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Sequence-Selective Metal Ion Binding to DNA Oligomers

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Sequence-selective interactions between DNA oligonucleotides and Pt(II) and Pd(II) complexes have been studied by 1D and 2D NMR spectroscopy. Titration of DNA oligomers with diamagnetic metal ion complexes induces chemical shifts of proton resonances close to the site of interaction. Conformational changes in the helical structure were monitored by measuring cross-peak intensities in 2D NOESY maps. Two duplex deoxynucleotides were studied, $[d(CGCGCG)]_2$ and $[d(CGCGAATTCGCG)]_2$, respectively. The hexamer was titrated in the duplex form with *cis*-$[Pt(NH_3)_2Cl_2]^{2+}$ (*cis*-DDP or cisplatin). The dodecamer was titrated with NO_3^--salts of $Pd(aq)^{2+}$, $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(dien)(H_2O)]^+$, respectively. The reaction between *cis*-DDP and the hexamer at conditions where the duplex form is retained proceeded exceedingly slowly. In the initial phase of the titration the NOESY map indicates conformational changes induced by non-covalent adduct formation between the intact hexamer and *cis*-DDP. The more reactive Pd compounds show a tendency towards sequence-selective binding to the duplex dodecamer. The 'naked' Pd ion is very reactive and exhibits selectivity towards the thymine T8 imino proton and N7 on G4. At a Pd(II)/dodecamer ratio of 4:1 the metal ions induce helix→coil transition. The mono- and bifunctional Pd complexes with 'bulky' ligands attack the dodecamer at the terminal GC base pairs, leaving the central Watson–Crick base pairs intact.

Platinum antitumor drugs have been reported to interact preferentially with guanine residues in DNA.[1–5] Several investigations have been aimed at determining the structural perturbation of DNA oligomers induced by platinum binding. The usual procedure has been to platinate the oligomer at a predetermined guanine site under single-strand conditions, extract the platinated species by HPLC and subsequently anneal the complex with the complementary unplatinated single strand to obtain the duplex form. In reactions between Pt complexes and intact double-helical oligonucleotides the duplex structure has been found to be disrupted under the conditions used.[5]

Over the past few years we have been interested in sequence-selective binding of transition-metal ions to DNA oligomers.[6–8] The first results from these investigations[6] indicated that Zn(II) and Mn(II) ions bind selectively to the oligonucleotide $[d(CGCGAATTCGCG)]_2$. The guanines were found to be the predominant binding sites in agreement with earlier metal–DNA studies. However, for the first time an unexpected sequence-selective metal binding pattern was revealed, where the guanine bases were affected in the order G4≫G2, G10 and G12. Further work[7] on manganese interaction with a series of ten different oligonucleotides showed that metal ions bind selectively to guanine residues on the 5′-side in the following order: 5′-GG > 5′-GA > 5′-GT ≫ 5′-GC. No clear evidence of binding to 5′-G in 5′-GC steps or to non-G residues was found.

Recently Frøystein and Sletten[8] have monitored titration experiments of Hg(II) ions to the dodecamer $[d(CGCGAATTCGCG)]_2$ by ^1H and ^{15}N NMR spectroscopy and found that Hg(II) interacts selectively with the AT tract of the oligomer. In a recent ^1H NMR chemical-shift study of the interaction between Zn(II) ions and the double helix $[d(ATGGGTACCCAT)]_2$ differential Zn(II) binding was reported.[9] Studies of interaction between Ca(II)[10,11] or Mg(II)[12] with DNA also indicate site-specific metal binding.

Numerous studies involving both *cis*- and *trans*-DDP interaction with DNA have focused on the intrastrand binding to guanine residues. The determination of binding sites has in most cases been deduced from ^1H NMR chemical-shift variations as a function of pH. Chelation of the type 5′-GG (N7–N7) and 5′-GNG (N7–N7) has been reported for *cis*-DDP, while only the latter mode of chelation has been found for *trans*-DDP.[2–5] However, in an investigation of *cis*-DDP binding to a series of dinucleotides (IpI, GpG, ApA, GpC and ApC) it was found that only the first three formed chelates while GpC and ApC led to mixtures of several complexes where cytosine

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rather than guanine showed the largest affinity towards *cis*-DDP.[13] This pattern is consistent with the proposed rule of sequence selectivity for Mn(II) and Zn(II) binding.[7]

Recently, Chottard has investigated sequence-selective kinetics of Pt(II) binding to oligomers[14] and found interesting differences in reaction patterns between $[Pt(NH_3)_3(H_2O)]^{2+}$, $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[PtCl(NH_3)_2(H_2O)]^{+}$. The first platination step involving guanine N7 in a 5′-GG context showed preference for the 5′-G compared to 3′-G for $[Pt(NH_3)_2(H_2O)_2]^{2+}$, while the chloro complex showed no selectivity between these two sites. Similar effects were observed for the intrastrand chelation step.

