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Sequence-Selective Interaction between Mercury(II) Ions and the DNA Dodecamer $[d(GCCGATATCGGC)]_2$ Studied by ^1H NMR Spectroscopy

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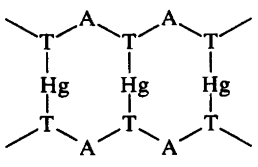
The palindromic dodecamer $[d(GCCGATATCGGC)]_2$ has been titrated by $Hg(ClO_4)_2$ in order to study sequence dependent Hg^{II} ion interactions. The titration pattern as monitored by 1D and 2D ^1H NMR is consistent with a transition to a new conformer of the dodecamer induced by Hg^{II} ions. At intermediate stages of the titration, the proton signals from the new conformer coexist with those of the original one, indicating slow exchange between the two forms on the NMR timescale. The data clearly show that there is no major alteration in the secondary structure, e.g. B→Z-form or duplex→hairpin transition. The intra- and inter-residue sequential walk is completed except for a break between T6 and A7. At a concentration level $r = [Hg^{II}]/[\text{nucleobase}] < 0.20$ all four central imino signals are present. This definitely excludes thymine N3 as a possible mercuration site. In the imino region of the spectra Hg^{II} ions induce a large upfield shift of the thymine imino resonance T8–N3H, while the other thymine resonance T6–N3H is unperturbed. The guanine imino signal G4–N1H shows a large downfield ring current shift caused by major conformational changes in the duplex. The complete titration experiment indicates that mercury, initially, binds selectively to the A5′–T8 base pair. A tentative model is proposed where mercury is cross-linking the two strands via thymine T8–O4 and the deprotonated amino group of the complementary adenine base A5′.

The search for new antitumor drugs[1] and the elucidation of the mechanisms involved in the toxic effects of certain metal ions, require a knowledge of the DNA–metal ion interaction. One of the first NMR spectroscopic studies on metal ion interaction with a double helical DNA oligomer, $[d(CGCGAATTCGCG)]_2$ showed that Zn^{II} and Mn^{II} ions bind selectively to the guanine residues in the order 5′-G4 > G2, G10 and G12.[2] In a similar study, Marzilli and co-workers[3] showed that Zn^{II} ions bind selectively to guanine residues in the oligomer sequence $[d(ATGGGTACCCAT)]_2$. Investigations on sequence-selective Zn^{II} and Mn^{II} ion binding to a series of oligonucleotides (10–12 base pairs) revealed an interesting binding pattern where the guanine residues on the 5′-side were affected in the order 5′-GG ≥ GA > GT > GC.[4] Titration of two 5′–3′ inverted DNA hexamers by Mn^{II} salt,[5] also showed base-selective binding to the guanines. The most probable binding site on the guanine residue is nitrogen G–N7. Sequence-selective metal binding may be explained by local variations in guanine N7 nucleophilicity, owing to base sequence dependent stacking. Qualitatively, the variation in molecular electrostatic potentials (MEP) along the oligonucleotide chain may be related to metal ion selectivity.[6]

Inorganic mercury, Hg^{II}, known to have toxic effects, is a stronger nucleobase binder than the other investigated metal ions and are found to bind reversibly to the DNA.[7] In contrast to metal ions like Mn^{II}, Cu^{II} and Zn^{II}, which prefer guanine bases, Hg^{II} ions seem to prefer adenine and thymine rich regions in DNA.[7] A chain slippage mechanism for the binding of Hg^{II} to poly(dA-dT)·poly(dA-dT) was proposed by Katz[8,9] whereby each Hg^{II} ion is linked to two thymine N3 on opposite strands (Scheme 1). The incorporation of a Hg^{II} ion requires proton release on N3 of each of the two bridging thymines, thus corrupting the conventional Watson-Crick



Scheme 1.

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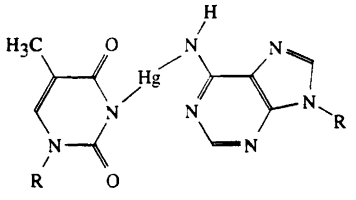
hydrogen bonds. The chain slippage model was further refined by Young *et al.*,[10] who suggested that Hg^{II} ions could be interstrand cross-linked to the thymines and that the adenines were self-base-pairing in poly(dA-dT)·poly(dA-dT). An alternative to the chain slippage model was proposed by Nandi *et al.*[11] in which the mercury ions cross-link the original complementary base pairs where hydrogen has been displaced from the amino group of adenine as well as from thymine N3H (Scheme 2).

