The Influence of the 5′-Nucleotide on the Binding of [PtCl(dien)]Cl to the Dinucleotides d(HpG), as Studied by Competition Experiments

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Abstract

The binding of the monofunctional platinum compound [PtCl(dien)]Cl to the dinucleotides d(HpG), with H = A, C or T, has been studied separately and in competition experiments. When using excess of d(HpG), only platinum coordination to the N7 of the 3′-guanine is observed, as deduced from the chemical shift of the non-exchangeable nucleobase protons and their pH dependence. Quantitation results obtained by proton NMR from the competition experiments indicate that the platinum compound does not react at random with the dinucleotides. A kinetic effect of the 5′-nucleotide on the binding to the guanine appears to be present. The relative reaction rate of the platinum compound with the dinucleotides decreases in the series: d(CpG), d(TpG) and d(ApG) (binding ratio 4:3:2).

Introduction

The interest in platinum–DNA interactions stems mainly from the antineoplastic activity exerted by certain platinum compounds. This biological activity is believed to be related to platinum interactions with the cellular DNA [1–3].

Platinum compounds appear to have a strong preference for guanine residues [4]. It is known that *in vitro* [5], at least four different platinum adducts can be formed with the well-known drug *cis*-$PtCl_2(NH_3)_2$ (cisplatin) for which in all cases guanine residues are involved in the binding: the intrastrand GG-chelate (most abundant), the AG-chelate, an adduct in which cisplatin is bound to two non-neighboring guanines and a monofuctional adduct of cisplatin with one bound guanine residue. The reaction of cisplatin with DNA is thought to occur via a two-step mechanism [6]. The first binding should occur exclusively with a guanine residue. The subsequent binding step – if any – should take place, largely with a guanine or to a smaller extent with an adenine residue. This hypothesis fully agrees with the above-mentioned *in vitro* results.

The first binding step of cisplatin to DNA does not seem to occur with a randomly present guanine. The high yield of GG-chelate (65%) found in the mentioned quantitation study [5] was far more than could be expected for statistical reasons (37%). This result suggests a kind of directing effect of cisplatin to GG-sequences in the DNA. This led us to see whether a similar kind of directing effect for the first binding step might also be present due to flanking DNA sequences. The simple model system of d(HpG) dinucleotides (H stands for A, C or T) has been chosen for this study. To mimic the first binding step, the monofunctional platinum compound [PtCl(dien)]Cl has been used. The results of these competition experiments are described below.

Materials and Methods

Starting Materials

The dinucleoside-monophosphates d(ApG), d(CpG) and d(TpG) were synthesized via an improved phosphotriester method [7], and used as sodium salts. The platinum compound [PtCl(dien)]Cl (dien: diethylenetriamine) was prepared according to Watt and Cude [8].

Competition Experiments

Equimolar amounts of two d(HpG) dinucleotides (about 3×10^{-6} mol; as determined by UV absorption) were incubated together with 0.6 equivalent of [PtCl(dien)]Cl in 300 ml doubly distilled water in the dark at pH 7.0 and 37 °C. The molar absorptions for d(ApG), d(CpG) and d(TpG) were estimated as 25 000, 19 000 and 19 500, respectively, at pH 7.0 and 254 nm. The three reactions were monitored by UV spectroscopy (Perkin-Elmer EPS-3T). After one week, the reaction mixtures were concentrated and lyophylized. A competition experiment in which equimolar amounts of the three dinucleotides were incubated with 1.2 equivalents of the platinum compound (*i.e.* 0.3 equivalents per dinucleotide, just as

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for the above-mentioned competition reactions) was carried out as described above. For reference purposes, the three dinucleotides were reacted each with a stoichiometric amount of [PtCl(dien)]Cl. After proton NMR measurements, the latter reference compounds were separated by gel filtration (Sephadex G25, Pharmacia) from the unreacted dinucleotide, and investigated again by proton NMR.

NMR Measurements

To the lyophylized samples, 0.5 ml D_2O (99.5%; Merck) was added. After adjusting the pH to 5.0 (uncorrected meter reading), a trace amount of tetramethyl ammonium nitrate (TMA, 3.18 ppm downfield from DSS) was added and the samples were lyophylized again. They were eventually dissolved in 0.4 ml D_2O (99.95%; Merck). Proton NMR spectra were recorded on a Bruker WM 300 NMR spectrometer, at 300 K. The spectra used for the quantitations were recorded with 16 K datapoints, using 1.8 s acquisition time. The quantitation of the products and the starting materials was performed by determination of the peak area of certain nucleobase proton signals. This determination was performed by electronic integration and by weighting peak areas of expanded resonances.

