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Conformational analysis of the adduct *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$ in aqueous solution. A high field (500-300 MHz) nuclear magnetic resonance investigation

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ABSTRACT A 500, 400 and 300 MHz proton NMR study of the reaction product of cis-$Pt(NH_3)_2Cl_2$ or cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ with the deoxydinucleotide d(GpG): cis-$[Pt(NH_3)_2\{d(GpG)\}]^+$ was carried out. Complete assignment of the proton resonances by decoupling experiments and computer simulation of the high field part of the spectrum yield proton-proton and proton-phosphorus coupling constants of high precision. Analysis of these coupling constants reveal a 100% N (C3'-endo) conformation for the deoxyribose ring at the 5'-terminal part of the chelated d(GpG) moiety. In contrast, the 3'-terminal -pG part of the molecule displays the normal behaviour for deoxyriboses: the sugar ring prefers to adopt an S (C2'-endo) conformation (about 70%). Extrapolating from this model compound, it is suggested that Pt chelation by a -dGpdG- sequence of DNA would require a S to N conformational change of one deoxyribose moiety as the main conformational alteration and lead to a kink in one strand of the double-helical structure of DNA.

INTRODUCTION[1]

The mechanism of action of the antitumor drug cis-$Pt(NH_3)_2Cl_2$ (cisPt) has been the subject of a large number of investigations[2,3]. A specific binding to DNA is supposed to be important for the observed antineoplastic activity of this compound[3]. The kinetically most favoured binding sites are known to be the N7 atoms of the guanine bases[4]. Interstrand crosslinks by cisPt appears to be a rare event and not specific for cisPt, since the inactive trans isomer is also able to form interstrand crosslinks[5]. Intrastrand crosslinking by cisPt of adjacent guanine bases is considered to be most likely[6]; recently it became evident that such a binding is possible in vitro in di- and in tetranucleotides[7,8]. Intrastrand crosslinking between adjacent guanines was also proposed to account for the gel-electrophoretic patterns obtained after BamHI[9] or exonucleaseIII[10] digestion of platinated DNA and for the selective inhibition of a cutting site of platinated pSM1 DNA by a restriction endonuclease[11].

A detailed conformational analysis of d(GpG)cisPt (a schematic structure is given in Figure 1) is of considerable interest. Thus it may provide a firm

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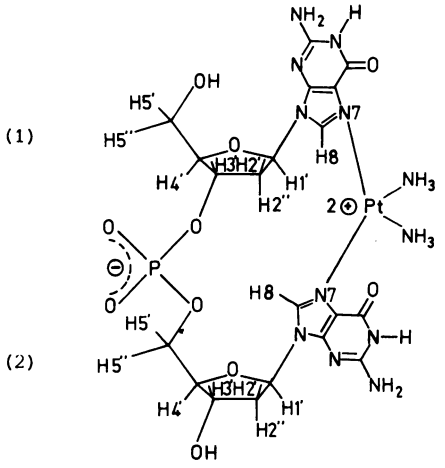

Figure 1. Schematic structure of d(GpG)cisPt. Please note that the orientation of the bases has no physical meaning.

basis for a deeper insight in the structural changes in DNA caused by this specific binding of cisPt. Nuclear Magnetic Resonance (NMR) spectroscopy is a very powerful physical technique to acquire information about a molecule in solution. This technique can yield intimate geometrical details of backbone and sugar rings by means of an analysis of the proton-proton and proton-phosphorus coupling constants. In addition, chemical shift differences due to increasing concentration and/or temperature can yield information about association and base stacking equilibria[12]. It should be stressed that in this particular case the stacking interaction between the guanine bases, chelated by cisPt, will be seriously diminished due to the fact that the planes of the bases cannot be parallel but are at angles between 45° and 90°, as has been found in several crystal structures of cis-bis(nucleotide)Pt complexes[13].

Up to date little attention has been devoted to the conformational changes in the sugar moieties of nucleotides coordinated to platinum compounds. Marcelis et al.[14] and Polissiou et al.[15] noted that the conformation of the riboses of 5'GMP coordinated to cisPt or $PtCl_4^{2-}$ can shift in the direction of the N conformation. Earlier work by some of us showed that chelation of cisPt by IpI, ApA, GpG, d(GpG) and d(pGpG) is accompanied by the disappearence of one H1'-H2' coupling constant, suggesting an N conformation for one furanose ring, supposed to be the 5' one from examination of CPK models[8]. However, no quantitative conclusions were drawn from these observations.

