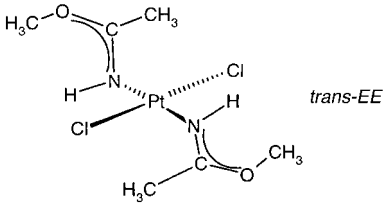
Antitumor *trans* Platinum Complexes can Form Cross-Links with Adjacent Purine Groups**

Yangzhong Liu, Concetta Pacifico, Giovanni Natile, and Einar Sletten*

The structure – activity relationship behind the high efficacy of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) in the treatment of cancers is still not fully understood (for recent reviews see reference [1]). There is a general consensus on the important role played by cisplatin in forming intrastrand (N7,N7) cross-links between adjacent purine bases. The clinically ineffective *trans* isomer cannot form these types of adducts. However, it has been shown recently that several analogues of *trans*-DDP can exhibit antitumor activity comparable to that of *cis*-DDP.[2–5] Of particular interest is a group of iminoether derivatives with the formula *trans*-[$PtCl_2$(iminoether)$_2$] which was shown to be endowed with significant antitumor activity in vivo.[3–6] Surprisingly, these compounds were much more cytotoxic than their *cis* isomers towards tumor cells.[2] The major DNA lesion formed by such iminoether compounds occurs through the formation of a monoadduct with the N7 nitrogen atom of a purine base. A detailed HPLC and NMR spectroscopic analysis of a DNA duplex consisting of 5′-d(CCTCG*CTCTC) and its complement 5′-d(GAGAGC-GAGG) platinated by *trans*-[$PtCl_2${(*E*)-HN=C(OMe)Me}$_2$] (*trans-EE*) has recently been reported.[7]



We have now characterized several adducts of *trans-EE* with the ribonucleotide r(ApG) by high-pressure liquid chromatography (HPLC) as well as by ^{1}H and ^{15}N NMR spectroscopy. Small fractions of the 1:1 reaction mixture of *trans-EE* and ApG were analyzed by HPLC at several time intervals. The distribution of the species is shown for two different time periods in Figure 1. Initially, one major species (**M1**) formed which attains a maximum concentration of 70% of the total products after about 8 h. This adduct is then transformed into two new species, **M2** and **M3**, which reach a steady-state equilibrium after about three weeks with a ratio of **M2**:**M3** of about 2:1.

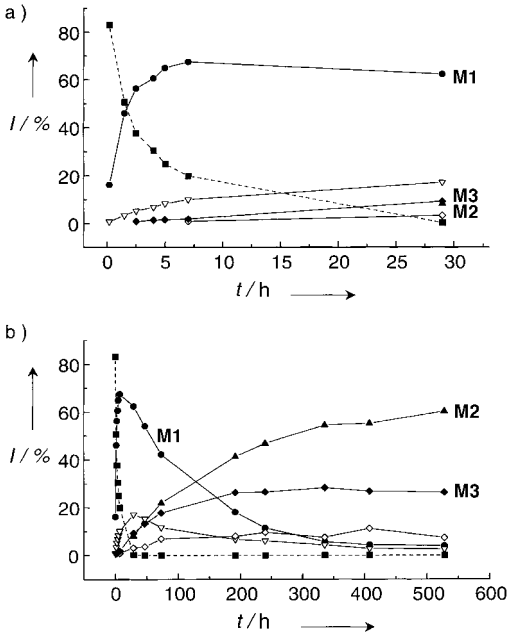


Figure 1. Distribution of the species in the reaction mixture for the platination of r(ApG) with *trans-EE* over 0–30 h (a) and 0–3 weeks (b).