The relative affinities of Hg^{II} binding to the monomer nucleosides have been shown by Simpson[12] to decrease in the order T > G > A, C. He also found that the affinity of calf thymus DNA for inorganic mercury is at least an order of magnitude greater than that of the nucleosides and that the mercuration of the primary amines compared to mercuration at other sites appears to be greater in polynucleotides than in the nucleosides. When native calf thymus DNA is exposed to increasing amounts of $Hg(ClO_4)_2$ an inversion of the CD spectra is observed indicating a transition of the secondary structure of DNA from B-form to Z-form DNA ($0.12 < r \leqslant 1.0$) as well as a decrease in the endonucleolytic DNA cleavage rate by staphylococcal nuclease at $0.08 < r < 0.5$.[13] In agreement with the results of Dove and Yamane[14] these changes can be reversed upon removal of Hg^{II} by NaCN. These observations are explained by interstrand cross-linking of DNA bases by Hg^{II}, thus assisting its complete renaturation.

Young *et al.*[10] noticed the disappearance of the thymine imino proton resonance in the ^1H NMR spectra of poly(dA-dT) upon the addition of Hg^{II}. This observation suggests that Hg–N3 bonds are established at these sites, or that Hg^{II}-binding occurs at other sites on adenine and thymine and thereby allowing the thymine N3H protons to exchange more rapidly with bulk water. Changes in ^1H NMR chemical shifts of monomer thymine base protons upon the addition of Hg^{II} were small, while those of the thymines of poly(dA-dT) displayed larger shift effects. These observations indicate that the latter effects are mainly due to changes in the secondary structure and that intrinsic shift changes due to the covalent binding of Hg^{II} to the nucleobase ring system are negligible.[10]

Recently, Frøystein and Sletten have carried out Hg^{II} titration of a buffered solution of the DNA duplex $[d(CGCGAATTCGCG)]_2$ and monitored the titration by ^1H and ^{15}N NMR spectroscopy.[15] The results show that Hg^{II} reacts selectively with the -AATT-tract of the DNA dodecamer, in a way that modifies the Watson–Crick hydrogen bonds of the AT base pairs. At intermediate stages of the titration both the native and the mercurated forms coexist in solution giving rise to two sets of NMR signals. At about five Hg^{II} ions added per dodecamer duplex, all original ^1H signals had vanished, i.e. the conformational transition was completed. The Hg^{II} ions did not discriminate between the two unique thymines involved in Hg^{II} binding as judged from the thymine imino spectra and the changes in the thymine methyl region. No large conformational change of the secondary structure such as transition to Z-DNA, or a hairpin structure could be detected. However, the Hg^{II} binding seemed to produce a 'bulge' in the AT-tract of the duplex. A model consistent with the ^1H and ^{15}N NMR data was postulated where four Hg^{II} ions establish interstrand A-Hg-T cross-links involving deprotonated adenine amino groups and oxygen O4 on the thymines.



Scheme 2.

In the present study, the dodecamer $[d(GCCGATATCGGC)]_2$ has been titrated by $Hg(ClO_4)_2$, and the effects of mercuration monitored by 1D and 2D ^1H NMR spectroscopy. The central part in this sequence contains the recognition sequence, -GATATC-, for the endonuclease EcoRV. The main purpose of the study was to (i) probe further the postulated cross-linking model, and (ii) investigate the possibility of sequence-selective Hg^{II} binding by comparing two different AT-tracts: -AATT-vs. -ATAT-.

Experimental

Sample preparation. The dodecamer 5′-d(GCCGATATCGGC) was synthesized by the solid-phase phosphite triester technique[16] and obtained from Laboratory of Biotechnology, University of Bergen. Purification of the synthetic dodecamer was carried out by chromatography in deionized and doubly destilled water on a 120 cm Sephadex G-25 column and lyophilized to dryness. The sample was dissolved in water, and was passed through a pipette filled with chelating resin (Sigma Inc.) to get rid of any paramagnetic impurities. The palindromic sequence readily forms a duplex. The dodecamer sample used for obtaining one-dimensional NMR spectra was prepared by lyophilization and subsequent addition of 0.4 ml H_2O, containing 10% D_2O, 200 mM NaH_2PO_4 buffer (pH 7), and 200 mM $NaClO_4$. The buffers were made from analytical reagents from E. Merck. The solutions were centrifuged to eliminate any solid particles and finally transferred to 5 mm NMR tubes (Wilmad, 528-PP). A stock solution of $Hg(ClO_4)_2$ was made up in H_2O, by dissolving 3.0089 g of $Hg(ClO_4)_2$ (99%+from Alpha Products) in 100 mL of H_2O. The solution was filtrated by a fine porosity (G4) filter. The clear solution was quite stable, when kept cool. The concentration of the Hg^{II} solution was determined by atomic absorption spectroscopy: $[Hg^{II}] = 0.15 \pm 0.01$ M. Aliquots of metal salt solution (5–80 µl) were added directly into the NMR tube with a micropipette. The exchangeable proton sig-