Recently a number of studies on several oligonucleotides were published[16-18]. The quantitative interpretation of the coupling constant data in

terms of N and S[19,20] and of C4'-C5' conformational populations was greatly improved by the use of a generalized Karplus equation[21-23]. This equation allows an explicit correction for the electronegativity and orientation of electronegative substituents. The present paper deals with a detailed conformational analysis of d(GpG)cisPt using high-field NMR techniques and purports to show that quantitative conclusions concerning several torsion angles of the backbone of the molecule can be drawn from the vicinal coupling constants.

CONFORMATIONAL NOTATION

The recently introduced α-ζ notation, recommended by IUPAC-IUB[24] for descriptions of conformations of polynucleotide chains is used throughout this paper. Torsion angles are labeled α to ζ (see Figure 2) in the direction of the polynucleotide chain starting with α at the P-O5' bond. The residue to which a given torsion angle belongs will be indicated between parentheses. Residues are numbered starting at the 5'-terminus of the oligonucleotide (see Figure 1). E.g., γ(2) denotes the C4'-C5' torsion angle of the -pG fragment in d(GpG). The conformation of the sugar ring will be described by the parameters P, the phase angle of pseudorotation and ψ (previously called τ), the amplitude of pucker[19,20]. We distinguish between N-type ($P=0^{o} \pm 90^{o}$, roughly C3'-endo) and S-type conformers ($P=180^{o} \pm 90^{o}$, roughly C2'-endo). This nomenclature adheres to the backbone notation system. The numbering is in the usual 5'OH → 3'OH direction. A clockwise torsional rotation of the rear backbone atom with respect to the front one is defined as positive, taking the eclipsed situation as the zero angle. The three possible rotamers along for example C4'-C5' of the Gp- fragment will be described as $\gamma^{+}(1)$, $\gamma^{-}(1)$

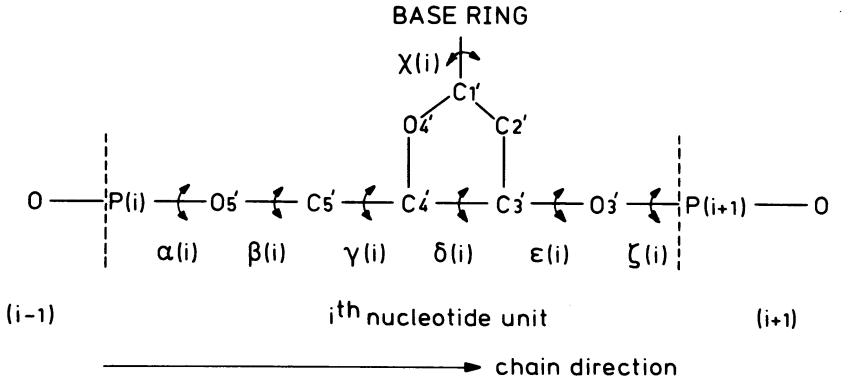

Figure 2. Conformational notation.

and γ^t(1) for respectively the gauche plus, gauche minus and the trans conformer. The glycosyl angle χ is defined by O4'-C1'-N9-C4.

MATERIALS AND METHODS

d(GpG) was synthesized by an improved phosphotriester method[25]. After purification the compound was treated with a Chelex-100 cation exchange resin to yield the sodium salt. CisPt was prepared by an established method[26]. The synthesis of the title compound followed the method of Chottard[8]: d(GpG) was reacted with one equivalent of cisPt (concentration 10^{-4} M, pH 6-6.5) for two weeks at room temperature. The reaction was followed by U.V. spectroscopy[27] and the product was used without any further purification, so the investigated compound is cis-[$Pt(NH_3)_2${d(GpG)}]Cl.NaCl. ^{1}H-NMR preliminary data were collected at 400 MHz and 300 MHz on a Bruker WM-400, -300 respectively. The compound was lyophilized three times from 99.75% ^2H_2O after adjustment of the p^{2}H to 6.5 and then redissolved in 99.95% ^2H_2O. Solutions of the samples were prepared in concentrations of 20, 10 and 2 mM. A trace of tetramethylammoniumchloride (TMA) was added to the samples as an internal reference (chemical shift relative to DSS is 3.18 ppm at 25°C). ^{1}H-NMR spectra were recorded at 500 MHz on a Bruker WM-500 spectrometer. The free induction decay signals (400-2000 accumulations, quadrature detection) were averaged on a Bruker Aspect-2000 data system, using spectral windows of 5000 Hz with 32k datapoints. Resolution enhancement was achieved by means of a Gaussian window technique according to Ernst[28]; resulting linewidths were about 1.0 Hz. ^{1}H-NMR spectra of d(GpG)cisPt were recorded at probe temperatures of 23°, 43°, 53° and 81°C for all concentrations and also at 60° and 70°C for the 10 mM sample. Extensive homonuclear decoupling experiments were carried out to assign the high-field deoxyribose signals (vide infra). Chemical shift and coupling-constant data of the 500 MHz spectra were obtained by simulation of the high-field spectral region using an expanded version of the computer program LAME[29]. ^{31}P-NMR spectra were recorded on a Bruker WM-300 spectrometer operating at 121.5 MHz, interfaced with a Bruker Aspect-2000 (16k datapoints) for data accumulation. The spectrometer was field frequency locked on the ^{2}H resonance of ^2H_2O, used as a solvent. Heteronuclear proton noise decoupling was used throughout. Spectra were recorded at several spectrometer setting temperatures. Tetramethylphosphonium bromide (TMPB) was used as an internal reference, shifts are reported relative to cyclic AMP[30].