Our previous work on the reaction of *trans-EE* with GMP and AMP shows that platinum binds to the N7 position to form monoadducts which are gradually transformed to diadducts in the presence of excess mononucleotides.[8] The reaction with GMP is much faster ($t_{1/2}$ = 3.5 h) than with AMP ($t_{1/2}$ = 23 h). The adduct **M1** is formed relatively fast ($t_{1/2}$ = 1.6 h) in the reaction between *trans-EE* and r(ApG), and is subsequently converted into **M2** within days ($t_{1/2}$ = 101 h). **M1** is characterized as a monochloro adduct at G-N7; a downfield shift of the G-H8 resonance by 0.45 ppm is in accord with platination at N7. The two minor components probably represent the solvated G-N7 and A-N7 monoadducts (the time-dependent HPLC patterns for this latter adduct and for **M1** are similar to those observed for the solvolysis of the monoadducts with AMP and GMP, respectively).[8]

The NMR spectrum of **M2** shows two unexpected features for the adenosine residue: an extreme downfield shift (3.68 ppm) for the anomeric proton A-H1′ to 9.55 ppm and a large downfield shift (0.82 ppm) of the aromatic proton A-H2 to 8.85 ppm (Figure 2). In addition, most of the other sugar protons on the adenosine residue exhibit significant downfield shifts. The assignments of these chemical shifts are based on COSY and ROESY spectra and also by comparison with the chemical shifts of free r(ApG), both in the present study and from that carried out by van Hemelryck et al.[9] (see Supporting Information). The assignments of A-H2 and A-H8 were also confirmed by measuring the T_1 relaxation times.

[*] Prof. Dr. E. Sletten, Y. Liu
Department of Chemistry
University of Bergen
Allegt. 41, 5007 Bergen (Norway)
Fax: (+47) 55589490
E-mail: einar.sletten@kj.uib.no
C. Pacifico, Prof. Dr. G. Natile
Dipartimento di Chimia Pharmaceutica
Università di Bari
Via E. Orabona 4, 70125, Bari (Italy)

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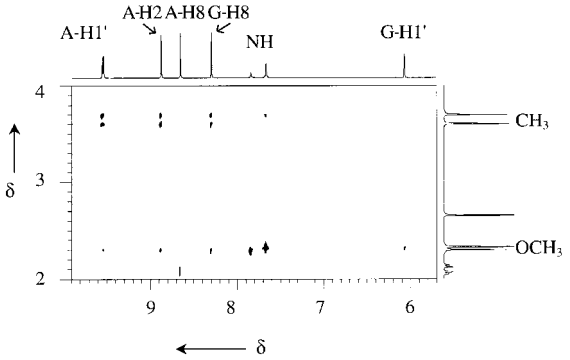

Figure 2. A section of the ROESY spectrum of adduct **M2** in D_2O (δ values are referenced to δ(HDO) = 4.952). A total of 2048 complex points in t_2 were collected for each of the 256 t_1 increments, and 72 transients were averaged for each increment. The iminoether NH signal is slowly exchanged with D_2O during one day at 280 K.

The plots of chemical shifts versus pH (Figure 3) show no significant changes for the A-H2 and A-H8 protons over the entire pH range (1 – 11). The pK_a value for a free A-N1 proton is normally found around 3.5 and 1.2 – 1.5 for an unplatinated

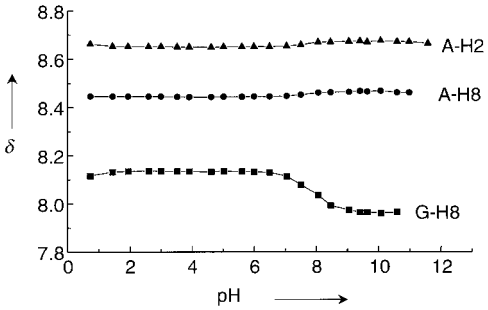

Figure 3. ^{1}H NMR chemical shifts versus pH for A-H2, A-H8, and G-H8 in adduct **M2**.

and N7-platinated adenine group, respectively. The absence of a shift change in this region may indicate either platination at A-N1 or, alternatively, platination at A-N3; the latter would produce a pK_a shift for A-N1 outside the pH range explored. The pK_a values estimated for N7 and N1 in N3-platinated *N*6,*N*6,*N*9-trimethyladenine were 0.3 and – 1.2, respectively.[10] Therefore, platination at A-N3 could account for both the titration experiment and the very large downfield shifts of A-H1′ and A-H2 since they would come right inside the deshielding cone of the platinum center. The deshielding effect of the platinum center could also account for the downfield shift of most of the sugar protons on the adenosine unit.