nals of the DNA oligomer were observed before and after successive additions of aliquots of $Hg(ClO_4)_2$. Samples for acquiring the NOESY spectra in D_2O were repeatedly lyophilized to dryness from 99.9% D_2O, and once from 99.96% D_2O. Finally the sample was dissolved in 0.4 mL of 99.996% D_2O and transferred to a 5 mm NMR tube.

During the NMR investigation two different DNA samples were used. The amounts of DNA were estimated from UV measurements at 260 nm assuming that the dodecamer adopts a single-strand random-coil conformation in aqueous nonbuffered solutions. Using an extinction coefficient $\varepsilon_{260}=1.107\times10^5\ M^{-1}\,cm^{-1}$ for one strand of the oligomer[17] the concentration of DNA in 0.4 ml samples were calculated as 5.5 ± 0.5 and 4.1 ± 0.5 mM, respectively. The relatively large error limits in concentration reflect the discrepancy between the amounts of DNA determined by UV and the amounts estimated by weighing the dried DNA samples. The samples were lyophilized directly in the NMR tubes to avoid the critical transfer step of the highly deuterated solvent. The residual water signal was used as an internal chemical shift standard. After completing the experiments on the native DNA samples that required D_2O as a solvent, the samples were lyophilized to dryness and redissolved in H_2O.

^1H NMR Spectroscopy. The ^1H NMR experiments were performed at 400.13 MHz on a Bruker AM-400 WB spectrometer. The temperature was 303 K in all experiments, if not stated otherwise. The 1D experiments in water were used to observe effects of mercuration upon the imino protons involved in Watson–Crick hydrogen bonds. For the 1D spectra, the water resonance was suppressed by selective excitation of the exchangeable and aromatic protons by a time-shared Redfield '2-1-4' pulse centred at 2567 Hz upfield from the water resonance.[18] The 15° pulse was 10.33 μs, and optimal values for the asymmetric delays were 28.2 μs. The spectral width was 10 000 Hz, the recycling delay 2 s, 8192 data points were acquired in quadrature detection mode, and 256 transients were averaged. The assignments of the exchangeable proton resonances were established by means of NOE by the same pulse-sequence as mentioned above. The NOE experiments were carried out at two temperatures, 303 and 277 K. The low temperature experiments were carried out in order to minimise the effects from exchange with water protons. The imino resonances were saturated by a weak irradiation field, ca. 15 Hz, using the decoupler. On the native dodecamer 192 transients were averaged, the recycling delay was 4 s, and the irradiation time was 624 ms at both temperatures. On the mercurated dodecamer the irradiation time was reduced to 300 ms, 1024 and 3200 transients were averaged at the sample temperatures of 303 and 277 K, respectively.

NOESY and COSY experiments were used to confirm previous proton assignments[19,20] of the nonexchangeable protons of the dodecamer. NOESY experiments were also used to obtain information on conformational and structural changes in the dodecamer upon mercuration. The ^1H NOESY spectra were collected in pure-phase absorption mode with quadrature detection into 1024 complex points for 450 t_1 values, using the TPPI method.[21,22] For each t_1-value 128 transients were used with a delay of 2 s between each transient. During the recycling delay, the water signal was suppressed with a weak irradiation pulse of 20 Hz. The NOESY spectra of the native dodecamer were carried out with mixing times of 60, 100 and 300 ms, whereas two NOESY spectra of the mercurated dodecamer were acquired with mixing times of 250 and 300 ms at $r=0.057$ and one NOESY experiment with a mixing time of 200 ms at $r=0.095$.