RESULTS AND DISCUSSION

Spectral assignment :

For the base protons in the low-field region of the spectrum the earlier assignment of Chottard et al.[8] agrees well with the present results.

The high-field region shows fourteen proton signals, those around 0.64 and 0.37 ppm downfield from TMA are the characteristic AB part of an ABX pattern of the H5'(1) and the H5"(1) of the 5' terminal part of the molecule. The connection between chemical shifts and stereochemistry was made according to Remin and Shugar[31]. The assignment of the remaining signals was performed using extensive decoupling experiments and each vicinally coupled pair of protons could be unambiguously identified. The assignment of the H2' and H2" protons followed from coupling-constant considerations[20,30].

Figure 3 shows the high-field protons of d(GpG)cisPt at 70°C. The excellent agreement between the observed and the simulated spectra suggest that the chemical shift data (Table 1) are accurate to 0.002 ppm. The coupling

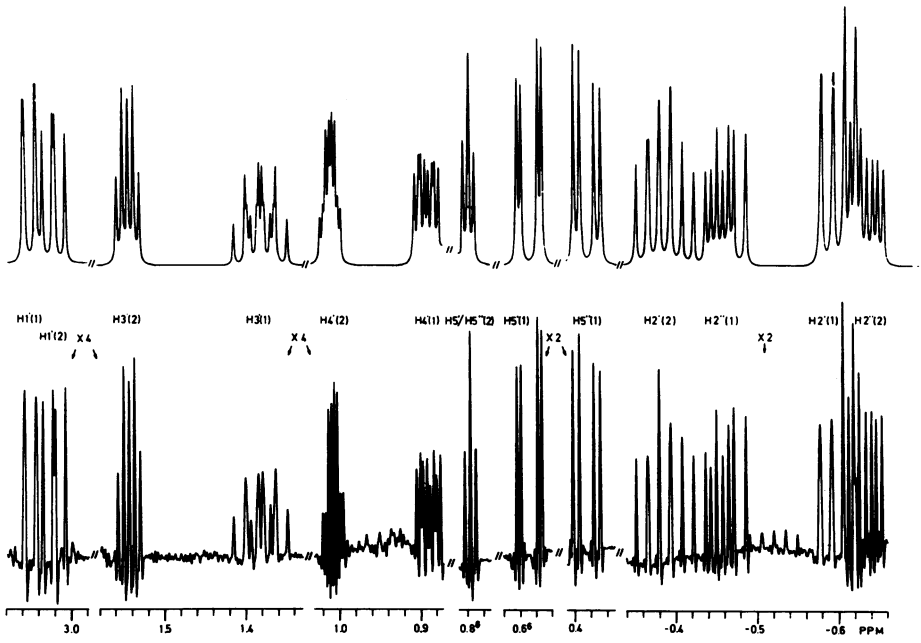

Figure 3. Deoxyribose resonances of the resolution enhanced 500 MHz NMR spectrum of d(GpG)cisPt ,10 mM, 70°C, with assignment (lower trace) and the corresponding computer simulated spectrum (upper trace). In order to obtain a relative intensity of the same magnitude the signals to the left of H5'/H5"(2) are multiplied by four, the signals to the right by two. Shifts are reported relative to TMA (δ = 3.18 ppm to DSS).