Nomenclature

The notation of the chain direction and the abbreviations is according to the recommendations [9, 10] of the IUPAC–IUB Joint Commission on Biochemical Nomenclature (JCNB), *i.e.* the dinucleotides are notated sequentially from the left to the right from the 5′-OH to the 3′-OH.

Results and Discussion

In Fig. 1, the structure of the four common nucleobases with the recommended numbering system is schematically depicted [9]. The non-exchangeable base protons which resonate in the aromatic part of the proton NMR spectrum are underlined. The possible sites for platinum binding to these nucleobases under the conditions used in this study are: guanine N7, adenine N1 and N7 and cytosine N3. Platinum coordination to these sites can induce changes in the chemical shift of nearby bound protons, up to 1 ppm [11].

The stoichiometric reaction at pH = 7 and 37 °C of the monofunctional platinum compound [PtCl-(dien)]Cl (see Fig. 2) with the separatedly incubated dinucleotides appeared to be complete within 5 days. After that period, changes in the UV absorption spectrum could no longer be observed. Proton NMR spectra of the crude reaction mixtures gave evidence for one reaction product, in addition to the starting

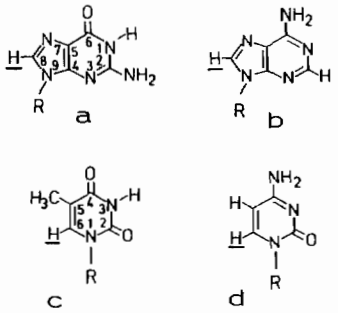

Fig. 1. Representation of the four common nucleobases (a) guanine, (b) adenine, (c) thymine and (d) cytosine which are present in DNA. The numbering convention [9] is depicted for the purines in guanine and for the pyrimidines in thymine. R denotes the attached deoxyribose moiety. The nucleobase protons that are used for the quantitation experiments are underlined.

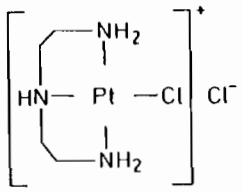

Fig. 2. Structure of the monofunctional platinum compound [PtCl(dien)]Cl.

dinucleotide. Gel permeation chromatography resulted indeed in the separation of two UV absorbing peaks, *i.e.* [Pt(dien)] [d(HpG)-*N*7(2)] and unreacted d(HpG).

The binding of the Pt(dien) moiety to the N7 of guanine was first indicated by the large chemical shift change of the guanine H8 proton signal (about 0.5 ppm downfield), which was less for the other nucleobase protons (about 0.2 ppm). This tentative assignment was confirmed by pH dependent chemical shift data of the non-exchangeable base protons (not shown). The spectra recorded at both pH 5.0 and 2.0 showed no large chemical shift difference for the H8 of the guanine. This can be expected for guanine residues which are platinated at the N7 site. For the unplatinated residues, an N7 protonation can be expected at pH 2.0, which induces a downfield shift of the guanine H8 proton [11]. This guanine N7 binding fashion was expected from the described preference of platinum compounds for guanine residues [4]. This preference seems to be even enhanced by the presence of a 5′-phosphate group [12], which is absent for the nucleobase H in the dinucleotide d(HpG). The chemical shift data of these stoichiometric reactions appeared to be very valuable for assigning the resonances in the spectra of the competition reactions.

The competition reactions appeared also to be complete within 5 days, as deduced from the UV

absorption spectra of the solutions. In these three reactions, stoichiometric amounts of two dinucleotides, *i.e.* d(ApG)/d(CpG), d(ApG)/d(TpG) and d(CpG)/d(TpG) were incubated with 0.6 equivalent of [PtCl(dien)]Cl. After the lyophilization, proton NMR spectra were recorded immediately after the second dissolution of the reaction mixture in D_2O. The aromatic region of the NMR spectrum of the competition reaction in which d(CpG) and d(TpG) were incubated is redrawn in Fig. 3.

