumin at 37°C and then compared by assay to untreated a2M. The results of the a2M activity assay are not altered by unbound cis-DDP nor does the drug inactivate trypsin or soybean trypsin inhibitor under the assay conditions (10, 28). Control experiments were performed in which a2M activity was assayed in the presence of up to 380 μM albumin (HSA or BSA). No significant difference was observed between these samples and others that contained only a2M. In separate control experiments, HSA and BSA were assayed alone and did not contain any inherent amidolytic activity.

![Fig. 1. Competition between BSA and a2M for reaction with cis-DDP. A, a2M activity remaining (%).](http://www.jbc.org/). B, Free cis-DDP at 6 h (μM).
of platinum added to the cells in the form of cis-DDP was varied in a range approximating the concentration of platinum complexed to albumin in the first experiment.

RESULTS

Characterization of a Platinum-Albumin Complex—Constant concentrations of cis-DDP and α-M were incubated with variable amounts of BSA. α-M activity was then assayed (Fig. 1). In the absence of a second protein, the inactivation of α-M by cis-DDP is dependent upon the drug concentration and time of incubation (10). Reaction conditions were selected (18–600 μM cis-DDP for 6 h) so that competition between BSA and α-M for cis-DDP binding might result in a decreased extent of α-M inactivation. This effect was not observed at any cis-DDP concentration, even at the highest molar ratio of albumin to α-M tested (270:1), which is comparable to the ratio found in normal human plasma.

One possible explanation for the results of the competition experiments is that albumin does not bind cis-DDP. Incubations were performed with BSA and cis-DDP under conditions equivalent to those presented above, except for the omission of the α-M. cis-DDP binding to protein was then assayed. Fig. 1 shows that after 6 h, a substantial level of reaction was measured in each experiment. Considerably less than 50% of the cis-DDP remained in an uncomplexed form (recovered in the supernatant after acid precipitation) when the concentration of albumin was greater than 300 μM.

The results of the binding studies from Fig. 1 are compared with similar experiments in Table I. Data are presented in the form, moles of cis-DDP bound/mol of albumin, for experiments in which the incubation ratio of drug to protein was greater than one. A single molecule of cis-DDP was readily bound by each BSA molecule by 6 h in most experiments. Additional studies performed for longer periods of time (600 μM for 16 or 24 h) or with a higher concentration of drug (1 mM for 6 h) showed that further cis-DDP binding by BSA was limited. The maximum level of binding was observed with 600 μM drug after a 16-h incubation (Table I). These studies suggest that a single binding site on BSA might be responsible for a large fraction of the observed reactivity.

CmAlb-1 and CmAlb-2 were prepared to study the role of the albumin cysteine residue in cis-DDP binding. The sulfhydryl group content of denatured BSA was 0.62 mol/mol, in good agreement with a previous study (20). CmAlb-1 and CmAlb-2 retained 0.06 mol (10%) and 0.03 mol (5%) of sulfhydryl group per molecule. Both preparations bound significantly less platinum after incubation with cis-DDP than did BSA under equivalent conditions (Fig. 2). CmAlb-1 demonstrated a slightly higher affinity for cis-DDP than did CmAlb-2 in some experiments; however, the difference was, at best, small.

<table>
<thead>
<tr>
<th>Binding ratios</th>
<th>Concentration of BSA μM</th>
<th>16-h incubation with 600 μM cis-DDP</th>
<th>6-h incubation with 600 μM cis-DDP</th>
<th>6-h incubation with 300 μM cis-DDP</th>
<th>6-h incubation with 80 μM cis-DDP</th>
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<tr>
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<td>1.2 (16)</td>
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<td>0.6 (2.1)</td>
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<tr>
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<td>1.3 (2.0)</td>
<td>1.1 (2.0)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>377</td>
<td>1.1 (1.6)</td>
<td>1.0 (1.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The values in parentheses represent the molar ratio of cis-DDP to BSA present in the incubation medium.

