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Reactions of Platinum(II) Complexes with Guanine Nucleosides and Nucleotides

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It is generally accepted that a possible mechanism leading to the antitumor activity of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) and analogs is a lesion in DNA of the replicating cells [1]. Considerable evidence points to guanine bases as the first site of attack on DNA for platinum complexes [2, 3]. Various authors have postulated modes of binding to guanine which may account for the fact that *cis*-DDP is an effective anticancer drug but its *trans* isomer is not. Among the more popular theories is one which states that *cis*-DDP may bind to guanine by chelation to the carbonyl oxygen atom on C(6) and the N(7) atom in the imidazole ring [4]. The *trans* isomer cannot do this. Another theory that has also gained acceptance [5–7] is that *cis*-platinum(II) complexes interact with DNA by binding to adjacent bases on the same strand. The distance between adjacent bases (*ca.* 3.5Å) is too short to accommodate linking by *trans*-DDP but is similar to the "bite" distance in *cis*-DDP [2].

We have demonstrated a selective interaction of *cis*-DDP with DNA molecules having a large percentage of adjacent guanine bases in the same strand [5]. In our attempt to isolate these platinum bound guanine bases from platinized DNA, we have prepared marker compounds to be used in a scheme of chromatographic separation for comparison purposes. Our products were kept in solution at closely monitored pH values to avoid complications such as oligomerization, which other workers have observed [8].

Preparation of 1:1 or 1:2 complexes between *cis*-DDP or $PtCl_2$(en) (DEP) and guanosine, deoxyguanosine, or deoxyguanosine-5′-monophosphate, disodium salt, was undertaken by mixing stoichiometric amounts of the reactants in water at pH 6.0–6.5. These mixtures were kept in the dark at 37 °C and reactions were judged to be complete when the U.V. spectrum of the mixture remained constant. In a typical reaction, this took one week to occur. Most reactions were done using a 7–10 m*M* solution of the platinum complex. The products formed are listed in the Table along with spectral parameters.

The crystal structure of the cation in complex B has been reported and shows that the metal is bound to guanosine through the N(7) positions on the imidazole ring [9]. No crystallography is available for the 1:1 complexes. Attempts to isolate similar 1:1 complexes from solution have resulted in mixtures of the starting materials [10].

Our reactions to form guanine nucleoside and nucleotide complexes of platinum differ from those previously reported in the following ways. The chloride ligands were not removed by Ag^+ prior to reaction. No buffers were used which would interfere with platinum binding by competing reaction. Reactions were done at 37 °C in the dark to mimic biologic conditions. Products were kept in solution and purified by Sephadex G-10 column chromatography. In addition, we have used deoxy nucleosides and nucleotides since these are present in DNA itself.

In order to characterize the chromatographic properties of these complexes for use as markers in studying DNA adducts with *cis*-DDP analogs, we have utilized a number of support systems. The best separation of complexes A and B occurred on thin layer cellulose using a solvent system containing n-butanol, methanol, H_2O, and aqueous NH_3 in a 60:20:19:1 volume ratio. Compound A had an R_f = .06 and the R_f for B was 0.16 with some streaking. In a 65:16.5:18.5 isopropanol, conc. HCl, H_2O system the R_f for A was 0.88 and R_f for B was 0.71. Complexes C and D were intractable to separation on the thin layer systems which we tried.

We also investigated the behavior of these complexes on columns using exclusion gel supports. With Biogel P-2, a polyacrylamide system, we observed that our platinum compounds reacted with the gel. This behavior was also noticed with other metal

TABLE. Spectral Properties for Platinum(II) Complexes of Guanosine and 5′-Deoxyguanosine Monophosphate.[a]

	Complex	Spectral Peaks (nm)	$A_{\lambda max}/A_{\lambda min}$
A	*cis*-$[(NH_3)_2PtGCl]Cl$	270(sh), 260, 230(sh)	A_{260}/A_{242} = 1.21
B	*cis*-$[(NH_3)_2PtG_2]Cl_2$	270(sh), 260	A_{260}/A_{240} = 1.37
C	Na[enPt(5′-dGMP)Cl]	272(sh), 257, 227(sh)	A_{257}/A_{243} = 1.09
D	Na_2[enPt(5′-dGMP)$_2$]	272(sh), 257	A_{257}/A_{234} = 1.42

[a]Abbreviations: G, guanosine; 5′-dGMP, deoxyguanosine-5′-monophosphate; en, ethylenediamine; sh, shoulder.