Table 1: Chemical shifts in ppm relative to TMA (δ = 3.18 ppm to DSS) of d(GpG)cisPt, 10 mM in ^2H_2O, p^{2}H = 6.5, recorded at 500 MHz

Proton	23°C Gp-	53°C	81°C	23°C -pG	53°C	81°C
1'	3.006	3.054	3.055	3.042	3.030	3.021
2'	-0.562	-0.586	-0.603	-0.468	-0.389	-0.368
2"	-0.445	-0.449	-0.456	-0.624	-0.623	-0.628
3'	1.443	1.396	1.377	1.544	1.550	1.546
4'	0.893	0.897	0.891	1.049	1.019	1.002
5'	0.621	0.648	0.652	0.863	0.862	0.859
5"	0.340	0.381	0.392	0.863	0.862	0.859
8	5.087	5.042	5.007	5.392	5.288	5.241

constant data (Table 2) are assumed to be accurate to 0.1 Hz unless indicated otherwise. ^{31}P-chemical shift values are given in Table 3.

Conformational analysis :

Earlier work[8] has shown that d(GpG) coordination to cisPt occurs through N7-N7 chelation and that both guanine bases have an anti configuration, corresponding to a head-to-head arrangement.

Deoxyribose conformations: Pseudorotation analysis of the two sugar rings in d(GpG)cisPt was performed in a standard Newton-Raphson least-squares minimalization procedure[23] (Program PSEUROT, de Leeuw, F.A.A.M., forthcoming thesis, Leiden). Five necessary parameters (ψ_N,P_N,ψ_S,P_S,X_N) were calculated, P and ψ denote the phase angle of rotation and the puckering amplitude[19,20], X_N is the mole fraction of the N-type conformer. The usual assumption was made that the geometrical parameters are independent of temperature between 10° and 90°C. The results are shown in Table 4.

Pilot calculations strongly indicate that the Gp- deoxyribose ring adopts a single N conformation in the whole temperature range, so X_N was constrained to 1.0. In order to estimate accuracy ψ_N was constrained to values ranging from 32° to 43° at intervals of 1° and the corresponding P values and root-mean-square (r.m.s.) deviations were computed. The final results, ψ_N = 38° and P_N = -1°, agree well with the parameter range deduced from an extensive amount of solid state data[33]. Apart from some cyclic phosphates with highly puckered deoxyribose rings (ψ = 43°, P = 32°, see ref 23),

Table 2: Coupling constants of d(GpG)cisPt in Hz, 10 mM in ^2H_2O, p^2H = 6.5, recorded at 500 MHz

Couple	23°C Gp-[a]	53°C	81°C	23°C -pG	53°C	81°C
1'2'	0.6	0.7	0.6	8.1	7.8	7.6
1'2"	7.3	7.3	7.2	6.0	6.2	6.3
2'2"	-13.9	-14.1	-14.1	-13.6	-13.8	-13.9
2'3'	6.8	7.1	7.1	6.1	6.7	6.8
2"3'	10.7	10.5	10.5	2.9	3.3	3.6
3'4'	8.1	8.3	8.1	3.2	3.4	3.6
3'P	7.0	7.0	7.2	-	-	-
4'5'	2.7	2.5	2.5	2.9 [b]	3.5 [b]	3.6 [b]
4'5"	3.4	3.9	4.1	2.9 [b]	3.5 [b]	3.6 [b]
4'P	-	-	-	2.8	2.1	1.8
5'5"	-12.8	-12.8	-12.8	x	x	x
5'P	-	-	-	3.2 [b]	3.3 [b]	3.5 [b]
5"P	-	-	-	3.2 [b]	3.3 [b]	3.5 [b]

(a) Estimated accuracy of the 3'P coupling constant is 0.3 Hz
(b) H5' and H5" are practically isochronous; only the sum of the coupling constants H4'H5' + H4'H5" and H5'P + H5"P can be obtained.

this study annotates the first pure N conformation of a deoxyribose ring in solution.

The -pG deoxyribose ring appears to exist in an N-S equilibrium. At three representative temperatures X_N was calculated while also P_N and P_S were refined. ψ_N and ψ_S were constrained ($\psi_N = \psi_S$) and the same procedure (vide supra) was followed. Pilot calculations showed that ψ_N and ψ_S are strongly correlated and the r.m.s. deviation did not decrease when one or both para-

Table 3: ^{31}P chemical shift data of d(GpG)cisPt relative to cyclic AMP, 20 mM in ^2H_2O, p^2H = 6.5.

chemical shift (δ) in ppm.					
Temperature	7°	27°	37°	57°	70°
chemical shift	1.46	1.35	1.29	1.31	1.33

Table 4: Pseudorotation parameters and mole fraction of N conformers in d(GpG)cisPt. The data of d(GpG) (unpublished results, for method see ref. 23) are shown for comparison, the standard deviation is given in parentheses.