The Activity of Platinum That Is Complexed to Albumin—The inability of BSA to protect α-M from inactivation by cis-DDP might be explained if α-M was inactivated in reactions which involved platinum-albumin complex as well as free drug. Chromatography experiments were performed, as described above, to test this hypothesis (Fig. 3). α-M was inactivated when incubated with elution fractions from the included volume of a chromatographed BSA-cis-DDP reaction mixture. This result most likely reflects reaction of the protease inhibitor with free drug. α-M also was inactivated when incubated with fractions that contained high concentrations of platinum-albumin complex. No effect was observed when the fractions between the two peaks were subjected to the same assay. It is, therefore, unlikely that the inactivation of α-M by the albumin-containing fractions resulted from reaction with loosely associated uncomplexed forms of cis-DDP. Platinum assays were performed with fractions 8, 9, and 10. The molar ratio of platinum to albumin in all three fractions was 1.0:1. This result is consistent with the acid precipitation studies already presented. The concentration of platinum (complexed to protein) in fractions 8 through 10 was 165 μM, 275 μM, and 202 μM, respectively. The extent of α-M inactivation caused by preparations of platinum-albumin complex was compared with the inactivation caused by comparable

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**TABLE I**

**Molar binding ratios for cis-DDP and BSA**

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**FIG. 2.** The binding of platinum to BSA, CmAlb-1, and CmAlb-2. The modified and unmodifed albumins were reacted with 600 μM cis-DDP for 6 h. Platinum binding was then assayed for BSA (A), CmAlb-1 (B), and CmAlb-2 (C). Details are provided under "Experimental Procedures."

**FIG. 3.** The reaction of platinum-BSA complex with α-M. BSA (900 μM) was reacted with 1.44 mM cis-DDP (total platinum, 0.80 μmol; volume, 550 μl). The solution was chromatographed on Sephadex G-25 and the recovered fractions were studied as described in the text.
concentrations of free cis-DDP. The protein-bound platinum demonstrated between 20 and 35% of the activity measured for unmodified drug in each case.

The reaction of cis-DDP with BSA may be divided into two categories based on the data presented, that involving an unmodified sulfhydryl group residue and that involving other sites. Chromatography experiments were performed with CmAlb-1 that was reacted with cis-DDP in order to determine whether the α₂M inactivation by platinum-BSA complex observed above was a function of sulfhydryl group bound platinum, platinum bound to other sites, or both. α₂M was inactivated when incubated with fractions from two well defined peaks in the elution profile (Fig. 4). A somewhat greater extent of inactivation was measured for fractions comprising the inclusion volume in this experiment as compared with the BSA-cis-DDP experiment. This result most likely reflects the higher concentration of reacted cis-DDP remaining in solution after incubation with alkylated albumin. Inactivation of α₂M by fractions containing platinum-CmAlb-1 complex was not as extensive as the inactivation caused by comparable concentrations of platinum-BSA complex; however, the molar ratio of platinum to protein in the CmAlb-1 complex was only 0.25. The reactivity of the platinum in platinum-CmAlb-1 with α₂M was approximately 50% of that demonstrated by an equivalent concentration of platinum in the form of cis-DDP.

**Competition Experiments with cis-DDP**—The low membrane permeability of aquated cis-DDP formed in the intracellular space and the high vascular free chloride concentration limit the amount of aquated cis-DDP in the plasma; however, the aquo ligand is extremely labile and aquated intermediates are very important in many cis-DDP reactions (4, 5, 7, 32). cis-DDP*, therefore, was studied in α₂M-BSA competition experiments similar to those presented in Fig. 1. BSA failed to inhibit α₂M inactivation by cis-DDP* for every combination of protein and drug tested (Table II). As previously reported (10), the extent of α₂M inactivation caused by comparable concentrations of cis-DDP and cis-DDP* was not significantly different.