In this paper we focus on sequence-selective binding of platinum(II) and palladium(II) compounds to intact oligonucleotide duplexes. This may be of importance in the search for nucleotide specific anti-cancer drugs. To form Pt(II) complexes it is necessary either to heat the solution or to allow it to stand for several days. Alternatively, we have employed Pd(II) ions which react approximately 10^5 times faster than Pt(II) ions. Both metal ions possess similar ionic radii, prefer nitrogen rather than oxygen donor atoms, and form strong tetragonal complexes.

The hexamer 5′-CGCGCG was chosen for these studies, since it forms an especially stable duplex form. It has been suggested that oligomers with six or less base pairs are easily converted to single strands by *cis*-platin.[14] According to previous results on single strands one might expect for this hexamer GCG (N7–N7) chelation.[15] The dodecamer chosen for the Pd(II) binding studies, 5′-CGCGAATTCGCG, forms a relatively stable double-helical structure which has been determined both in the solid state by X-ray crystallography[16] and in solution by NMR methods.[17]

Experimental

Oligonucleotides. Two different DNA oligomers were employed for the binding studies, $[d(CGCGCG)]_2$ (I) and $[d(CGCGAATTCGCG)]_2$ (II). They were synthesised by the usual solid-phase phosphite triester techniques,[18] purified by chromatography in distilled water on a 120 cm Sephadex G-25 column and lyophilised to dryness. The sequences are palindromes which readily form duplexes. The lyophilised samples were dissolved in 0.4 mL solution containing 0.15 mM EDTA and 20 mM sodium phosphate adjusted to pH 7.0 with NaOH. EDTA was added to prevent effects from paramagnetic impurities. The DNA samples were lyophilised to dryness from D_2O and redissolved in 99.96% D_2O. Finally the samples were dissolved in 0.4 mL of 99.996% D_2O and transferred to 5 mm NMR tubes.

Chemicals. The activated form of *cis*-DDP, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, was prepared by reacting the drug with $AgNO_3$ and filtering off the precipitate prior to reaction with the oligonucleotide. Three different sources of Pd(II) were used, K_2PdCl_4, $[Pd(en)(H_2O)_2](NO_3)_2$ and $[Pd(dien)(H_2O)](NO_3)_2$; the last two were prepared as described earlier.[19,20]

NMR spectroscopy. All spectra were recorded on a Bruker AM-400 MHz wide-bore spectrometer at 400.13 MHz. The temperature was 303 K in all experiments. One-dimensional spectra were typically recorded into 4096 complex points with a 3479 spectral width. The spectra were obtained after 128 transients with a pulse width of 8.5 ms and a recycling delay of 5 s. COSY and NOESY experiments were used to confirm previous proton assignments of the oligomers.[18,21] NOESY experiments were also used to examine the effects of metal-ion-induced conformational changes. COSY and NOESY spectra were collected in phase-sensitive mode with quadrature detection into 1024 complex points for 730 t_1 values, using the TPPI method.[22,23] For each t_1 value 64 transients were used with a delay of 2 s between each transient. During the recycle delay the water signal was suppressed with a weak irradiation pulse of 20 Hz. In all experiments a mixing time of 400 ms was used.

The data were processed with the program FELIX from Hare Research, Inc. on a SGI-4D/35 computer. The one-dimensional FIDs were Fourier-transformed and baseline-corrected with a first-order polynomial fit. The water signal was set to 4.7 ppm and the other signals were referenced to this signal. Linewidths were measured directly from the spectra. The 2D data were zero-filled to 2048 complex points along t_2, multiplied with an exponential function increasing the linewidth by 2 Hz. The spectra were baseline-corrected with a second-order polynomial fit. The FIDs along t_1 were zero-filled to 4096 complex points and lightly apodized with a 90° phase-shifted sine-bell function.

