Kinetic preference of 5'GMP over 3'GMP in reactions with platinum amine compounds as studied by competition reactions

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Abstract

Competitive binding of 5'GMP and 3'GMP with [PtCl(dien)]Cl has been performed as a function of pH. The identity and relative amounts of the reaction products have been determined by ^{1}H NMR spectroscopy. The ratio of the formed products, [Pt(dien)(5'GMP-N7)]:[Pt(dien)(3'GMP-N7)], increases from 3.5 at pH 4 to 7.5 at pH 9. An apparent pK_a of 6.6 is observed which corresponds to a phosphate (de)hydronation. The kinetic preference for 5'GMP is caused by the position and the charge of the phosphate group.

Introduction

It is generally accepted that DNA is the primary molecular target of the antitumor drug [*cis*-$PtCl_2(NH_3)_2$] (*cis*-Pt) [1]. Several studies show that bifunctional coordination is favoured at –GpG– sites [2, 3].

The rate of platination of (oligo)nucleotides at guanine-N7 is enhanced by a 5'phosphate group, as has been observed in numerous studies [4–12]. As proposed by Marcelis *et al.* [6] an electrostatic interaction with the 5'phosphate directs the platinum complex to the N7 position of the purine, resulting in an increased rate of platination compared to, for example, guanosine. For geometric reasons, a similar interaction with a 3'phosphate will not direct the platinum complex to the N7 position of the corresponding guanine (see Fig. 1).

If electrostatic interactions with the 5'phosphate do occur, the ratio of the reactivity of 5'GMP over 3'GMP would be dependent on the charge of the phosphate group (pK_a 6.23 [13]). In the competition experiments between 5'GMP and 3'GMP with [PtCl(dien)]Cl (dien stands for diethylenetriamine) forming [Pt(dien)(5'GMP-N7)] and [Pt(dien)-(3'GMP-N7)], respectively, the pH effect on this directing effect has not yet been investigated [6]. Therefore, in the present study, the reaction of [PtCl(dien)Cl with a mixture of 5'GMP and 3'GMP is carried out as a function of pH. These experiments have been performed at low concentrations (6.7×10^{-5} M) in order to exclude the contribution of intermolecular interactions.

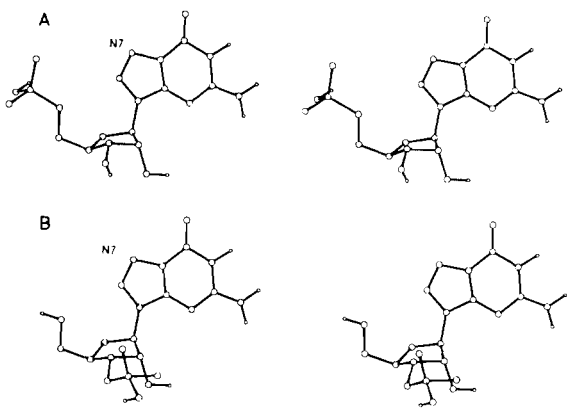

Fig. 1. Stereoview of the 5'GMP (A) and 3'GMP (B) structures believed to be present in solution (sugar conformations are taken as N).

Experimental

Starting materials

The mononucleotides 5'GMP and 3'GMP were obtained from SIGMA chemicals and used without further purification. The platinum complex [PtCl(dien)]Cl was prepared according to Watt and Cude [14].

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Reactions

Stock solutions of 5'GMP, 3'GMP and [PtCl(dien)Cl were prepared. 5'GMP (2.04 mg, 5 μmol) and 3'GMP (2.04 mg, 5 μmol) were dissolved in 75 ml doubly distilled water. [PtCl(dien)]Cl (1.85 mg, 5 μmol in 1 ml) was added and the reactions were performed at room temperature for 24 h in the dark. The pH values of the various reaction solutions (pH 4–9) were adjusted by NaOH or HCl and kept constant during the reaction by a titrator (type TTT1C radiometer Copenhagen) with NaOH. After completion of the reaction the pH was adjusted to 5.3 and the solvent was removed by vacuum evaporation.

To confirm that the competition reactions were completed after 24 h, reactions were performed at pH 4 and 9 (i.e. the two extreme pH values) between [PtCl(dien)]Cl and two equivalents of 3'GMP under the same conditions (NB: It is assumed that replacement of 5'GMP by the less reactive 3'GMP will slow down the reaction compared to the actual competition experiments). These two reactions were monitored by UV spectroscopy (Perkin-Elmer EPS-3T). For reference purposes, [Pt(dien)(5'GMP-N7)] and [Pt(dien)(3'GMP-N7)] were prepared by reacting 5'GMP and 3'GMP, respectively, with a stoichiometric amount of [PtCl(dien)]Cl.