**The Reaction of HSA with cis-DDP**—HSA was allowed to react with cis-DDP for 6 h. The amount of platinum bound to protein was then determined. In separate experiments variable amounts of HSA were incubated with α₂M and cis-DDP. α₂M activity was then measured. The results of these studies are summarized in Table III. The reaction of cis-DDP with HSA was indistinguishable from the reaction with BSA. This result

![Fig. 4. The reaction of platinum-CmAlb-1 complex with α₂M. CmAlb-1 (740 µM) was reacted with 1.18 mm cis-DDP (total platinum, 0.71 µmol; volume, 590 µl). The solution was chromatographed on Sephadex G-25 and the recovered fractions studied as described in the text.](http://www.jbc.org/)

**TABLE II**

<table>
<thead>
<tr>
<th>Concentration of BSA (µM)</th>
<th>α₂M activity remaining</th>
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<td>54%</td>
</tr>
<tr>
<td>377</td>
<td>51%</td>
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</table>

**TABLE III**

<table>
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<th>Concentration of albumin (µM)</th>
<th>Platinum bound (nmol)</th>
<th>Molar ratio (platinum to HSA)</th>
<th>α₂M activity remaining</th>
</tr>
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<td>0 (0)</td>
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<td>12%</td>
</tr>
<tr>
<td>377 (15.1)</td>
<td>15.0</td>
<td>1.0</td>
<td>12%</td>
</tr>
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</table>

*The values in parentheses represent the number of nanomoles of HSA present.

![Fig. 5. Dialysis of platinum-albumin complex and free cis-DDP against α₂M. A, chamber one contained 100 µM cis-DDP and chamber two contained α₂M. B, chamber one contained 150 µM platinum-BSA complex. The second chamber contained α₂M. The volumes of the dialysis solutions were 1 ml. Samples were removed from the chamber containing α₂M at the following times: (a) 0 h; (b) 1.5 h; (c) 3.5 h; (d) 6.0 h; (e) 8.5 h; (f) 11 h.](http://www.jbc.org/)

is not unexpected since the primary and tertiary structures of HSA and BSA are extremely similar (12, 13).

**Dissociation of Platinum-Albumin Complex**—When unreduced α₂M is electrophoresed under denaturing conditions, a single major band (Mₒ ~ 360,000) corresponding to the half-molecule (two disulfide bonded subunits) is observed (33). cis-DDP reacts with α₂M and forms intersubunit cross-links that prevent polypeptide dissociation in denaturant (10). Dialysis experiments were performed to determine whether the inactivation of α₂M by platinum-BSA complex resulted from
The Reaction of Albumin with cis-DDP

reversible dissociation of platinum from the albumin (Fig. 5). Free cis-DDP readily crossed a dialysis membrane and reacted with \( \alpha_M \) in the adjacent chamber, forming interalbumin cross-links (initial concentration of cis-DDP in single chamber: 70 \( \mu \)M, 100 \( \mu \)M, 200 \( \mu \)M). The cross-linked \( \alpha_M \) migrated at a rate typical of whole molecules (\( M_\text{r} \sim 718,000 \)) in a denaturing gel. Cross-linking was not observed when \( \alpha_M \) was dialyzed against platinum-BSA complex (initial concentration of platinum in single chamber approximately 150 \( \mu \)M in two trials). These data suggest that cis-[Pt(NH\(_3\))\(_2\)]\(^2+\), where \( A \) represents Cl\(^-\), OH\(^-\), \( H_2O \), etc., is not released from platinum-albumin complex at a significant rate.

Discussion

Many investigators have demonstrated significant binding of platinum by plasma proteins soon after cis-DDP is injected into the circulation. As described above, the majority of the cis-DDP remains in the less reactive dichloro form while in the plasma. The number of nucleophiles that might function as an entering group and replace a Cl\(^-\) is limited when compared with the group that might replace an \( H_2O \) ligand (4). It is, therefore, considered unlikely that cis-DDP binds randomly to all plasma proteins at levels proportional to their concentrations, as has been suggested (34). The reaction of cis-DDP with albumin demonstrates an expected specificity. The majority of the interaction involves a single cysteine residue. Stable association of platinum with other sites on the protein is limited even after long periods of incubation. The low plasma concentration of cis-DDP present immediately after an injection (less than 50 \( \mu \)M) and the comparatively high concentration of plasma proteins (albumin alone approximately 670 \( \mu \)M) make it probable that only the cysteine residue competes favorably for drug binding in vivo. Sulphydryl groups in proteins, other than albumin, have been shown to react readily with cis-DDP (35, 36). \( \alpha_M \) is a notable exception in that it has no free sulphydryl groups yet still binds platinum with high affinity (10).