M2 also exhibits a downfield shift of 0.45 ppm for the G-H8 proton, which supports the coordination of platinum at G-N7. The plot of the G-H8 signal against pH (Figure 3) shows an inflection point at pH 7.5 which corresponds to N1 (de)protonation of a platinated G-N7 residue. In the adduct of *cis*-DDP with r(ApG), where platinum coordinates at A-N7 and G-N7, the pK_a value measured for G-N1 was 7.8,[9] which is thus in good agreement with our results.

For stereochemical reasons *trans* Pt compounds are unable to coordinate to two sites simultaneously if they involve either A-N7/G-N7 or A-N1/G-N7. Consequently, if G-N7 is one of the binding sites the second binding site must be at A-N3. Although this model might appear rather unusual (considering that N1 is appreciably more basic than N3), support is also provided by the natural abundance 2D [^{1}H,^{15}N] HMBC NMR spectra (see Supporting Information), which show that the resonances for G-N7 and A-N3 are shifted upfield by 99 and 83 ppm, respectively, while the other nitrogen atoms exhibit only minor downfield shifts. These nitrogen shifts compare nicely with the platinum-induced shifts of GMP and AMP adducts.[8]

The dicoordination model was optimized by energy-minimization calculations (BIOSYM)[11] using distance constraints obtained from the ROESY spectra. The *trans-EE* ligand was initially fixed in the conformation determined by an X-ray structure analysis.[12] In the final calculation only the platinum and the four nitrogen atoms were fixed in a square-planar arrangement. The resultant geometry of the structure was in qualitative agreement with the experimental data (Figure 4).

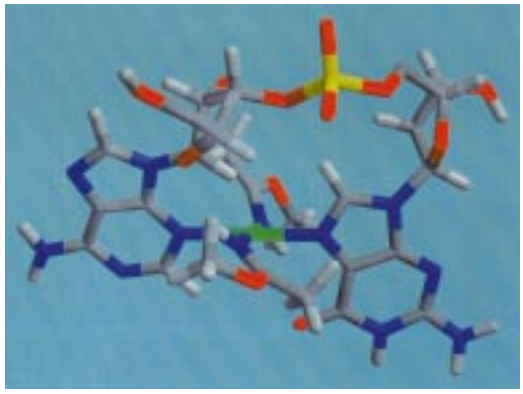

Figure 4. Energy-minimized structure of the *trans-EE*/r(ApG)(N3,N7) chelate.

In this model A-H1′ is located close to the platinum center (2.29 Å), which explains the very large (3.68 ppm) metal-induced downfield shift resulting from the paramagnetic anisotropy of platinum. Large downfield shifts are typical for protons at a pseudoaxial position above the platinum center in a square-planar complex.[13] A short Pt ··· H distance of 2.53 Å was observed in the crystal structure of *trans*-[$PtCl_2$(benzoquinoline)(PEt_3)] which corresponded to the downfield shift (in solution) of 2.75 ppm for the signal in the NMR spectrum.[13]

The NMR spectra of **M3**, the second major component formed at long reaction times, are distinctly different from those obtained for **M1** and **M2**. The integration of the 1D spectra indicates that the composition of **M3** corresponds to a *trans-EE*:r(ApG) ratio of 2:1. Moreover, the A-H8 and G-H8 signals are shifted downfield by 0.83 and 0.70 ppm, respectively, which clearly indicates platination of both residues at N7. Therefore the formation of **M3** can be explained by the presence of a slight excess of the platinum complex in the reaction sample which leads to the formation of a dimetalated r(ApG).