The distance geometry program[17,24] DSPACE (Hare Research, Inc.) was used in modelling the structure of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ complexed to the $[d(CGCGCG)]_2$ hexamer. The starting structure for the calculations was $[Pt(NH_3)_2(H_2O)_2]^{2+}$ separated by 10 Å from the DNA. Observed structural changes in the DNA conformation (from NOESY spectra) in the presence of the platinum compound were implemented on the $[d(CGCGCG)]_2$ hexamer in the idealized coordinates.[25] The structure of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ is based on X-ray crystallographic data.[26,27] The DNA coordinates were kept fixed during the calculations, and the $[Pt(NH_3)_2(H_2O)_2]^{2+}$ coordinates were moved (optimized) by simulated annealing and conjugate gradient refinement.[24]

Results

The hexamer. The assignment of the 400 MHz ^1H resonances of (I) were based on COSY and NOESY spectra and are shown for the downfield base proton/anomeric

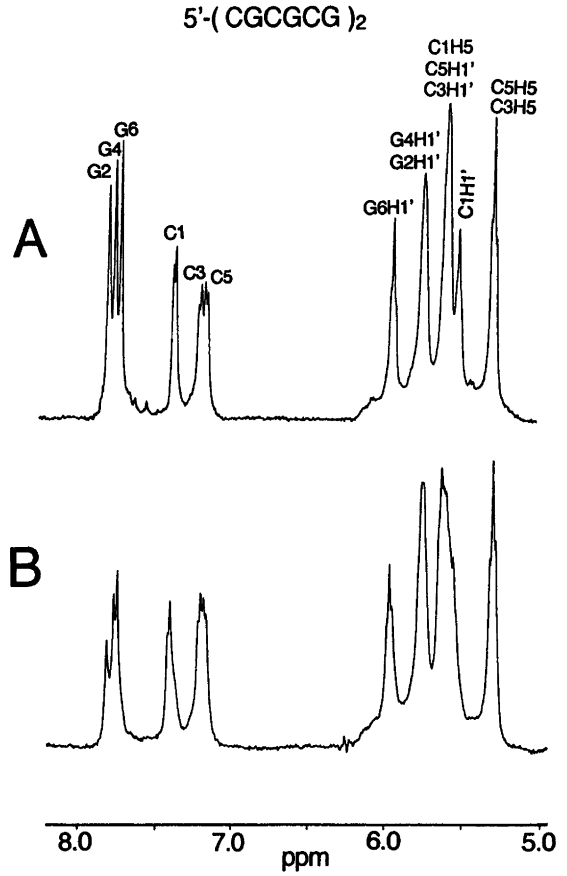

Fig. 1. 400 MHz ^{1}H NMR spectrum of the down-field part of the non-exchangeable protons of 14 mM $[d(CGCGCG)]_2$ in a 200 mM phosphate D_2O solution: (A) without *cis*-DDP, (B) with *cis*-DDP/duplex ratio of 0.14. The residual water signal was used as chemical-shift reference (4.70 ppm) and the sample temperature was set to 303 K in both spectra.

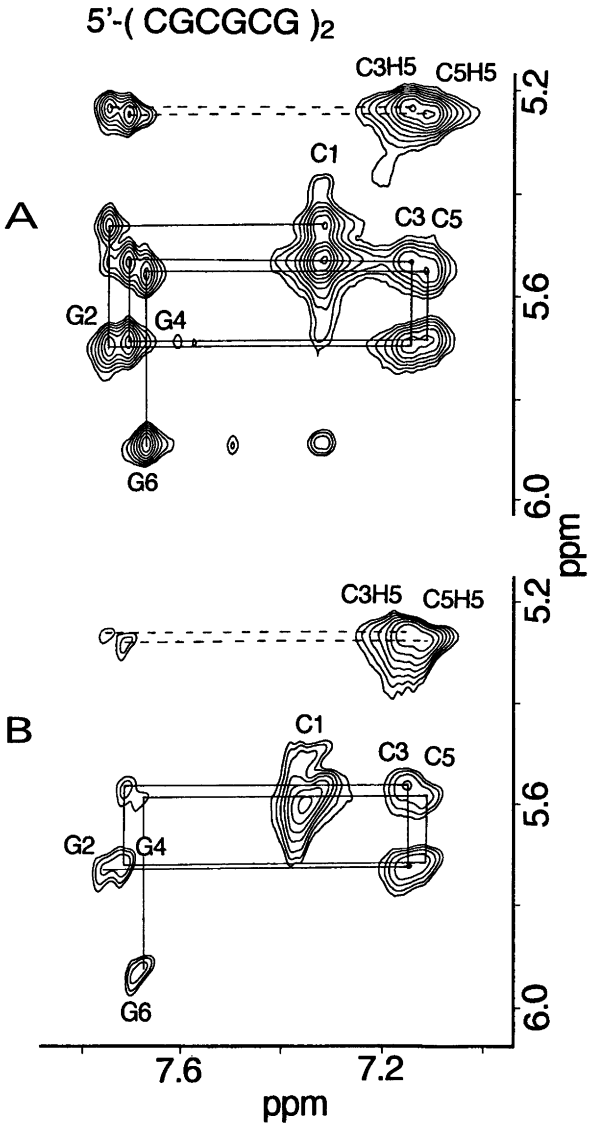

Fig. 2. Contour map of the H8/H6–H1′/H5 region in the 400 MHz NOESY spectrum of $[d(CGCGCG)]_2$ (400 ms mixing time). The intra-residue H8/H6–H1′ cross-peaks are labelled with the residue number. The sequential connectivity is indicated with a solid line. (A) Without *cis*-DDP, (B) with *cis*-DDP/duplex ratio of 0.14.

