The Aquated Form of Cisplatin Selectively Attacks at the G[3] Site in d(GTG)

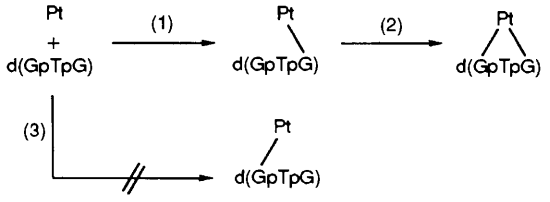
Mark Garner,[a] (the late) Michael Green,[a] Nelleke Boogaard[b] and Jan Reedijk*[b]

[a] *The Department of Chemistry, University of York, Heslington, York Y01 5DD, UK*
[b] *Department of Chemistry, Gorlaeus Laboratories, Leiden University, 2300 RA Leiden, The Netherlands*

While d(GpTpG) reacts with *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ eventually to form a G[1]N(7), G[3](7) chelate, the initial attack is exclusively at G[3], (rate constants for the initial step and cyclisation being 21.6 $dm^3\ mol^{-1}\ s^{-1}$ and $4.4 \times 10^{-4}\ s^{-1}$, respectively at 37.0 °C).

Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, the anticancer drug, attacks DNA very selectively at d(GpG) sites.[1] Turning to simple systems, one finds that in the reaction of the *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$/*cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ system with guanine containing mononucleo-sides and -tides, there is also a selectivity; our groups[2,3] have observed that pG and in particular dpG (*i.e.* 5′-GMP and 5′-dGMP) react more quickly than do G, dG and Gp (3′-GMP). In contrast in the case of dinucleotides the selectivity is much less striking; $[Pt(dien)Cl]^+$ (dien = diethylenetriamine) attacks the G moiety in d(ApG), d(TpG), d(CpG), d(GpA), d(GpT) and d(GpC) at only slighlty different rates,[4] a similar effect being observed for $[Pt(NH_3)_3(OH_2)]^{2+}$ reacting with GpG, ApG, CpG, GpA and GpC.[5] In all these cases there appears to be no large selectivity for pG over Gp. In contrast when cisplatin attacks DNA nearly 80% of the platinum finishes as *cis*-$[Pt(NH_3)_2d(\cdots GpG\cdots)]$ chelates. Apart from nearly 20% bonding in *cis*-$[Pt(NH_3)_2d(\cdots ApG\cdots)]$ units, very little of the platinum ultimately binds to single G sites.[1] The contrast between mono- and di-nucleotides and DNA itself raises the question of how trinucleotides behave.

We have therefore investigated the trinucleotide d(GpTpG) on the basis that T does not react with cisplatin and its relatives in neutral or mildly acidic conditions[6] so that the affinities of the G[1] and G[3] units, which are Gp and pG moieties, respectively, towards the platinum centre can be studied separately.



^{1}H NMR studies at 300 MHz were made on 1:1 to 1:3 mixtures of *cis*-$[Pt(NH_3)_2(OH_2)_2](CF_3SO_3)_2$ and the disodium salt of d(GpTpG) at 27 °C reacting with each other. {The pH varies from 6.27 to 6.43 so that the predominant reacting platinum species[7] was *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$, [d(GpTpG)] *ca.* 10^{-3} mol dm^{-3}}. The platinum ion attacks the G units at N(7) as in Scheme 1, the sites of binding being identified by shifts in δ-values during reactions (1) and (2) G[1]H(8): 7.89 → 7.92 → 8.36, G[3]H(8): 8.00 → 8.50 → 8.38. No evidence was found for reaction (3). Clearly the initial attack occurs only at G[3], and not at G[1].