The NMR data were processed on a Silicon Graphics IRIS 4D/35 using the program FELIX (Hare Research, Inc.). The one-dimensional FIDs were multiplied with an exponential function increasing the linewidth by 1 Hz followed by Fourier-transform and a base-line correction using a first-order polynomial fit. The residual water signal was used as a chemical shift reference and set to 4.7 ppm at 303 K. The 2D data were zero-filled to 2048 complex points along t_2, apodized with a skew (0.7) and 30° phase-shifted and squared sine-bell function. The spectra were base-line corrected with a third order polynomial fit. The FIDs along t_1 were zero-filled to 4096 complex points and apodized with a skew (0.7) and 30° phase shifted and squared sine-bell function.

Results

Since the dodecamer $[d(GCCGATATCGGC)]_2$ is self-complementary, the following number system was used for one of the symmetrical strands: 5′-d(G1-C2-C3-G4-A5-T6-A7-T8-C9-G10-G11-C12)-3′. Residues on the complementary strand are primed (′). The relative amounts of Hg^{II} vs. oligomer are given as $r=[Hg^{II}]/$[mononucleotide]. The spectral assignments of most of the nonexchangeable and exchangeable protons of the dodecamer has been made by the use of NOESY, COSY and 1D NOE.[19,20]

Hg^{II} titration and 1D ^1H NMR spectra in water. The aromatic region of a series of 1D proton spectra of the dodecamer with increasing amounts of Hg^{II} salt added are shown in Fig. 1(sample B). Almost identical results were obtained for samples A and B. The EcoRV sequence is well suited for 1D experiments since most of the aromatic proton signals (H8, H6 and H2) are clearly resolved with the exception of G11-H8/A5-H2 and C3-H6/C9-H6 (Table 1). The signals of amino protons involved in Watson–Crick base-pair hydrogen bonding typically fall in the lower part of the aromatic region while the amino protons facing the solvent appear in the upper part. At 303 K, only the amino signals belonging to the cytosines C3 and C9 were observed.

The addition of mercury to the DNA samples produces

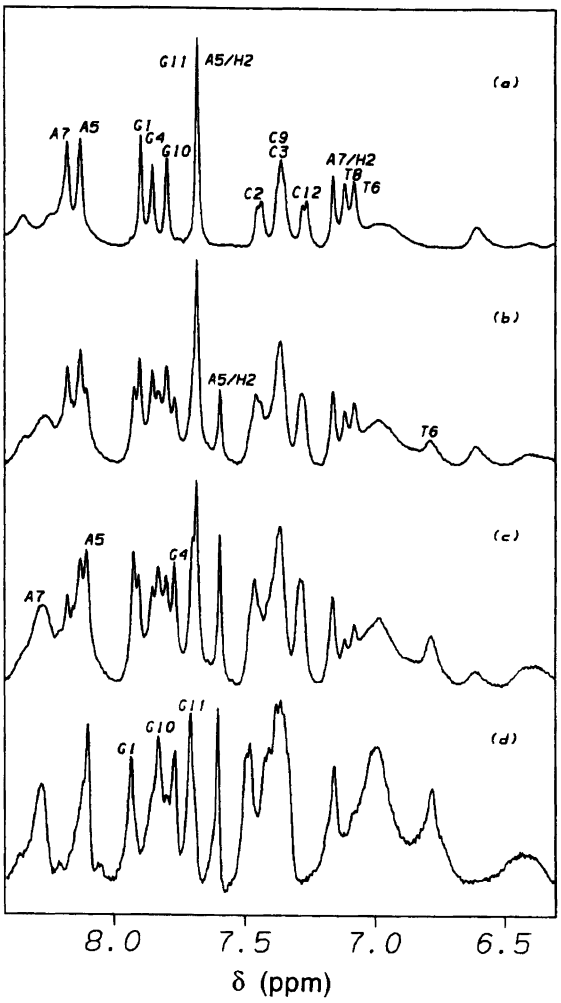


Fig. 1. 400 MHz ^{1}H NMR spectra obtained from a 4.1 mM sample of the dodecamer [d(GCCGATATCGGC)]$_2$ containing 200 mM NaH_2PO_4 buffer(pH 7) and 200 mM $NaClO_4$ salt in 90% H_2O/10% D_2O and with successive amounts of $Hg(ClO_4)_2$ added at 303 K. The Hg^{II}/nucleotide ratios were as follows: (a) $r=0$, (b) $r=0.038$, (c) $r=0.057$, (d) $r=0.095$. Only the nonexchangeable protons are annotated.