Parameter deoxyribose	ψ_N	P_N	ψ_S	P_S	overall r.m.s. (Hz)
Gp-.cisPt	38(2)	-1(7)	-	-	0.38
-pG.cisPt	34(2)	-1(6)	34(2)	149(2)	0.12
Gp-	38[a]	0[a]	37(1)	165(4)	0.10
-pG	35[a]	0[a]	34(1)	155(3)	0.02

Mole fractions of N conformer				
Temperature	23°	34°	43°	81°
Gp-.cisPt	100[b]		100[b]	100[b]
-pG.cisPt	23(2)		25(2)	29(2)
Gp-		11(1)		
-pG		37(1)		

a) Constrained value, see ref. 23
b) Constrained value, see text.

meters were included in the iteration. The results, $\psi_N = \psi_S = 34^o$ and $P_N = -7^o$, $P_S = 149^o$ are within the range observed in crystal structures of nucleic acid constituents[33], although both P values of the present compound are about 15^o smaller than the average P values. The lower values of ψ agree with a proposed effect of a 5' phosphate[33]. Contrary to the Gp- deoxyribose ring the conformational freedom appears to be quite large for the -pG deoxyribose ring and for the C4'-C5' bond. Such a freedom has been reported before for the 3'end of free oligonucleotides[17], but it is surprisingly large in this "constrained" chelating dinucleotide.

Conformation around C4'-C5': $\gamma(1)$ and $\gamma(2)$: The population distribution of the three classical rotamers ($\gamma^+, \gamma^-, \gamma^t$) around the C4'-C5' bond can be easily calculated from the observed coupling constants J4'5' and J4'5" by using the limiting coupling constants reported by Haasnoot et al.[21]. This approach yields for $\gamma(1)$ an increasing population $\gamma^t(1)$ with increasing temperature at the expense of $\gamma^+(1)$ and a negligible amount $\gamma^-(1)$ (see Table 5). In those cases where only the sum Σ of J4'5' and J4'5" can be obtained from experiments (because of the accidental chemical shift equivalence of H5' and H5") the γ^+ population is calculated with the approximate[18] sum rule, eqn. (1)

Table 5: Population distribution (%) along the backbone angles γ and β at several temperatures.

Torsion angle	γ(1)[a]	γ(2)[b]	β(2)
Conformer	γ^+	γ^+	β^t
Temperature			
23°	77	79	93
53°	72	67	92
81°	70	65	89

a) Percentage trans isomer is respectively 23, 28 and 30%.
b) In the applied sum rule the value of γ(2) amounts to 52° (the average value from crystal structure data), calculations with γ(2) = 58° (from the model) resulted in the same rotamer population distributions.

$$\gamma^+ = (13.75 - \Sigma)/10.05 \qquad (1)$$

Along γ(2) the amount of $\gamma^+(2)$ decreases with increasing temperature which indicates more conformational freedom at higher temperatures (see Table 5, second entry).

Conformation around C5'-O5': β(2), and C3'-O3': ε(1): Proton-phosphorus coupling constants are converted into conformational information by means of an approximate Karplus equation, proposed by Altona[34], eqn. (2)

$$^3J_{HP} = 17.6 \cos^2\theta - 3.8 \cos\theta \qquad (2)$$

where θ is the proton-phosphorus torsion angle. To compute the fractional population β^t of the preferred trans rotamer along β a sum rule can be extracted assuming a classical rotamer model, eqn. (3)

$$\beta^t = (23.9 - \Sigma')/18.9 \qquad (3)$$

where $\Sigma' = J_{5'P} + J_{5''P}$. The calculation (Table 5) yields a 93% pure trans conformation. The relatively large long-range coupling constant H4'-P (2.8 Hz at 26°C) also indicates a large conformational purity around β(2).

Along the C3'-O3' bond the ε^+ conformation can be a priori excluded from X-ray data on related compounds[33]. As is explained elsewhere[36,37] the experimentally observed values of $^3J_{H3'P}$ do not allow an unambiguous distinction between ε^t ($\varepsilon \simeq 210°$) and ε^- ($\varepsilon \simeq 280°$) conformers. In this case an $\varepsilon^-(1)$ conformation seems very unlikely because of the very small nonbonded distance H5'(1).OP (approximately 1.5 Å, vide infra), which would occur because of the N conformation of the deoxyribosering. Therefore, an $\varepsilon^t(1)$ conformation is expected, in this case an ε(1) of ∿198° may be computed from the proton-

phosphorus coupling constant.

