DALTON COMMUNICATION

Intramolecular migration of co-ordinated platinum from a sulfur to N^7 in the nucleopeptide Met-d(TpG) (5′-*O*-methioninate-*N*-ylcarbonylthymidine 2′-deoxyguanosine monophosphate) †

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The nucleopeptide Met-d(TpG) (5′-*O*-methioninate-*N*-ylcarbonylthymidine 2′-deoxyguanosine monophosphate), containing a methionine moiety covalently linked to a TpG dinucleotide, upon reaction with platinum complexes, initially yielded platinum co-ordination to the sulfur atom, which subsequently is substituted by the N^7 atom of guanine for the reaction with monofunctional [Pt(dien)Cl]Cl (dien = diethylenetriamine); in the case of the cisplatin analogue [Pt(en)Cl_2] (en = ethane-1,2-diamine) formation of a stable S,N chelate takes place.

Platinum anticancer drugs are believed to exert their therapeutic action through interactions with DNA, the ultimate target being the N^7 of guanine.[1,2] At present it is not clear how the Pt species reaches the DNA, as platinum(II) complexes are known to react rapidly with sulfur-donor ligands, such as those found in the amino acids cysteine and methionine. Currently there is much interest in studying the interaction of platinum complexes with sulfur-containing proteins and peptides, since these interactions are likely to play an important role in platinum metabolism.[3,4] Platinum–sulfur interactions have been associated with the negative side effects of platinum treatment, as well as with the development of resistance.[5]

It has been shown recently that both inter- and intramolecular displacement of platinum(II) of the S-bound thioethers by guanine N^7 can occur,[3,4,6] implying that DNA platination might occur *in vivo* after intermediate interactions with thioether-containing biomolecules.

However, the molecules used so far, *S*-guanosyl-L-homocysteine and methionine, suffer from the fact that at pH 7 other groups may also compete, *e.g.* NH_2 and COO^-. Therefore a molecule is required in which only thioether and nucleobase atoms are available. Consequently the nucleopeptide model Met-d(TpG) **I** (5′-*O*-methioninate-*N*-ylcarbonylthymidine 2′-deoxyguanosine monophosphate) (Fig. 1) has been designed and synthesized;‡ this model species contains methionine which is covalently linked through a carbamate linkage to a bis(2′-deoxynucleotide). A competition study was undertaken in platinum co-ordination between the sulfur and the N^7 of guanine at physiological pH.

The nucleopeptide **I** (3 mM) was first reacted with [Pt(dien)Cl]Cl (dien = diethylenetriamine) in a 2:1 ratio to avoid a possible excess of the platinum complex. The reaction was carried out in D_2O in an NMR tube (310 K, pH 7) and monitored by ^1H NMR spectroscopy.§ The signals corresponding to the H^8 of guanine and the SCH_3 provided information on the course of the reaction.

Two monofunctional complexes [Pt(dien){Met-d(TpG)-*S*}]$^+$ **1** and [Pt(dien){Met-d(TpG)-N^7}]$^+$ **2** were found to be formed subsequently. The H^8 of the guanine of free Met-d(TpG), observed at δ 8.04, and the SCH_3 signal at δ 2.06, are taken as monitors for the reaction. Within 2 h of incubation with [Pt(dien)Cl]Cl the intensity of the SCH_3 peak had decreased to half its original intensity and an additional peak appeared at δ 2.52 with equal intensity, and the intensity of the H^8 signal also decreased by 50% and an additional peak appeared 0.02 ppm downfield, both being indicative for a 1:1 adduct. These signals have been assigned to the SCH_3 and H^8 of complex **1**, clearly indicating that the platinum is co-ordinated to the thioether function. After prolonged incubation the intensity of the peak at δ 2.52 gradually decreased, whilst the intensity of the singlet at δ 2.06 returned to its original value. The H^8 region shows the simultaneous appearance of a singlet at δ 8.45, 0.39 ppm downfield from the free H^8 signal. This signal has been assigned to the H^8 of complex **2**. The H^6 signal of thymine was found to be slightly shifted downfield to δ 7.45 (from δ 7.30). Co-ordination to the N^7 of guanine was confirmed by a pH titration, showing the absence of a N^7 protonation effect on the H^8 resonance at low pH. The rearrangement of complex **1** into **2** was found to the complete after 6 d at room temperature. Complex **2** proved to be a stable end product of the reaction.