Carboxamidomethylated BSA was prepared by two methods, one to minimize reaction of the alkylating reagent with nucleophilic groups other than cysteine (CmAlb-1) and one to ensure maximal modification of cysteine residues (CmAlb-2). Methionine and histidine residues that may be modified by excess iodoacetamide (as in CmAlb-2) also react with square planar platinum complexes (37). CmAlb-1 and CmAlb-2 incorporated nearly the same amount of platinum when reacted with cis-DDP. These data suggest that albumin does not contain a second highly reactive methionine or histidine that alone accounts for the observed nonsulphydryl group mediated platinum binding.

The cysteine residues in BSA and HSA form disulfide bonds with a variety of sulphydryl group-containing molecules in the plasma, including cysteine, glutathione, and other albumin monomers (10, 14, 21). This disulfide bonding explains why less than one mol of sulphydryl group per mol of albumin was detected with DTNB titrations. The experiments presented in this study suggest that the fraction of serum albumin lacking a free sulphydryl group may be less reactive with cis-DDP.

The antitumor activity of cis-DDP probably results from reaction with DNA; however, the nature of the reaction remains unclear (5). Experiments were performed in which solutions of platinum-albumin complex were dialyzed against solutions containing \( \alpha_M \). This technique permitted an evaluation of two of the factors that might be critical if platinum-albumin complex was to have cytotoxic activity in vivo. Cross-linking of \( \alpha_M \) required that 1) the platinum dissociate from the albumin so that it could cross the dialysis membrane (the protein complex was impermeable) and 2) the dissociated platinum remain bifunctional (two somewhat labile leaving groups). The absence of cross-linking probably indicates that these conditions were not completely satisfied. If similar conditions are important for cytotoxicity, these studies suggest that platinum complexed to albumin is therapeutically inactive.

A recent investigation has provided evidence suggesting that cis-DDP may be cytotoxic without crossing the cell membrane (7). The proposed mechanism for cytotoxicity involves interaction of the drug with DNA associated with the surfaces of cells. This hypothesis is supported by studies that demonstrate the presence of DNA on the membranes of tumor cells (38, 39) and reaction of cis-DDP with these nucleic acids (39). When platinum-albumin complex was incubated with \( \alpha_M \) in the same solution, the \( \alpha_M \) was partially inactivated. The concentration of free cis-DDP required to demonstrate \( \alpha_M \) inactivation is higher than the concentration necessary to demonstrate cross-linking (less than 8 \( \mu \)M) (10). The inactivation of \( \alpha_M \) by platinum-albumin complex in these experiments and the absence of cross-linking in the dialysis experiments are consistent with a mechanism for \( \alpha_M \) inactivation involving direct reaction of the \( \alpha_M \) with platinum-albumin complex, perhaps via the second labile site of the platinum. Other high affinity binding sites such as those present on membrane DNA might react with platinum-albumin complex similarly. Experiments with tumor cells in culture and platinum complexes formed with albumin will be necessary to better address this issue.

Note Added in Proof—in a recent publication, Lottenberg and Jackson (1980) Biochim. Biophys. Acta 742, 558-562 reported that high concentrations of albumin alter the extinction coefficient of p-nitroaniline generated during substrate hydrolysis in enzyme assays such as the \( \alpha_M \)-trypsin activity assay. In the present manuscript, the highest concentration of albumin that was incubated with \( \alpha_M \) was 380 \( \mu \)M. After addition of trypsin, soybean trypsin inhibitor, 0.8 ml of buffer, and 1.0 ml of substrate, as required for the activity assay, the albumin was diluted to 8 \( \mu \)M (0.5 mg/ml). This concentration of albumin, according to the work of Lottenberg and Jackson, affects the extinction coefficient of p-nitroaniline by less than 0.3%, in good agreement with the control experiments presented under "Experimental Procedures."

References

The Reaction of Albumin with cis-DDP

Complexes of serum albumin and cis-dichlorodiammineplatinum (II). The role of cysteine 34 as a nucleophilic entering group and evidence for reaction between bound platinum and a second macromolecule.

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