NMR measurements

The dried reaction products were dissolved in 0.5 ml D_2O (99.8%, Merck) and a trace amount of tetramethylammonium nitrate (TMA) was added. The pH was again adjusted to 5.3 by addition of NaOD (0.1 M) and DCl (0.1 M) and the samples were lyophilized. After dissolving in 0.5 ml D_2O (99.95%, Merck) the ^1H NMR spectra were recorded on a Bruker WM 300 spectrometer at 294 K. The pH values reported as pH* have not been corrected for deuterium isotope effects. The quantitation of the products and the starting materials was obtained by integration of their H1' resonances. The ^1H NMR spectra were recorded with an acquisition time of 2 s and a relaxation delay of 1.5 s. A planimeter was used to obtain accurate integrals (OTT planimeter Ahrend).

Results

The reactions between [PtCl(dien)]Cl and two equivalents 3'GMP, both at pH 4 and 9, are completed after 24 h, as no further changes in the UV spectra were observed. This was confirmed by ^1H NMR spectroscopy (i.e. no more unreacted [PtCl(dien)]Cl was present). Therefore, the (expected faster) competition reactions between 5'GMP and 3'GMP were estimated to be completed also within 24 h. During the competition reactions NaOH had to be added in order to keep the pH constant; small pH changes may occur as a result of the reduced ionization constant of guanine-N1 after N7 platination [15, 16].

The reactions of 5'GMP and 3'GMP with [PtCl(dien)]Cl, when carried out separately, both form one platinated product. The H8 proton chemical shifts of both products are downfield compared to the free nucleotides (Table 1). In addition the position of these signals is independent of pH between pH 2 and 5 (i.e. no N7 protonation effect anymore) and shows a pH dependence around pH 8.5, due to the (de)protonation of N1 (data not shown). These observations confirm that both 5'GMP and 3'GMP are platinated at their N7 atoms [15, 16] forming [Pt(dien)(5'GMP-N7)] and [Pt(dien)(3'GMP-N7)], respectively. In comparing the products, formed during the competition experiments, with the reference products, it is obvious that the same complexes are formed.

One should realize that H8 protons of platinated guanine residues are susceptible to exchange with deuterium [17]. In addition these signals are broad, due to unresolved coupling with the ^{195}Pt nucleus [18]. As a result, H8 signals are not suitable for obtaining accurate integrals; therefore, the H1' resonances had to be used. The ^1H NMR spectra of the products from the competition reactions were recorded at pH* 5.3, because at that value no overlap of the H1' resonances is present (Table 1).

In Fig. 2 the ratio of the products [Pt(dien)(5'GMP-N7)] and [Pt(dien)(3'GMP-N7)] is depicted as function of pH (corrected for the initial ratio 5'GMP:3'GMP, (1.1)). In all cases, as expected, about 50% of the total amount of mononucleotides has reacted with the platinum compound. Some guanosine was formed through degradation of GMP during the reactions (0–3%), but this is considered to have no serious influence on the observed ratio.

TABLE 1. Chemical shift of the H8 and the H1' protons of the reaction products and the unreacted mononucleotides, recorded at pH* 5.3

Compound	H1' (ppm)	H8 (ppm)
Guanosine	2.72	4.80
5'GMP	2.75	4.91
3'GMP	2.76	4.81
[Pt(dien)(3'GMP-N7)]	2.81	5.30
[Pt(dien)(5'GMP-N7)]	2.85	5.48

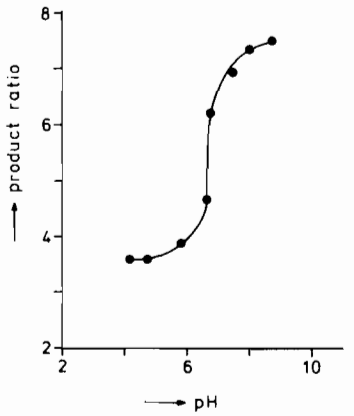

Fig. 2. Plot showing the pH dependence of the product ratio [Pt(dien)(5'GMP-N7)]: [Pt(dien)(3'GMP-N7)], as determined by integration of the H1' resonances of starting material and complexes (pH* 5.3).

Discussion

The phosphodiester group in the DNA backbone is thought to be important both from a thermodynamic point of view, i.e. stabilization of the d(pGpG)-N7,N7 adduct by means of a hydrogen bonding to the platinum coordinated amine [19–21], and from a kinetic point of view, i.e. directing the platinum complex to the N7 position of the guanine [4–12]. As proposed by Marcelis *et al.* [6] the kinetic directing effect is interpreted in terms of an electrostatic interaction between Pt(II) and the negatively charged phosphodiester group.

The preference for binding at 5'GMP over 3'GMP [6] is confirmed in the present study and has now been investigated in more detail, as a function of pH. An electronic directing effect (especially effective for 5'GMP, see Fig. 1) will depend on the charge of the phosphate group, leading to an even larger preference for [Pt(dien)(5'GMP-N7)] compared to [Pt(dien)(3'GMP-N7)], upon increasing pH; this is indeed observed with an apparent pK_a of 6.6. It is assumed that migration of platinum from 5'GMP to 3'GMP or vice versa does not occur, since ligand-exchange reactions of such Pt(II) compounds are known to be very slow [22]. The observed effect nicely correlates with the pK_a (6.23) of the phosphate [13]*.