Repeating the above experiment in a ratio 1:2 {for **I**:[Pt(dien)Cl]Cl} revealed that within 2 h the methionine moiety was platinated; in this case subsequent platination of N^7 was found to be completed after 15 h. On the basis of the downfield shift of both H^8, H^6 and SCH_3 the end product has unambiguously been determined to be complex **3**, [{Pt(dien)}$_2${Met-d(TpG)-N^7,*S*}]$^{3+}$, in which both the thioether and the N^7 are platinated. Formation of these complexes is schematically depicted in Scheme 1.

In a next step the behaviour of a bifunctional platinum compound was investigated. The reaction of nucleopeptide **I** with

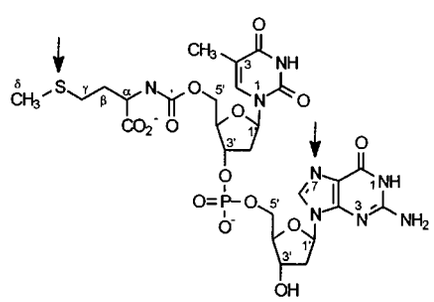


Fig. 1 Schematic structure of nucleopeptide Met-d(TpG) **I**, arrows indicate possible platination sites

† Based on the presentation given at Dalton Discussion No. 2, 2nd–5th September 1997, University of East Anglia, UK.

‡ Met-d(TpG) has been synthesized from methionine, 5′-deoxyribosylthymine and 5′-deoxyguanosine. Successful introduction of the carbamate linkage required easily removable protecting groups and use of 1-hydroxybenzotriazole hydrate in the carbonyldiimidazole-mediated coupling reaction. The synthesis has been described in more detail elsewhere.[6]

§ The complexes [Pt(dien)Cl]Cl and [Pt(en)Cl_2] (en = ethane-1,2-diamine) were prepared by methods previously described.[7,8] All platination reactions were performed at nucleopeptide concentrations of 3 mM in D_2O at 310 K, pH was adjusted to 7. The pH meter readings were not corrected for deuterium isotope effects. The NMR spectra were recorded on a 300 MHz Bruker DPX300 spectrometer in D_2O at 310 K at pH 7, unless indicated otherwise. Proton NMR spectra were calibrated against NMe_4NO_3 [at δ 3.18 with respect to sodium 3-(trimethylsilyl)propanesulfonate].

Table 1 Relevant ^{13}C chemical shifts for **I** and complex **4** in ppm relative to sodium phosphate in D_2O at 293 K at pH 7 ($t_{\frac{1}{2}}$ = 100 min)

	G^2-C	G^4-C	G^8-C	α-C	β-C	γ-C	δ-C	CO
I Met-d(TpG)$^-$	154.6	152.0	137.3	54.7	30.2	31.2	14.8	178.0
4 [Pt(en){Met-d(TpG)-*S*,N^7}]$^+$	155.7	155.8	141.5 141.9	54.5	30.0	38.3 39.4	19.5 20.3	175.9

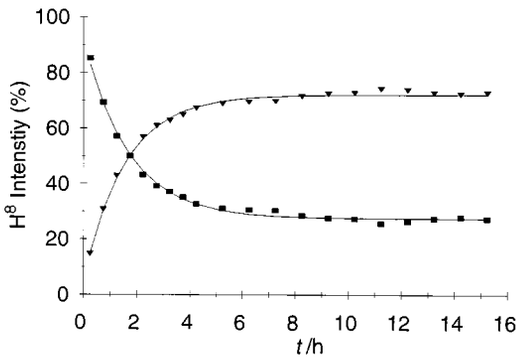
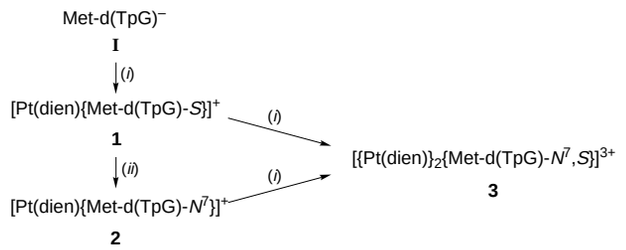
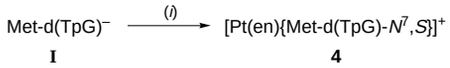


Fig. 2 Plot of relative intensity of H^8 signals *versus* time for the reaction of Met-d(TpG) **I** (■) with [Pt(en)Cl_2] to give complex **4** (▼) at pH 7 at 310 K