region in Fig. 1A. A sequential walk in the same region is indicated in Fig. 2A. The duplexes are associated end-on in solution producing a strong NOE cross-peak between C1–H1′ and G6–H8. In the first set of titrations using regular *cis*-DDP, the expected covalent interaction between guanine N7 of the intact duplex and platinum did not take place. However, a significant broadening of the guanine H8 protons relative to the cytosine H6 protons are observed (Fig. 1B).

In the NOESY map distinct differential decrease in the H6/H8–H1′ cross-peak intensities indicates conformational changes induced by *cis*-DDP (Fig. 2). The same pattern is observed in the sugar region where the intensities of the inter-residue cross-peaks between the H6/H8 protons and H2′/2″ protons are appreciably reduced (data not shown). Specificly the G2–H8 to the C1–H1′, the G2–H1′, the C1–H2′r and the C1–H2′s distances were increased from the corresponding distances in the naked DNA (no Pt added). Calculating the distances from the corresponding NOESY cross-peak volumes showed that the G2–H8 to the C1–H1′, the G2–H1′, the C1–H2′r and the C1–H2′s distances had increased to 4.19 Å (3.87 Å in idealized (id.) B-DNA), 4.19 Å (3.86 Å in id. B-DNA), 3.48 Å (3.18 Å in id. B-DNA) and 2.10 Å (1.8 Å in id. B-DNA), respectively. This increase in the described proton–proton distances was carried out in our DNA model by changing the torsion angles along the DNA backbone for the residues C1 and G2. Reedijk[28] has pointed out the stabilizing effect of the formation of a hydrogen bond between the amine ligand and guanine-O6. The altered DNA structure (as described above) may be obtained by accomodating such a H bond between *cis*-DDP and G2–O6. Once this H bond is establilshed, the most likely candidate for H bonding to the second amine in *cis*-DDP is C1–O2 rather than a DNA phosphate group.

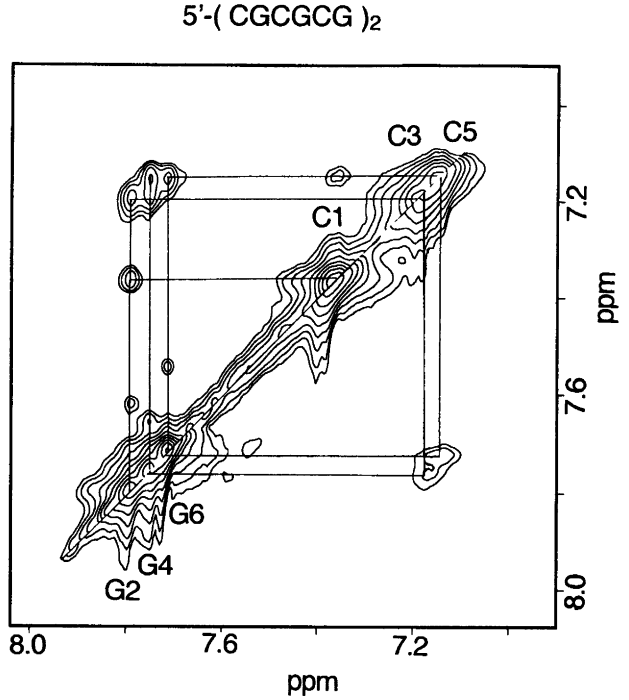

Fig. 3. Contour map of the H8/H6–H8/H6 region in the 400 MHz NOESY spectrum of $[d(CGCGCG)]_2$. (i) Above the diagonal without *cis*-DDP and (ii) below the diagonal with *cis*-DDP/duplex ratio of 0.14.

Figure 4 shows the Pt compound with both amine groups forming H bonds to the DNA, one to G2–O6 and the other amine to C1–O2. Furthermore, in the aromatic–aromatic crosspeak region (Fig. 3) the stacking pattern is retained after the addition of *cis*-DDP. The imino proton resonances of the hexamer occur as a single, overlapping peak both before and after the titration (data not shown).

In order to enhance the reactivity the activated species, $[Pt(NH_3)_2(H_2O)_2]^{2+}$ of *cis*-DDP, was used in the second series of titration. However, even after the reaction mixtures had been left standing for several days only a small amount of duplex did react covalently with platinum. Apparently, the intact helical duplex structure is relatively inert towards *cis*-DDP, and only when the oligomer occurs as single strand will the reaction take place at an appreciable rate. However, even then the yield of platinated single strand is usually less than 50–60%.