In conclusion it can be stated that the kinetic preference for platination of 5'GMP, compared to 3'GMP, is the result of the location and charge of the phosphate group. The kinetic direction influence in oligonucleotides most likely will be smaller, because of the presence of a shielded phosphodiester group (i.e. in 5'GMP the phosphate group is more flexible and therefore has better directing properties), but still enhancement of the rate of platination can be expected, as has been observed before [9, 10, 12]. An exception is the deoxydinucleotide d(GpG) where coordination is favoured at the 5' guanine [23]. In this case, probably, a highly accessible terminal 5' guanine (i.e. more reactive) can compete effectively with the phosphate directing effect. In the former studies [10, 12] the more flexible ribodinucleotide r(GpG) was used, with increased phosphate directing properties compared to d(GpG).

Since, in DNA all the bases have a phosphodiester group at the 5' site, an overall rate accelerating effect can be expected, which will be important for the amount of platinum binding to DNA over other potential ligands (especially sulfur-containing ligands, like cysteine and methionine). Therefore the phosphate directing effect is an important feature in the mechanism of action of platinum antitumor compounds. At the moment it is difficult to predict if such a kinetic effect will also lead to a preference for a particular base. Further research is necessary to examine this in detail.

*The small difference in the pK_a values as measured by us and Martin *et al.* is probably related to the different concentrations used in the two studies, i.e. 6.7×10^{-5} and 0.1 M, respectively.

Acknowledgements

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References

1 J. Reedijk, A. M. J. Fichtinger-Schepman, A. T. van Oosterom and P. van de Putte, *Struct. Bonding (Berlin), 67* (1987) 53.
2 A. C. M. Plooy, A. M. J. Fichtinger-Schepman, H. H. Schutte, M. van Dijk and P. H. M. Lohman, *Carcinogenesis, 6* (1985) 561.
3 A. M. J. Fichtinger-Schepman, J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry, 24* (1985) 707.
4 J.-P. Girault, G. Chottard, J.-Y. Lallemand and J.-C. Chottard, *Biochemistry, 21* (1982) 1352.
5 K. Inagaki, K. Kasuya and Y. Kidani, *Chem. Let.*, (1983) 1345.

6 A. T. M. Marcelis, C. Erkelens and J. Reedijk, *Inorg. Chim. Acta, 91* (1984) 129.
7 S. Eapen, M. Green and I. M. Ismael, *J. Inorg. Biochem., 24* (1985) 233.
8 D. J. Evans, N. R. Ford and M. Green, *Inorg. Chim. Acta, 125* (1986) L39.
9 J. L. van der Veer, H. van den Elst, J. H. J. den Hartog, A. M. J. Fichtinger-Schepman and J. Reedijk, *Inorg. Chem., 25* (1986) 4657.
10 B. Van Hemelryck, J.-P. Girault, G. Chottard, P. Valadon, A. Laoui and J.-C. Chottard, *Inorg. Chem., 26* (1987) 787.
11 J. L. van der Veer, G. A. van der Marel, H. van den Elst and J. Reedijk, *Inorg. Chem., 26* (1987) 2272.
12 A. Laoui, J. Kozelka and J.-C. Chottard, *Inorg. Chem., 27* (1988) 2751.
13 K. H. Scheller, V. Scheller-Krattiger and R. B. Martin, *J. Am. Chem. Soc., 103* (1981) 6833.
14 G. Watt and W. Cude, *Inorg. Chem., 7* (1968) 335.
15 A. T. M. Marcelis, G. W. Canters and J. Reedijk, *Recl. Trav. Chim. Pays-Bas,* (1981) 391.
16 J.-C. Chottard, J.-P. Girault, G. Chottard, J.-Y. Lallemand and D. Mansuy, *J. Am. Chem. Soc., 102* (1980) 5565.
17 J.-P. Girault, J.-C. Chottard, E. R. Guittet, J.-Y. Lallemand, T. Huynh-Dinh and J. Igolen, *Biochem. Biophys. Res. Commun., 109* (1982) 1157.
18 J.-Y. Lallemand, J. Soulie and J.-C. Chottard, *J. Chem. Soc., Chem. Commun.,* (1980) 436.
19 G. Admiraal, J. van der Veer, R. A. G. de Graaff, J. H. J. den Hartog and J. Reedijk, *J. Am. Chem. Soc., 109* (1987) 592.
20 S. E. Sherman, D. Gibson, A. H. J. Wang and S. J. Lippard, *J. Am. Chem. Soc., 110* (1988) 7368.
21 C. S. Fouts, L. G. Marzilli, R. A. Byrd, M. F. Summers, G. Zon and K. Shinozuka, *Inorg. Chem., 27* (1988) 366.
22 J. D. Atwood, *Inorganic and Organometallic Reaction Mechanisms,* Brooks/Cole, Monterey, CA, 1985.
23 E. L. M. Lempers, M. J. Bloemink, J. Brouwer, Y. Kidani and J. Reedijk, *J. Inorg. Biochem.,* (1990) in press.