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The platination of oligonucleotides by *trans*-DDP has been shown to produce intrastrand 1,3-(N7,N7) cross-links with d(GpTpG),[14] d(GpCpG),[15] d(GpApG),[16] and a 1,3-(N1,N7) cross-link with d(ApGpG).[17] Monoadducts and interstrand cross-links were mainly formed in the reaction with double-stranded DNA;[18] *trans*-DDP was considered unable to form 1,2-intrastrand d(GpG) or d(ApG) adducts as a result of its structure.[17] Thus, the stable adduct **M2** represents a new and unprecedented chelation geometry for a *trans* platinum complex. Although the chelation process is relatively slow, the N3-Pt-N7 structure may be of biological relevance since A-N3 is readily exposed to platination in the minor groove. The involvement of the N3 atom of adenine as an alkylation site[19] and in the cleavage reaction of hammerhead ribozyme has recently been demonstrated.[20] This study is presently being extended to other ribo- and deoxy-dinucleotides.

Experimental Section

trans-EE was synthesized according to a literature procedure.[12] r(ApG) was purchased from Sigma. *trans-EE* (2.3 mg, 5.6 μmol) was added to 5 mL of a 1 mM solution of r(ApG) (5 μmol, pH 4.0) at room temperature in the dark. Samples were collected for HPLC detection at various time intervals. Samples were lyophilized and dissolved in D_2O (0.5 mL) and the ROESY NMR spectra (mixing time 300 ms, 3 mM sample, pH 3.9, $T = 280$ K) were recorded on a Bruker DRX 600 instrument. The pH values were measured on a Philips PW 9420 pH meter. HPLC was performed on a Waters 626 LC instrument using Millennium 32 software and a reverse-phase Waters Symmetry C8 column with gradient elution (0–50% methanol in 50 mM $NaClO_4$ at a flow rate of 0.8 mL min^{-1}).

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Molecular Modulation of Surfactant Aggregation in Water: Effect of the Incorporation of Multiple Headgroups on Micellar Properties**

Jayanta Haldar, Vinod K. Aswal, Prem S. Goyal, and Santanu Bhattacharya*

Surfactant molecules self-organize in water,[1] often producing nearly spherical aggregates called micelles in dilute solutions, and lyotropic mesophases at higher concentrations. The polar headgroups of these aggregates lie near the bulk aqueous phase, whereas the hydrocarbon chains extend inwardly to avoid unfavorable water contacts. Electrostatic interactions between the headgroups determine their relative positions and separations in an aggregate. The correlation of the molecular structure of various surfactants with the aggregate type produced upon self-assembly is important as surfactant solutions are useful in a number of household, industrial, and scientific applications.[2]

Molecular design of surfactants of widely varying architectures offers an excellent opportunity for tailoring surfactant aggregation behavior and the resulting complex fluid properties. For instance, dimeric or oligomeric surfactants that consist of two or more conventional surfactant units connected at the headgroup by a suitable spacer are attracting attention because of the number of unusual properties that they manifest.[3]

In ionic micelles, surfactant molecules such as cetyltrimethylammonium bromide (CTAB) also ionize in aqueous solution and the corresponding micelles are aggregates of

[*] Prof. Dr. S. Bhattacharya, J. Haldar
Department of Organic Chemistry
Indian Institute of Science
Bangalore 560012 (India)
Fax: (+91) 80-360-0529
E-mail: sb@orgchem.iisc.ernet.in
Dr. V. K. Aswal
Solid State Physics Division, Mumbai Centre
Bhabha Atomic Research Centre, Mumbai 400085 (India)
Dr. P. S. Goyal
IUC-DAEF, Mumbai Centre
Bhabha Atomic Research Centre, Mumbai 400085 (India)

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