The dodecamer. The assignments of proton resonances are in accordance with those published in the report on the complete solution structure of the dodecamer based on NMR data.[17] In the first series of titration experiments monitored by 1D and 2D ^1H NMR aliquots of the monofunctional complex, $[Pd(dien)(H_2O)]^{2+}$, were added to the dodecamer. In the aromatic/anomeric region (Fig. 5A) the assignments of the chemically shifted guanine H8 resonances were based on a NOESY map recorded at a Pd(II)/duplex ratio of approximately 4:1 (data not shown). In the first part of the titration the intensities of the H8 protons of both G2 and G12 decrease, while simultaneously new chemically shifted signals appear at 8.26 and 8.28 ppm corresponding to the DNA–Pd(dien) adduct. On further addition of Pd(dien) G10–H8 and subsequently G4–H8 are affected. The chemical shifts of the cytosine signals are compatible with those of the adjacent guanines; H6 of C3 and C11 shift down-field 0.13 and 0.14 ppm, respectively. Of the two aromatic thymine signals only H6 of T8 is shifted in accordance with the observations in the imino proton region (Fig. 5B). The adenine resonances H2 and H8 show only minor chemical shifts.

In the imino proton spectra (Fig. 5B) a pronounced sequence-selective exchange broadening is observed for the T8 imino proton, while T7 exhibits almost no broadening. Also the imino protons of the penultimate guanines G2 and G10 are influenced strongly by monofunctional adduct formation. The dodecamer gradually undergoes a partial duplex→coil transition.

Interactions between the $-CH_2-CH_2-$protons of the diethylenetriamine ligand and the oligomer are manifested as strong exchange peaks in the NOESY map. Most no-

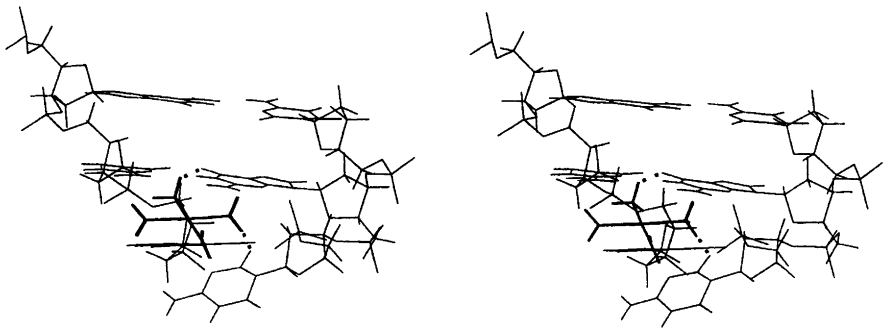

Fig. 4. Stereo-view of the molecular model of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (bold lines), located in the major groove of the $[d(CGCGCG)]_2$ hexamer, where only half of the DNA is shown. The structure satisfies observed changes in the intramolecular DNA NOESY cross-peaks for the C1 and G2 residues. The two dotted lines indicate the possible intermolecular hydrogen bonds between an amine hydrogen and O6 of G2 (upper dotted line) and an amine hydrogen and O2 of C1 (lower dotted line).