The ^{1}H NMR work was complemented by kinetic studies at 37.0 °C on changes in UV spectra on 1:1 and 1:10–20 mixtures of the reactants (*i.e.* under second- and pseudo- first-order conditions, respectively, in which the trinucleotide was in excess), ([Pt] = 10^{-5} mol dm^{-3}). k_1 = 21.6 $dm^3\ mol^{-1}\ s^{-1}$ and $k_2 = 4.4 \times 10^{-4}\ s^{-1}$. k_1 is comparable with the analogous rate constants for dpG and pG which are 7.4 and 13.0

$dm^3\ mol^{-1}\ s^{-1}$, respectively.[8] The fact that reaction (3) could not be detected means that $k_3 \leqslant 1\ dm^3\ mol^{-1}\ s^{-1}$. No rate constant for the attack of *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ on dGp [*i.e.* the reaction analogous to (3)] appears to have been measured, but that for Gp is known to be *ca.* 0.1 $dm^3\ mol^{-1}\ s^{-1}$ at 37.0 °C.[8] Thus, in the d(GpTpG) trinucleotide, the dGp and dpG units appear to react at rates comparable with their mononucleotide counterparts, Gp and pG. In other words they react independently of each other. The 5′-phosphate group of a pG unit, as opposed to the 3′-phosphate of Gp, can approach the N(7) of its G closely. It has been suggested that electrostatic attraction between the 5′-phosphate group and the platinum centre[2,9] and also hydrogen bonding between the 5′-phosphate and NH_3 or OH_2 ligands[10,11] can stabilize the transition state. The reaction of d(GTG) manifests this effect.

However, this neat, simple result for d(GpTpG) is not reflected in the reactions of d(GpApG). The absence of reaction (3) for d(GpTpG) provides an interesting contrast with d(GpApG). *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ attacks G[1] as well as G[3] during the initial reaction,[12] the parameter analogous to k_1/k_3 being 1.9 in comparison to $\geqslant 21$ found here for d(GpTpG). The presence of the 5′-phosphate in the pG unit in d(GpTpG) seems to control the attack by the platinum centre. While this could be true for d(GpApG) to some extent, the activity of its G[1] suggests that other effects such as conformation of the trinucleotide and loose bonding between N(7) of A in this case to the fifth coordination site in the platinum complex are important.

To conclude, there is a considerable difference in behaviour of cisplatin type compounds towards G dependent not only on immediate neighbours but also on the type of compound or chain in which the G lie.

Our thanks to the Yorkshire Cancer Research Campaign and to Johnson Matthey for support.

Received, 19th November 1992; Com. 2/06178C

References

1 A.-M. J. Fichtinger-Schepman, J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry*, 1985, **24**, 707.
2 A. T. M. Marcelis, C. Erkelens and J. Reedijk, *Inorg. Chim. Acta*, 1984, **91**, 129.
3 D. J. Evans, N. R. Ford and M. Green, *Inorg. Chim. Acta*, 1987, **125**, 239.
4 J. L. van der Veer, H. P. J. M. Noteborn, H. van den Elst and J. Reedijk, *Inorg. Chem. Acta*, 1987, **131**, 221.
5 A. Laoui, J. Kozelka and J.-C. Chottard, *Inorg. Chem.*, 1988, **27**, 2751.
6 S. S. Eapen, M. Green and I. M. Ismail, *J. Inorg. Biochem.*, 1985, **24**, 233.
7 M. Green, M. Garner and D. M. Orton, *Transition Metal Chem.*, 1992, **17**, 164 and references cited therein.
8 S. S. Eapen, D. J. Evans and M. Green, unpublished work.
9 M. J. Bloemink, E. L. M. Lempers and J. Reedijk, *Inorg. Chim. Acta*, 1990, **176**, 317.
10 M. Green and J. Miller, *J. Chem. Soc., Chem. Commun.*, 1987, 1864 and corrigendum, 1988, 404.
11 D. M. Orton and M. Green, *J. Chem. Soc., Chem. Commun.*, 1991, 1612.
12 J. L. van der Veer, H. van den Elst, J. H. J. den Hartog, A.-M. J. Fichtinger-Schepman and J. Reedjik, *Inorg. Chem.*, 1986, **25**, 4657.