a double set of resonances. This implies that there is a slow exchange on the NMR time scale between the native and the mercurated DNA. By increasing the concentration of Hg^{II} to $r=0.09$ (ca. 2 Hg per duplex) one major component is present in the solution. A general observation is that the DNA NMR signal to noise ratio is lower when metal ions are added than when the the DNA is unperturbed by metal interaction.[4,5,15] The most prominent chemical shift changes of the aromatic protons are the large upfield shifts of T6-H6 (−0.27 ppm) and C9–H5 (0.24 ppm) (Table 1). The methyl resonances of thymine T6 and T8 are shifted upfield (−0.11 ppm) and downfield (0.17 ppm), respectively. The aromatic proton resonances of the flanking residues (G1, C2, C3, G10, G11, C12) hardly experience any chemical shift changes upon mercuration. For the sugar protons H1′, H2′/2″, H3′, H4′ one may notice relatively large shift changes for the mercurated species, especially for residues G4 and T6.

The imino proton regions of the spectra at different levels of mercuration are shown in Fig. 2. Only the four imino signals of the central part of the duplex are observed at 303 K. The imino signals involved in the two end-base pairs are not detected due to rapid exchange with bulk water (terminal fraying). At the higher magnetic field (500 MHz) and lower sample temperature (292 K) the G11 imino signal is observed close to the G4 imino signal. The assignments of the imino resonances have been made previously[19] and are in accordance with the values expected for double helical DNA. The signals of the imino protons of the two thymines (T6/T8) appear downfield and those of the two guanines (G4/G10), upfield. No signal was observed around 11–12 ppm, the region typical for hairpin species.

The addition of Hg^{II} to the dodecamer produces dramatic chemical shift changes for the imino signals of guanine G4 and thymine T8 (Table 2). The intensity of the G4 signal is gradually reduced with a concomitant build-up of a new signal 0.15 ppm downfield of the original signal. At a titration level corresponding to approximately two Hg^{II} per duplex a 90/10 equilibrium is reached between the new and old signals. Further increase in the Hg^{II} concentration does not significantly alter the 90/10 ratio. The thymine T8 imino signal undergoes a gradual reduction in intensity and vanishes completely at a mercuration level of about 2 Hg^{II} per duplex. Upon further additions of mercury the resonance re-emerges −0.12 ppm upfield from the old position reaching maximum signal intensity at $r=0.18$ (ca. 4 Hg/duplex). Since the T6 and G10 imino resonances are partly overlapping it is difficult to follow chemical shift changes of these two signals by 1D NOE measurements during the full titration experiment. The spectra recorded at 277 K were poorly resolved owing to excessive line broadening as a result of increased viscosity at this temperature. Thus we were not able to assign the resonances of the amino protons of adenines and guanines.

2D ^{1}H NMR in D_2O. Contour plots of the aromatic/H1′ cross-peak region of the NOESY maps recorded for (a) the native form, (b) 1.4 Hg/duplex ($r=0.057$), and (c) 2.3 Hg/duplex ($r=0.095$) are presented in Fig. 3. The corresponding maps of the aromatic methyl region are displayed in Fig. 4. The intraresidue cross-peaks are labelled according to the sequential numbering of the residues. Since in regular B-form DNA the intra- and inter-residue distances between H1′ and the base protons are <5 Å, sequential assignments are possible as indicated by solid lines connecting alternating intra- and inter-residue cross-peaks. The remaining cross-peaks, not connected by solid lines correspond to cytosine H5/H6 and H5/H1′ distances and adenine H2/H1′ inter-and intra-strand distances. One additional cross-peak (top of Fig. 3) between G1–H8 and G12–H1′ originates from end-to-end association of the dodecamers in solution.

The NOESY map corresponding to 1.4 Hg/duplex [Fig. 3(b)] displays a double set of cross-peaks which

Table 1. ^{1}H Resonance assignments for the nonexchangeable protons of the DNA dodecamer [d(GCCGATATCGGC)]$_2$. Sample B in D_2O (i) without Hg^{II} and (ii) in the presence of Hg^{II}.[a]