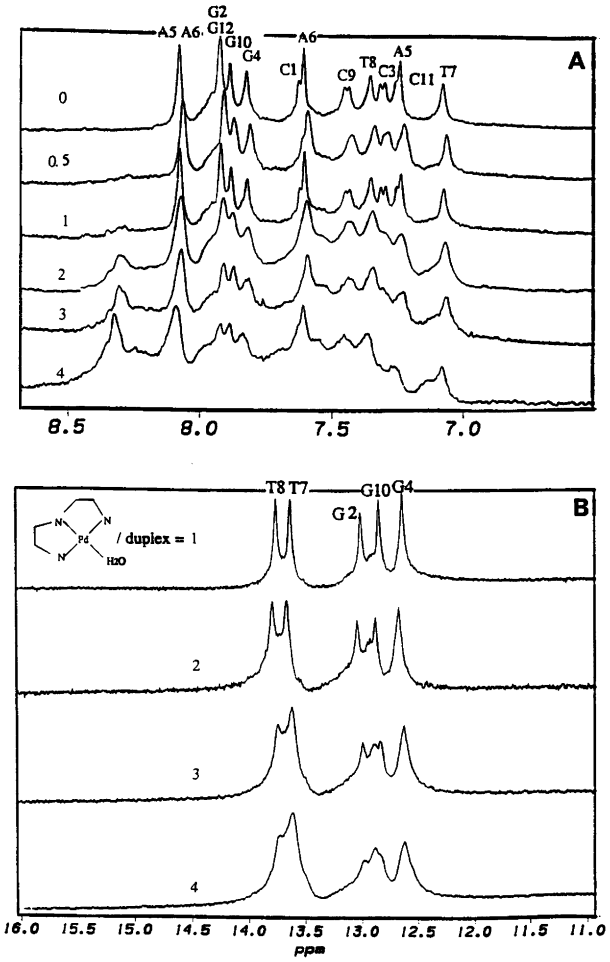

Fig. 5. The imino proton region (A) and the base proton region (B) of the 400 MHz ^{1}H NMR spectra of (Pd-dien+[d(CGCGAATTCGCG)]$_2$) in 200 mM phosphate buffer (H_2O/D_2O 90/10) at 292 K.

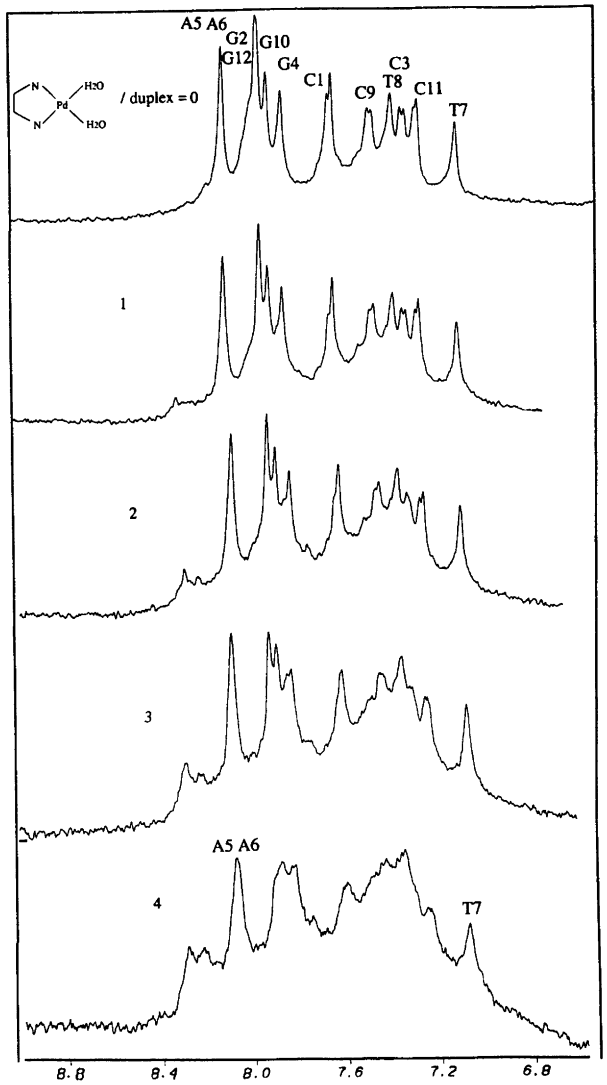

Fig. 6. The base proton region of the 400 MHz ^{1}H NMR spectra of (Pd-en+[d(CGCGAATTCGCG)]$_2$). Concentration of oligomer: 0.4 mM in 200 mM D_2O phosphate buffer.

ticeable are the interstrand interactions observed between dien and H8, H1′, and H2′ of adenine A6.

In the second series of titrations the bifunctional complex, $[Pd(en)(H_2O)_2]^{2+}$, is expected to mimic the binding mode of *cis*-$[Pt(NH_3)(H_2O)_2]^{2+}$. The effect of adding Pd(en) to the dodecamer is shown for the proton resonances in the base region (Fig. 6). Severe overlap of the H8 resonances prevents an accurate assessment of the interaction pattern. However, integration of the deconvoluted peaks indicates that the two new signals appearing at 8.2 and 8.3 ppm correspond to the chemically shifted G2/G12–H8 proton signals. The NOESY spectra of the sample at a Pd(II)/duplex ratio = 4 were not of sufficient quality to provide any useful information concerning sequence-selective binding.

In the final titration series aliquots of K_2PdCl_6 solution were added to the oligomer to a Pd(II)/duplex ratio of approximately 4. Only 1D spectra were recorded, owing to rapidly deteriorating signals during the titration. Two separate spectral regions are shown corresponding to the imino protons (Fig. 7A) and the methyl protons (Fig. 7B).

The resonances of imino protons involved in Watson–Crick hydrogen bonding are shown to broaden gradually and merge into two separate regions corresponding to thymine protons and guanine protons, respectively. An initial sequence-selectivity may be detected indicating a slight preference for thymine T8 rather than thymine T7. A similar tendency is observed for guanine G2 vs. G4 and G10.