Scheme 1 (*i*) Pt(dien)$^{2+}$; (*ii*) Pt migration



Scheme 2 (*i*) Pt(en)$^{2+}$

[Pt(en)Cl_2] (4:3 ratio, *i.e.* a slight excess of the nucleopeptide) was monitored under the same conditions as mentioned above. Co-ordination of the Pt(en) unit to the sulfur atom of the thioether function was found to proceed slower than for Pt(dien) and appeared to be completed within 10 h, resulting in a broadened peak for SCH_3 (δ 2.51, +0.45 ppm). The intensity of the free H^8 signal decreased simultaneously with that of the free SCH_3 signal, and a new signal appeared 0.51 ppm downfield from the free H^8 signal. The variation in intensity of the H^8 protons *versus* time is plotted in Fig. 2 ($t_{\frac{1}{2}}$ = 100 min). The nearly simultaneous decrease of intensity of the peaks corresponding to the free SCH_3 and H^8 indicates platination of the sulfur atom to be the rate-limiting step, followed by a fast chelation step yielding a stable chelate between the sulfur atom and the N^7 of the guanine moiety, complex **4**, [Pt(en){Met-d(TpG)-N^7,*S*}]$^+$. No subsequent displacement of the S-bound thioether by N^7 was observed even after prolonged standing in the presence of unreacted nucleopeptide **I**. The reaction is depicted in Scheme 2.

The preparation of complex **4** was also repeated on a larger scale in a 1:1 ratio. Platination of N^7 was confirmed by monitoring the pH-dependent behaviour of proton H^8 in the NMR spectrum. Carbon-13 NMR spectroscopy confirmed chelation of N^7 and the sulfur in the methionine moiety. Carbon-13 chemical shifts most affected by platination are listed in Table 1 for **I** and for complex **4**. Most apparent are the splittings and downfield shifts by 8.2/7.1 ppm and 5.5/4.7 ppm for the γ-C and δ-C respectively. The G^8-C signal is also split and shifted downfield by 4.2/4.6 ppm. Co-ordination of the platinum to the sulfur atom in methionine should result in the formation of two diastereomers, giving rise to a doubling of these signals. This splitting is indeed observed, but not for carbon atoms further away from the Pt co-ordination site; although many signals are severely broadened. In fact the broadening of the SCH_3 signal in the ^1H NMR spectrum can also be attributed to the occurrence of the two diastereomers.

The results of the reaction **I** with Pt(dien) are in agreement with previous competition studies using the nucleopeptide model *S*-guanosyl-L-homocysteine[3] and intermolecular studies using methionine and 5′-GMP (guanosine 5′-monophosphate),[4] and using *S*-methyl-γ-glutamylcysteinylglycine and 5′-GMP[6] at lower pH, showing that the thioether function in a platinum–sulfur adduct can be substituted by the N^7 of guanine. Reaction of **I** with bifunctional Pt(en), however, results in formation of a stable S,N^7 chelate even in the presence of extra unplatinated N^7. This seemingly contrasting finding is in agreement with previous reports that S,N^7 chelates are remarkably stable,[8,9] and in fact suggests a biological role for sulfur-containing molecules in the formation of protein–DNA cross links formed by DNA.[10]

We are currently investigating reactions with the nucleopeptide Met-d(TpGpG), containing an extra guanosine. This model will enable the study of the competition in co-ordination of bifunctional platinum compounds between thioethers and the highly reactive GpG sequence.

In summary, we have shown that monofunctional Pt migration from S to N^7 can occur in a nucleopeptide under physiological conditions. However, the chelate containing a Pt–(S,N^7) bond is stable towards Pt–S dissociation and migration.

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References

1 J. Reedijk, *Inorg. Chim. Acta*, 1992, **198–200**, 873.
2 M. J. Bloemink and J. Reedijk, in *Metal ions in biological systems*, eds. H. Sigel and A. Sigel, M. Dekker, New York, 1996, vol. 32, pp. 641–685.
3 S. S. G. E. van Boom and J. Reedijk, *J. Chem. Soc., Chem. Commun.*, 1993, 1397.
4 K. J. Barnham, M. I. Djuran, P. del Socorrro Murdoch and P. J. Sadler, *J. Chem. Soc., Chem. Commun.*, 1994, 721.
5 S. L. Kelley, A. Basu, B. A. Teicher, M. P. Hacker, D. H. Hamer and J. S. Lazo, *Science*, 1988, **241**, 1813.
6 S. S. G. E. van Boom, Ph.D. Thesis, Leiden University, 1995.
7 G. W. Watt and W. A. Cude, *Inorg. Chem.*, 1968, **7**, 335.
8 M. Iwamoto, S. Mukundan and L. G. Marzilli, *J. Am. Chem. Soc.*, 1994, **116**, 6233.
9 K. J. Barnham, Z. Guo and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1996, 2867.
10 J. Reedijk, *Chem. Commun.*, 1996, 801.

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