Conformation around P-O3': ζ(1), and P-O5': α(2): Unfortunately the torsion angles ζ and α cannot be determined from coupling constants. From ^{31}P-NMR shift data a distinction can be made between $\zeta^{t}\alpha^{+/-}$ or $\zeta^{+/-}\alpha^{t}$ and $\zeta^{+/-}\alpha^{+/-}$ conformers, because of the expected large deshielding effect of a trans conformation[38]. Compared to the signals in e.g. d(CpCpGpG)[39] the phosphorus NMR signal in d(GpG)cisPt (Table 3) is shifted slightly (0.2 ppm) to low field which strongly suggests no trans conformation around ζ(1) and α(2). Model building shows that only the $\zeta^{-}(1)\alpha^{-}(1)$ combination is allowed because of the constraints imposed by the Pt bridge.

Chemical shift considerations :

In order to distinguish between associative shift effects and internal temperature dependent shift effects, temperature-concentration profiles were obtained. Table 6 represents the protons that show chemical-shift effects larger than 0.01 ppm. The temperature-concentration profiles of both H2' and H2" protons are shown in Figure 4 as an example. H2'(2) exhibits only an internal shift effect in contrast to H2'(1) which shows a concentration and a temperature-dependent effect.

Associative shift effects: Both sites of d(GpG)cisPt are likely to show intermolecular association behaviour. The large shift effect on H1'(1) is ascribed to association at G(1) (the "A-side"). The H8(1) does not show any association shift effect, so probably the association is limited to the six-membered ring of the guanine residue. This effect may be rationalized on the basis of steric effects: hindrance due to an NH_3 at the platinum. Association

Table 6: Differential shieldings in ppm, larger than 0.01 ppm, observed for d(GpG)cisPt. Differences due to decreasing association: δ(2 mM) - δ(20 mM) were observed at 23°C, differences due to decreasing temperature: δ(81°C) - δ(23°C) at 10 mM. Positive values denote an upfield shift.

proton	1'	2'	2"	3'	4'	5'	5"	8
associative shift								
Gp-	0.11	0.03	-	-	-	-	-	-
-pG	-0.01	-	-	-0.01	-	-	-	-0.05
Internal shift								
Gp-	-	-0.05	-	-0.07	-	0.03	0.05	-0.10
-pG	-0.01	0.10	-	-	-0.05	-	-	-0.15

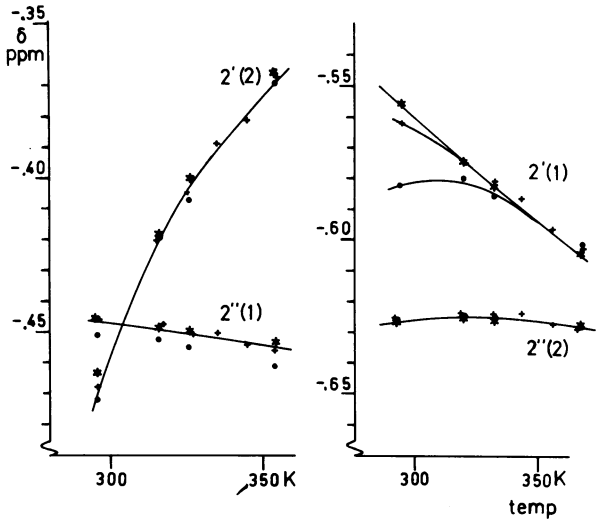

Figure 4. Temperature-concentration profiles of H2' and H2" protons. Solid lines connect the data points obtained at 10 mM for H2'(2), H2"(1) and H2"(2). H2'(1) shows a clear temperature-concentration dependence; legend: • : 20 mM, + : 10 mM, * : 2 mM. Shifts are reported relative to TMA.

at the B-side of the molecule (at G(2)) is less strong or has less influence upon the NMR spectrum. The H8(2) chemical shift change amounts to only 0.05 ppm and changes for H1'(2) and H3'(2) are much smaller. Noteworthy is the opposite influence at the chemical shifts due to association at the two sites of the molecule. This phenomenon also occurs in $m^6_2Apm^6_2A$ (Olsthoorn, C.S.M. and Altona, C. unpublished results) where the H2'(2) resonance exhibits deshielding due to association.

Internal shift effects: Proton chemical-shift changes in deoxyribonucleotides are usually ascribed to changes in conformational composition leading to different shielding or deshielding influences of the aromatic bases and of the oxygen atoms[40]. In our particular case the effects exerted by the bases are supposedly constant. Significant rotations about the glycosyl angles (χ) or large changes in the average Pt-N7 torsion angles are considered unlikely, because neither H1'(1), H1'(2), H2"(1) nor H2"(2) show more than marginal internal chemical shift changes with decreasing temperature.