In the base region of the non-exchangeable protons (not shown) the largest chemical shifts are observed for H8 of the four guanines. At a Pd(II)/duplex ratio of 4 a mixture of complexed and uncomplexed species is present. The spectral resolution is not sufficient to distinguish any tendency for sequence-selective binding. The methyl signals of thymine T7/T8 (Fig. 7A) experience large upfield shifts in the complexed form of the dodecamer. This is contrary to the large down-field shifts

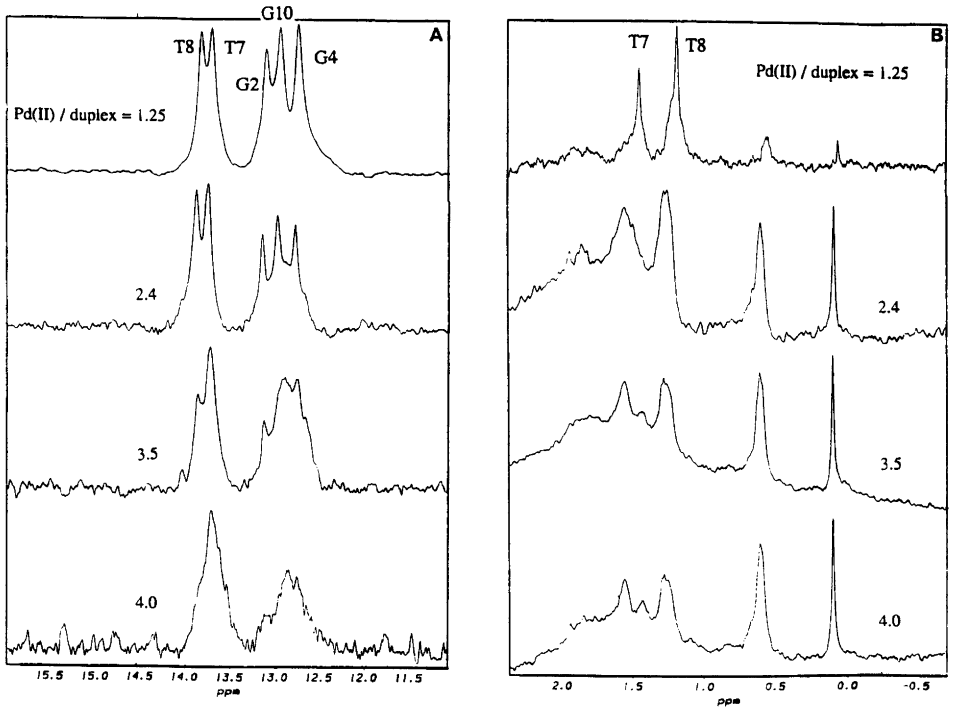

Fig. 7. 400 MHz ^{1}H NMR spectra of $(Pd^{2+}+[d(CGCGAATTCGCG)]_2)$. (A) The imino proton region. (B) The upfield thymine methyl region.

observed for the methyl groups in the thermally induced helix-coil transition.[29]

Discussion

Platinum binding to the hexamer. Platinum coordination to guanine N7 has been reported to cause H8 downfield shifts of the order of 0.60 ppm as a result of the inductive effect of platinum(II).[30] However, in oligonucleotides the magnitude of these metal-ion-induced shifts is also dependent on base-stacking variations giving rise to changes in ring current. In cases where large distortion and even complete strand separation are induced by metal-ion coordination it is difficult to predict the net chemical-shift effects.

The extremely low reactivity of *cis*-DDP towards the intact double-helical hexamer even after standing for several days was somewhat unexpected. The situation may be related to the 5′GC dinucleotide–*cis*-DDP system where guanine exhibited total lack of reactivity.[31] The absence of mono-dentate G-binding to 5′-GC is in accordance with the proposed selectivity rule.[8] This in contrast to the monofunctional 5′-G binding in *cis*-DDP-soaked crystals of the double-stranded dodecamer d(CGCGAATTCGCG).[26] Here the platinum prefers guanine in the 5′-GA step.

The 1,3 binding mode, in which *cis*-DDP coordinates to next-neighboring guanines, has been observed for short, single-stranded oligonucleotides such as d(GCG).[15,32] To accomodate a 1,3 chelate the intervening nucleotide has to be destacked in a 'bulged-out' fashion. In the intact hexamer duplex the trippel Watson–Crick GC hydrogen bonds have to be disrupted in such a destacking process. Evidently, this geometric constrain prevents the formation of a d(GCG)(N7, N7) chelate.