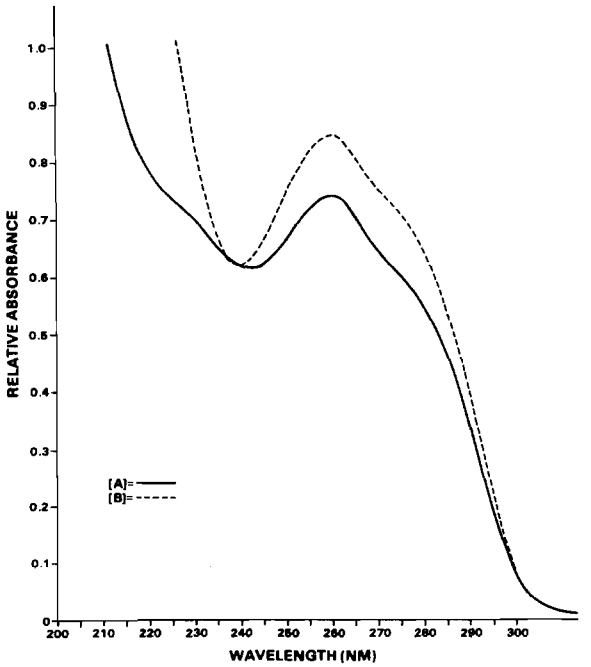

Figure 1. U.V. spectra of 1:1 complexes.

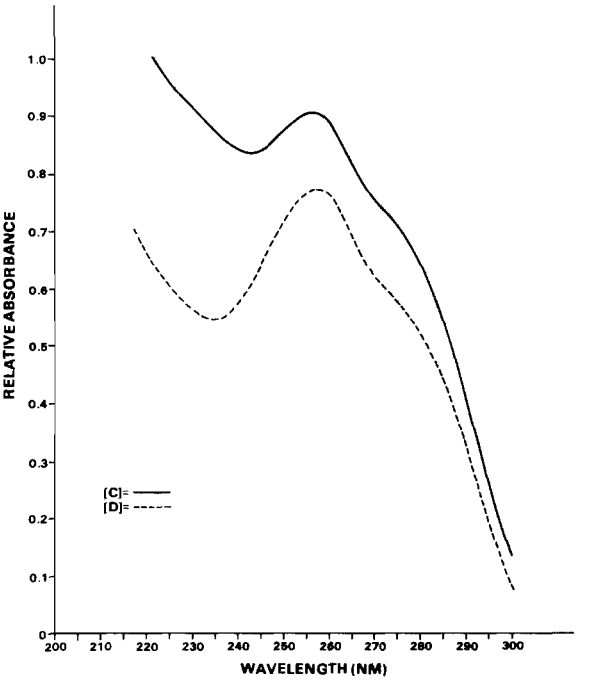

Figure 2. U.V. Spectra of 1:2 complexes.

complexes [11]. Therefore, we tried a number of Sephadex (polyagarose) gels. A 90 cm × 1.5 cm Sephadex G-25 superfine gel system using 0.1 M ammonium formate pH 6.5 as the eluent and ascending chromatography at 5.8 to 6.0 ml/hour was able to discriminate between C and D. The 1:1 complex (C) was isolated with maximal exclusion in fraction 130 while the 1:2 complex (D) appeared in fraction 118. Minor trailing of the peaks occurred, indicating slight interaction with the bed support. We are observing more clearly discerned peaks on a Sephadex G-15 system using descending flow of eluent.

An interesting conclusion may be made from comparison of the ultraviolet spectra of the 1:1 complexes with the 1:2 complexes. The spectra of complexes with a 1:1 mol ratio of Pt to base showed a shoulder near 230 nm of intensity about equal to the peak near 260 nm. This shoulder was absent in the 1:2 complexes. Figures 1 and 2 illustrate these spectra. The presence of this peak in chloroammine complexes of platinum(II) has been assigned to a charge transfer transition arising from a platinum-chloride interaction [12].

In summary, we have prepared in solution 1:1 and 1:2 complexes of *cis* platinum amines and guanine base containing deoxy nucleotides and nucleosides. The U.V. spectra indicate that the 1:1 complexes contain chloride ion bound to platinum, thus ruling out any bidentate interaction with the guanine base. We have identified some properties of these on a number of thin layer and column chromatographic systems. Although we and others [10] have been unable to isolate the 1:1 complexes from solution at this time, our data clearly discriminate between monofunctional and bifunctional binding to guanine. Further studies are in progress.

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