The H8(2) proton in d(GpG)cisPt shows different chemical shift change with decreasing temperature compared to base protons in non-complexed oligonucleotides. Base protons of the 3'-terminal residue display shielding effects due to stacking interactions. In contrast, the H8 protons of the 5'-terminal residue in $m^6_2Apm^6_2A$ and in m^6_2ApU are deshielded when these dimers assume the

stacked conformation. In d(GpG)cisPt both H8 protons are deshielded at lower temperatures. In complexes of Pt(II) with mononucleotides similar deshieldings have been noted[14,41]. Deshielding of H8 protons can be related tentativaly to the increasing population of $\gamma^+(2)$ at lower temperatures, vide supra, which means that the time-averaged distance between the C5'-O5' bond and H8 becomes smaller with decreasing temperature, resulting in a deshielding of the H8 resonances.

In d(GpG)cisPt the chemical shift changes of H5'(1), H5"(1) and H3'(1) also appear to be correlated with an increasing population of $\gamma^+(1)$ at lower temperatures. In a γ^+ conformation, H3'(1) is close to the C5'-O5'(1) bond and this gives rise to deshielding[18]. In a $\gamma^t(1)$ conformation both H5(1) protons are close to one of the phosphate oxygens, therefore a decreasing population $\gamma^t(1)$ (i.e. increasing $\gamma^+(1)$) will result in a shielding effect. The internal chemical shift change observed for H2'(1) can be rationalized by taking an increasing influence of O5'(2) at lower temperatures into account. From the model of the preferred conformation of d(GpG)cisPt ($\gamma^+(2)$ and S conformation for sugar ring(2), vide infra) we calculate a relatively short non-bonded distance (2.5 Å) for O5'(2).H2'(1). Model considerations show that O5'(2) has a different orientation and a greater distance to H2'(1) in the remaining conformational species (either $\gamma^t(2)$ or N conformation for the furanose ring in the -pG moiety). Therefore, O5'(2) causes a larger deshielding effect on H2'(1) at lower temperatures. The shielding of H2'(2) cannot be easily explained, but see ref. 18.

A model for d(GpG)cisPt:

The amount of quantitative data brings in the possibility to construct a model of the most abundant conformer of the adduct using program BUILDER (van Kampen, P.N., de Leeuw, F.A.A.M. and Altona, C. private communication) for the d(GpG) part and program UTAH[42] for the cisPt part, assuming Pt-N7 distances of 2.0 ± 0.1 Å and N-Pt-N bond angles of 90° ± 3°. Thus an impression of the spatial structure can be obtained and non-bonded distances can be computed to yield a reasonable molecular structure. Pilot calculations show that an $\varepsilon^-(1)$ (280° along C3'-O3') conformation is not likely due to the non-bonded distance of approximate 1.5 Å between H5'(1) and the pro(R) phosphate oxygen of unit (2). In Figure 5 the ORTEP[43] drawings which resulted are shown. It should be stressed that this structure of d(GpG)cisPt is but one of the possible conformers whose features appear to fit the NMR data of the most abundant species in solution. The model has the following characteristics: deoxyribose ring(1) has the pseudorotational parameters: P = -1°, ψ = 38°,

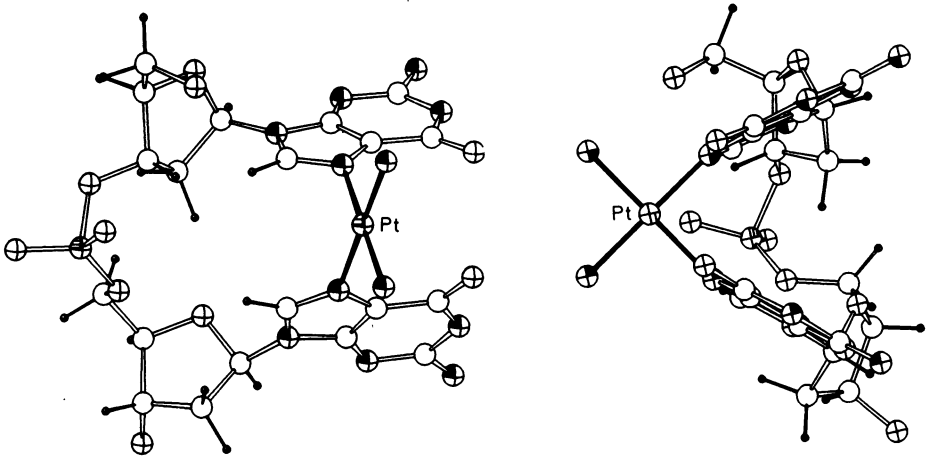

Figure 5. ORTEP drawings of a possible conformer of d(GpG)cisPt in solution. The exchangable protons are omitted. O : C, ⊕ : N, ⊕ : O, ⊕ : P, ⊕ : Pt, ● : H.