In the present case less than 5% of the oligomer was platinated under duplex conditions. However, a comparison of NOESY maps (Fig. 2) recorded before and after addition of *cis*-DDP clearly reveals conformational changes. The cross-peak between G2–H8 and C1–H1′ is missing, and the intensity of the corresponding cross-peak between G6 and C5 is considerably reduced. To explain these observations one may invoke outer-sphere, non-covalent interaction between *cis*-DDP and the duplex.

Palladium binding to the dodecamer. The recently discovered sequence-selective metal-ion coordination to oligonucleotides was first noticed for this dodecamer, 5′-CGCGAATTCGCG. It was found[6,7] that Mn(II) and Zn(II) prefer N7 of G4 while Hg(II) binds in the AT region.[8] Studies on the kinetics of platinum(II) binding to oligonucleotides have indicated discrimination between 5′ and 3′ guanine sites for different Pt(II) species.[13] In the present investigation we have used palladium(II) instead of platinum(II) in order to speed up the reaction.

In the first series of titrations the monofunctional Pd-dien species are found to exert a certain degree of sequence-selective binding in the AT region. The imino signal of thymine T8 is seen to be broadened and disappear due to rapid exchange with bulk water, while the T7 imino

signal is much less affected (Fig. 5A). Also the outermost GC base pairs represented by the imino signals of G2 and G10 are seen to be more affected than the interior G4 signal. The bulky Pd-dien species attack the duplex at the ends, opening the outer Watson–Crick base pairs. At a Pd-dien/duplex ratio of 4:1 the innermost base pairs involving 5′-GAAT- are still intact.

In the spectral region of the non-exchangeable base protons (Fig. 5B) the most noticeable effect of Pd-dien is the large downfield shifts of guanine H8 protons. The composite peak at 8.28 ppm is assigned to H8 of G2/G12 based on comparison of the integrated intensities at the original and new peak positions. These findings are consistent with the spectral data of the imino protons and support a binding mechanism where the initial attack of Pd-dien is located in the terminal region of the duplex. This mode of action is different from that observed for hexaaqua species of Mn(II) and Zn(II), which involved selective binding to the central part of the duplex.[6,7]

In the titration series involving the bifunctional Pd-en complex the binding-induced spectral variations in the non-exchangeable base proton region (Fig. 6) are seen to be rather complex. The pronounced effect on G4–H8 in the initial part of the titration may indicate sequence selectivity; however, at a 4:1 ratio all four guanines are seen to be almost equally affected.

In the final set of titrations a solution of K_2PdCl_4 was added to the oligomer to check if the hexaaqua Pd(II) species, Pd(aq), exhibited similar sequence-selective binding as reported for other transition metals. In the imino region (Fig. 7A) we observe the same T8 vs. T7 selectivity as was found for Pd-dien (Fig. 5A). However, while the G2 and G10 imino protons were affected equally by Pd-dien, only G2 is selectively influenced by Pd(aq). Eventually, at a Pd(II)/duplex ratio of 4:1 the thymine and the guanine imino resonances broadened into two separate peaks indicating complete disruption of the duplex structure. This transition to a single-stranded disordered structure is clearly demonstrated in the methyl region (Fig. 7B). The methyl groups of both thymines experience large upfield shifts of the type observed in the thermal melting of the duplex.[2] The Pd(aq) species is an aggressive reagent and at a ratio of 2.4 most of the non-exchangeable base protons are strongly affected. Only in the initial stage (Pd/duplex = 1.25) is it possible to detect a slight preferential line broadening of G4–H8 (data not shown).

Conclusion

In the present paper we have shown that the double helical $[d(CGCGCG)]_2$ is found to be relatively inert towards *cis*-DDP at the given electrolyte, buffer and temperature conditions. Monodentate 5′-G coordination in the 5′-GC context is known to be less favourable and, furthermore, 1,3 GCG chelation has not been observed for intact duplexes. However, the binding mechanism of *cis*-DDP in the cell may involve attack on the DNA in an open single-stranded conformation.

The interactions between the dodecamer, $[d(CGCGAATTCGCG)]_2$ and different mono- and bifunctional Pd(II) species exhibit a certain degree of sequence selectivity. However, in this short sequence end effects tend to obscure preferential coordination of complexes with bulky ligands. Both donor strength of the ligating atoms and the preferred coordination geometry of the metal ions are important factors for determining metal ion–DNA selectivity. The sequence-dependent molecular electrostatic potential (MEP)[33] of the binding sites is found to be of key importance when hexa- and tetragonal metal hydrates are involved.

We are presently checking out the selectivity pattern for several transition metals. This work may be of importance in the search for better metal-based anti-tumor drugs which are assumed to interact selectively at the nucleic acid level. Several non-platinum-metal complexes have shown promising anti-tumor activity and are presently subjected to clinical trials.[34]

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