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Reactions of Cisplatin with Sulfur-containing Amino Acids and Peptides I. Cysteine and Glutathione

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Introduction

Cisplatin, cisdichlorodiammineplatinum(II), a potent antitumor agent [1] interacts in biological systems with both proteins and DNA. The binding to proteins appears to be irreversible [2] which suggests that a strong bond is formed between the central platinum atom of the square planar complex and one or more of the amino acids in the protein molecule. While extensive work has been carried out in many laboratories on the interaction of cisplatin with DNA [3], very little work has been reported on the nature of the interaction of cisplatin with proteins [4].

The sulfur containing amino acids present in the protein molecule could serve as possible binding sites for cisplatin or a metabolite thereof, since it is well known that platinum(II), a soft acid, is more likely to form a stable bond with sulfur, a soft base, rather than with oxygen or nitrogen, both hard bases [5]. As a possible model for understanding the nature and the mechanisms whereby cisplatin is bound to proteins, we have studied the reaction of this anti-tumor agent with cysteine, both as a single amino acid and as part of a small peptide, glutathione.

Experimental

Materials

Samples of cisplatin were obtained from either Bristol Laboratories, from Dr James Hoeschele (Oak Ridge National Laboratory), or synthesized in our laboratories by Dr. Randall C. Manaka, according to the modified Kauffman–Dhara procedures previously described [6] and were used without further purification. Cysteine hydrochloride monohydrate was purchased from Matheson, Coleman and Bell, and glutathione (reduced form) from Sigma Chemical Co. Both products were reagent grade and were used without further purification.

TABLE I. Analysis of the Product Resulting from the Reaction of Cisplatin with Cysteine, 1.1.

	I	II	III	Average
C	11.80	11.58	11.42	11.60 ± 0.19
H	2.32	2.34	2 32	2.32 ± 0.01
N	5.73	5.68	5.85	5.76 ± 0.07
Pt	–	55.4	52.6	54.0 ± 1.40
Cl	2.5	1.4	1.7	1.87 ± 0.46
S	–	–	7.39	7.39 ± 0 00

Preparation of the Complexes

Cisplatin-cysteine 1:1

Cisplatin (0.050 g, 0.167 mMol) was dissolved in 50 ml of 0.9% saline, and to this solution was was added 0.0293 g (0.167 mMol) of cysteine hydrochloride monohydrate as a powder while stirring. The resulting solution was placed in a constant temperature water bath at 37 °C, and allowed to incubate for 4 to 5 days. The very fine, light yellow, amorphous precipitate was centrifuged down, washed with water and methanol and then allowed to air-dry overnight. This material was further dried under vacuum at 76 °C. The elemental analysis data are reported in Table I.

Cisplatin-glutathione 1:2

Cisplatin (0.100 g, 0.333 mMol), was dissolved in 100 ml of 0.9% saline and to this solution was added 0.205 g (0.667 mMol) of glutathione (reduced form) in a powder, while stirring. The resulting solution was placed in a constant temperature water bath at 37 °C and allowed to incubate at that temperature for 4 to 5 days. The solution, initially of a very light yellow color, turned to deep yellow overnight; a yellow crystalline precipitate formed slowly, which was centrifuged down, washed with methanol and ether, and allowed to dry overnight. It was then dried further under vacuum at 76 °C. The elemental analysis corresponded to a product of composition C 27.56%, H 4.22%, N 9.54%, Pt 22.55% and S 8.09%. Calculated for $C_{20}H_{32}N_6O_{12}PtS_2 \cdot 3H_2O$. C 27.87%, H 4.44%, N 9.75%, Pt 22.63% and S 7.44%.

The IR spectra of the solid compounds were obtained using a Beckman IR 4240 Spectrophotometer (4000–200 cm^{-1}) using fluorolube and nujol mulls between NaCl plates. pH measurements were made using a Beckman Model 3500 digital pH meter.

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TABLE II. Estimated Values for Various Possible Products of the Reaction of Cisplatin with Cysteine.

Possible Structure	Elemental Composition
$Pt(NH_3)_2$ • cysteine	$PtC_3H_{11}N_3S$
$Pt(Cyst)_2$	$PtC_6H_{10}N_2S_3$
$[Pt(NH_3)_2]$ • $(Cyst)_3$	$Pt_2C_9H_{21}N_4S_3$
$[Pt(NH_3)(Cyst)_3]_n$	$[Pt_2C_9H_{21}N_5S_3]_n$
Best fit to experimental data	$Pt_6C_{21}H_{51}N_9S_5$

Results and Discussion

Cysteine contains three possible coordination sites, the sulfhydryl, the amino and the carboxyl groups. It is known to react rapidly with tetrachloroplatinate, forming bis(cysteinato)platinum(II), which, according to Chandrasekharan *et al.* [7] is coordinated to platinum through the oxygen and the sulfur atoms oxygen and the sulfur atoms. Volshtein and Krylova [8] reported the formation of a bridged 1:1 complex between $[PtCl_4]^{-2}$ and cysteine, with the cysteine being bound to platinum through the nitrogen and sulfur atoms. In addition they assigned a dinuclear structure to the bis-(cysteinato)platinum(II) originally reported by Chandrasekharan *et al.* [7], with cysteine being coordinated to the platinum both through the nitrogen and sulfur atoms and cysteine also acting as a bridge connecting two platinum atoms through the sulfur and oxygen atoms. In a recent paper Pneumatikakis and Hadjiliadis [9] proposed a polymeric structure for the 1:2 complex formed between $[PtCl_4]^{-2}$ and cysteine. In all these reports, it appears that the sulfur atom participates in the binding of platinum in accordance to the theory that soft acids (platinum) form the most stable bonds with soft bases (sulfur).