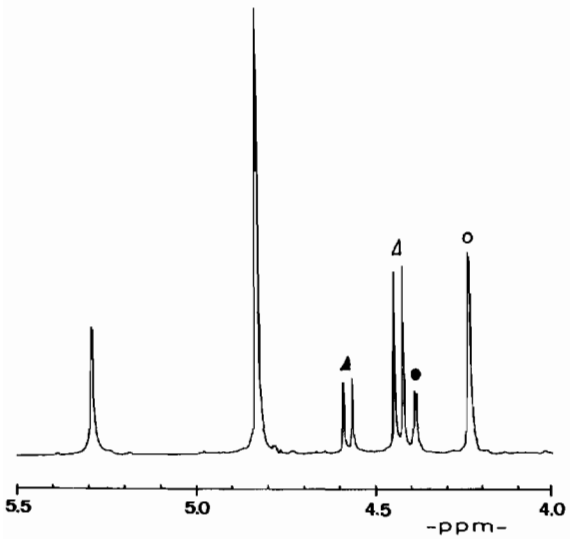

Fig. 3. Aromatic part of the 300 MHz spectrum of the competition mixture, containing an equimolar amount of d(CpG)/d(TpG) after the reaction with 0.6 equivalent of [PtCl(dien)]Cl. The resonances used for the integrations are indicated by open and closed figures for the unbound and the bound dinucleotide, respectively (C-H6: △/▲; T-H6: ○/●).

The fact that platinum binding to the guanine residues did not only influence the chemical shift of the H8 of these guanines, but also of the nucleobase protons of the neighboring nucleotide H, allowed integration of these proton resonances. Integration of the guanine H8 signal is not advisable, since the H8 proton is susceptible to exchange with deuterium [13], which can introduce errors in the calculations. For the d(CpG)/d(TpG) competition, moreover, the guanine H8 resonances strongly overlap, both for the platinated and for the unreacted dinucleotides (see Fig. 3). Therefore the cytosine and thymine H6 resonances were used for the integrations. For the competition experiments with d(ApG), the adenine H2 signal was integrated. The discrimination between the H8 and the H2 signal of adenine was based upon the fact that in an unplatinated adenine, the H8 proton resonates invariably at lower field than the H2 proton [14, 15].

The areas of the mentioned resonances were determined both by electronic integration and by weighing of the expanded signals. The difference between these two methods fell within experimental error (5%). The percentage of binding (P_x) of [PtCl(dien)]Cl to a certain dinucleotide X in the competition reaction with the dinucleotide Y was calculated using the formula

$$P_x\,(\%) = 100(P(X)/[P(X) + P(Y)])$$

In this formula, P(X) stands for the percentage of bound dinucleotide X, which is determined by the formula:

$$P(X)\,(\%) = 100(A_b(X)/[A_b(X) + A_u(X)])$$

$A_b(X)$ and $A_u(X)$ denote the integrated areas of the resonances of the bound and the unbound dinucleotide X, respectively. The percentages of the three different competition experiments are given in Table I. These values are averaged from three experiments, giving an experimental error below 5%.

It can be seen that the 5′-neighboring nucleobase has indeed an effect on the binding of [PtCl(dien)]Cl to the 3′-guanine. The binding rate of the platinum compound to the dinucleotide decreases in the row: d(CpG), d(TpG) and d(ApG). It should be noted that the percentages depicted in Table I are consistent among themselves. This means that the percentages of a third competition reaction, can be calculated from the other two competitions. This makes secondary effects upon the platination rate, due to for example intermolecular stacking between the dinucleotides, less likely. In another experiment, equimolar amounts of the three dinucleotides were incubated with 1.2 equivalent of [PtCl(dien)]Cl under identical conditions as described above for the other competitions. The quantitation results from this competition (not shown) also agrees with the data from Table I.

TABLE I. Quantitation Results of the Competition Experiments Between the d(HpG) Dinucleotides

Competition of	Percentage of bound dinucleotide		
	d(ApG)	d(CpG)	d(TpG)
d(CpG)/d(TpG)	–	58	42
d(ApG)/d(TpG)	40	–	60
d(CpG)/d(ApG)	33	67	–

From these results, it can be concluded that kinetic effects upon the binding of platinum compounds to DNA as a result of neighboring sequences are indeed possible. It should be noted, however,

that the results of these quantitations cannot be extrapolated immediately to larger DNA fragments. It is known that a 3'-terminal nucleotide in an oligonucleotide behaves differently from the other nucleotides. Moreover, the intramolecular stacking in the dinucleotides will be much smaller than when they are incorporated into larger, double-stranded DNA fragments. Nevertheless, the results obtained in this dinucleotide system are expected to be helpful for future studies to understand the binding directing effect which are involved in platinum DNA interactions and which cause preferential binding of *cis*-Pt to GG-sequences.

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