this results in torsion angle $\delta(1)$ of 87^{o}, deoxyribose ring(2) is characterized by P = 150^{o}, $\psi = 35^{o}$, yielding $\delta(2)$ as 137^{o}. The other torsion angles have the following values: $\gamma(1)$: 52^{o}, $\varepsilon(1)$: 210^{o}, $\zeta(1)$: 300^{o}, $\alpha(2)$: 295^{o}, $\beta(2)$: 182^{o}, $\gamma(2)$, 58^{o}, $\chi(1)$: 110^{o} and $\chi(2)$: 115^{o}. The differences between these angles and those obtained by NMR data are within the range that can be expected by describing a compound with such different approaches. The structure around the platinum atom agrees well with several crystal structures[13]. The angle between the planes of the bases amounts to 53^{o} i.e. are within the range deduced from solid state data[13] (in between 45^{o} and 90^{o}). The distances between the platinum atom on the one hand and the planes of the bases of G(1) and G(2) on the other hand are respectively 0.65 and 0.56 Å. Unfortunately, accidental isochronicity of H5'(2) and H5"(2) precludes the calculation of $\beta(2)$ and $\gamma(2)$ from the NMR data.

CONCLUSIONS

Detailed conformational analysis of the non-base protons of d(GpG)cisPt shows a complete population shift of deoxyribose ring(1) (the ring at the 5'-free end) from the S (C2'-endo) to the N (C3'-endo) conformer due to the chelation of cisPt by the d(GpG) N7 atoms. Compared with a B-DNA like structure no large alterations are observed in the remaining torsion angles. The most abundant conformer at room temperature is characterized by the following

torsion angles as deduced from the spin-spin couplings:

O5'-C5'-C4'-C3': $\gamma^{+}(1)$, 52^{o}; C5'-C4'-C3'-O3': $\delta^{+}(1)$, 87^{o} (3)
C4'-C3'-O3'-P : $\varepsilon^{t}(1)$, 198^{o} (4) C3'-O3'-P -O5': $\zeta^{-}(1)$;
O3'-P -O5'-C5': $\alpha^{-}(2)$; P -O5'-C5'-C4': $\beta^{t}(2)$, $\sim 180^{o}$;
O5'-C5'-C4'-C3': $\gamma^{+}(2)$, $50\text{-}60^{o}$; C5'-C4'-C3'-O3': $\delta^{t}(2)$, 137^{o} (2)

A model of a probable conformer which fits aforementioned data could be build. The torsion angles $\zeta(1)$ and $\alpha(2)$ are in the usually found range (300^{o} and 295^{o} respectively). The torsion angles $\chi(1)$ and $\chi(2)$ amount to approximately 115^{o}, i.e. an anti configuration. An unexpected feature is the conformational freedom which appears in this molecule. This freedom makes it likely that such a lesion can also be formed in DNA. After a reaction with one guanine base, the local destabilisation which is required for the next reaction step is only an S to N conformational alteration of the 5' deoxyribose moiety; other significant torsion angle changes are not required for this chelation. If such a d(GpG)cisPt chelate is present in DNA, a kink in one strand would be formed. If the opposite strand does not conform itself to this unusual conformation a local denaturation will occur.

The present in depth study of d(GpG)cisPt allows a comparison of this possible lesion in DNA with e.g. a thymidine dimer (T(p)T) due to U.V. irradiation of DNA. In both compounds the bases are not coplanar, the angles between the planes of the bases are roughly 60^{o} in both cases. Some striking differences are however apparent. In T(p)T the largest changes compared to TpT are found at the 3'-end of the molecule[44] and no complete S to N population shift is observed, thus e.g. intramolecular phosphate distances to neighbours will be largely different for these two compounds in DNA. It is therefore likely that a repair enzyme for a T(p)T lesion in DNA will react differently with a d(GpG)cisPt lesion than with a T(p)T lesion.

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1. Abbreviations used:
NMR, nuclear magnetic resonance; TMA, tetramethylammoniumchloride; TMPB, Tetramethylphosphoniumbromide; d(GpG), deoxyguanylyl(3'-5')deoxyguanosine; cisPt, cis-diamminedichloroplatinum(II); transPt, trans-diamminedichloroplatinum(II); d(GpG)cisPt, cis-deoxyguanylyl(3'-5')deoxyguanosinediammineplatinum(II); Gp-, 5'-OH end part of d(GpG)cisPt; -pG, 3'-OH end part of d(GpG)cisPt.
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