In contrast to $[PtCl_4]^{-2}$ which reacts rapidly with cysteine to form a precipitate at room temperature [7, 8], cisplatin, which has two carrier ligands (the NH_3 groups) and two leaving ligands (Cl), reacts very slowly with cysteine. An incubation period of several days at 37 °C is necessary to obtain a pale yellow amorphous precipitate which is insoluble in most common solvents such as water, methanol, acetone, DMF and carbon tetrachloride. The results of the elemental analysis suggest that a material of constant elemental composition has been formed, although there appears to be no simple molar ratio between the components of the complex (see Table I). The infrared spectrum shows a broad band in the region 3500–2600 cm^{-1}. These results lead us to suggest that the substance we isolated is of a polymeric nature. The absence of the SH band at 2568 cm^{-1} is consistent with the formation of a

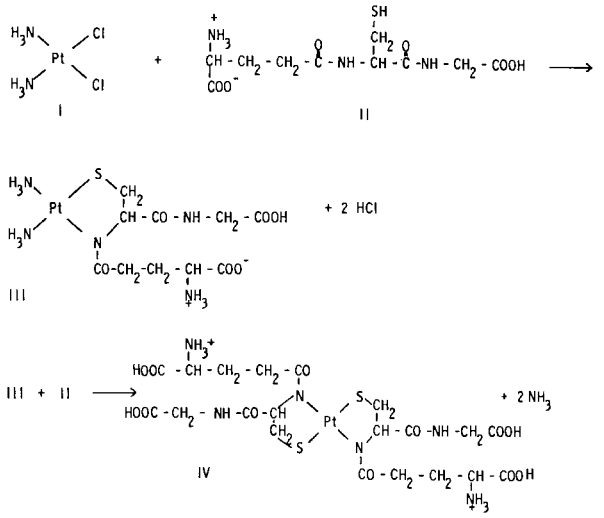

Fig. 1. Representation of possible reaction mechanisms for interaction of cisplatin with glutathione.

Pt–S bond. While no single product or polymer with a simple molar ratio appears to correspond to the analytical data obtained, we have represented on Table II various estimations of possible structures. None of these appear to correspond to the experimental results obtained.

When cysteine is incorporated into a protein molecule, it rarely sits at either the terminal carboxyl or terminal amino end; both, these functions are blocked through peptidic linkages. Glutathione is a tripeptide that is therefore both a good model compound and also an important biochemical agent that might itself be a target for reaction with cisplatin. Unlike, its reaction with cysteine, cisplatin appears to yield a well defined compound with glutathione when the molar ratio between these two reactants is 1:2. The elemental analysis of this product leads to the empirical formula $PtC_{20}H_{32}N_6O_{12}S_2$ • $3H_2O$, which is consistent with a bis-(glutathionato)-platinum(II) (IV). The infrared spectrum shows that the SH band at 2515 cm^{-1} had disappeared, consistent with the formation of a Pt–S bond. The results of the elemental analysis indicate that all four original ligands of cisplatin have been displaced. A possible mechanism for the reactions that occur upon interaction of cisplatin with glutathione is presented in Fig. 1. The molecule of glutathione acts as a bidentate chelating ligand, coordinating to the platinum via the cysteinyl sulfur and nitrogen atoms (the latter, part of the peptide bond to glutamic acid), forming a five membered ring. Similar coordination behaviour has been proposed for palladium(II) [10] and for nickel(II) [11] on reaction with glutathione. The release of the peptide proton, when platinum is coordinated to a peptidic amido group, was documented in an X-ray crystallographic analysis of

chloroglycyl-L-methionato-platinum(II) monohydrate by Freeman and Golomb [12].

Once the sulfur is coordinated to platinum, it appears that it labilizes the *trans* ammine ligand [8] leading to its replacement by a second glutathione molecule through formation of a further Pt–S bond, followed by ring closure. The evidence available at present does not allow us to conclude whether *trans*-labilization precedes or follows closure of the first ring.

The loss of an ammine group from cisplatin on reaction with methionine has been reported by Volshtein *et al.* [13]. They postulate that the initial step involves coordination of methionine to platinum through sulfur and nitrogen, following which the ammine ligand *trans* to the Pt–S bond is labilized, resulting in the release of ammonia and the binding of an additional methionine molecule to platinum through the formation of a second Pt–S bond.

The *trans* labilization of the ammine ligand following the binding of platinum to a deprotonated amidate nitrogen [14] or to a heterocyclic nitrogen [15, 16] has been proposed by Lippard and his coworkers. Their findings led them to suggest that the ammine ligands from *cis*-diammine platinum(II) compounds are released when these agents bind to DNA [17].

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