	^{1}H Chemical shift (ppm)						
Nucleotide	H6 H8	H2 H5 CH3	H1′	H2′	H2″	H3′	H4′
G1	7.96		5.98	2.65	2.79	4.88	*3.78*
	0.05	0.04	0.04	0.05	0.04	*0.06*	
C2	7.52	5.39	6.02	2.17	2.51	4.88	4.17
	0.04	0.08	0.03	0.03	0.03	0.06	*0.03*
C3	7.44	5.61	5.58	*2.03*	2.39	*4.88*	*4.14*
	0.03	0.10	0.04	*−0.06*	−0.08	*0.00*	*−0.04*
G4	7.92	5.66	2.74	2.85	5.06	*4.06*	
	−0.05	−0.34	*−0.22*	*−0.21*	*−0.06*	*0.06*	
A5	8.19	7.76	6.26	2.65	2.97	5.05	4.29
	0.00	−0.06	−0.08	−0.02	−0.14	−0.04	−0.05
T6	7.15	1.47	5.72	2.11	2.52	4.91	4.22
	−0.27	−0.11	−0.25	−0.50	−0.16	−0.15	−0.10
A7	8.24	7.25	6.27	2.64	2.96	5.03	4.22
	0.13	[b]	−0.02	*−0.10*	0.00	0.05	0.02
T8	7.19	1.34	5.95	2.06	2.47	4.88	*4.17*
	0.06	0.17	−0.12	0.08	0.04	0.06	*0.07*
C9	7.42	5.59	*5.59*	*1.97*	*2.37*	*4.87*	*4.14*
	0.09	*0.24*	*0.22*	*0.03*	*0.05*	*0.02*	*0.04*
G10	7.87	5.67	*2.76*	*2.69*	5.02	4.06	
	0.05	−0.02	*−0.03*	*0.14*	0.03	*0.02*	
G11	7.74	5.99	2.54	2.74	5.01	*4.22*	
	0.05	0.03	0.06	0.06	0.04	*0.01*	
C12	7.33	5.19	6.16	2.26	2.75	5.00	4.06
	0.05	0.07	0.05	0.04	−0.23	0.03	0.04

[a] For each residue the first line lists the chemical shifts prior to Hg^{II} addition, whereas the second line represents the changes in the chemical shifts at a mercury level of two Hg^{II}/duplex. The values are referenced to the residual HDO peak, for which the chemical shift has been set to 4.70 ppm. The H5′/H5″ resonances are not included, due to poor resolution of the resonances. Chemical shifts printed in italics are from poorly resolved or overlapping resonances, where the exact chemical shift values are impossible to measure. [b] Not assigned.

merge to a single set at 2.3 Hg/duplex. The most dramatic effect of mercuration is the break in the inter-residue sequential 'walk' between thymine T6–H1′ and adenine A7–H8. A corresponding break is observed between T6–H2′/2″ and A7–H8. All the other residues may be sequentially linked in the complexed species indicating that the major part of the native, right-handed B-form duplex is still intact.

The NOESY spectrum of the sample containing 4 Hg^{II}/duplex ($r=0.168$) had a low signal-to-noise level, and consequently yielded less useful information (data not shown). However, the main features from the NOESY spectrum of the 2.3 Hg per duplex sample concerning the flanking residues are still present. The cross-peak involving T6 has vanished due to motional averaging and/or structural changes.

Discussion

The present study confirms some important aspects of the previous findings concerning the nature of Hg^{II} ion reaction with DNA oligo-nucleotides that contain both AT base pairs as well as GC base pairs.[15] The most important observation is that the thymine N3H imino protons are not displaced by Hg^{II} ions as implied in the chain-slippage model.[8,9] The fact that all the imino resonances are present in short hetero-oligonucleotides does confirm that the oligomer is a hydrogen bonded double helix at a mercuration level of four Hg^{II} per duplex. However, one aspect which is difficult to rationalize is the temporary disappearance of the T8 imino signal at the intermediate level of 2.3 Hg^{II} per duplex. On the other hand, the slight decrease in the NMR signal intensity of the G10 imino proton can be attributed to the fact that G10 is the across-strand nearest neighbour to G4 and the establishment of a new resonance position of the G4-imino signal do have some influence on the G10 imino signal.

At this titration stage there exists only one duplex species in solution as confirmed by a single set of resonances in the NOESY map. This duplex evidently has adopted a 'bulged' structure where the A5′–N1···HN3–T8 hydrogen bond is in rapid exchange with the solvent water. The distortion of the duplex is most clearly reflected in the NOESY map where the cross-peak, T6–H6/H′1–A7, is missing, indicating that this inter-residue distance is increased from the normal 3.7 Å in regular B-form DNA to a distance >5 Å or

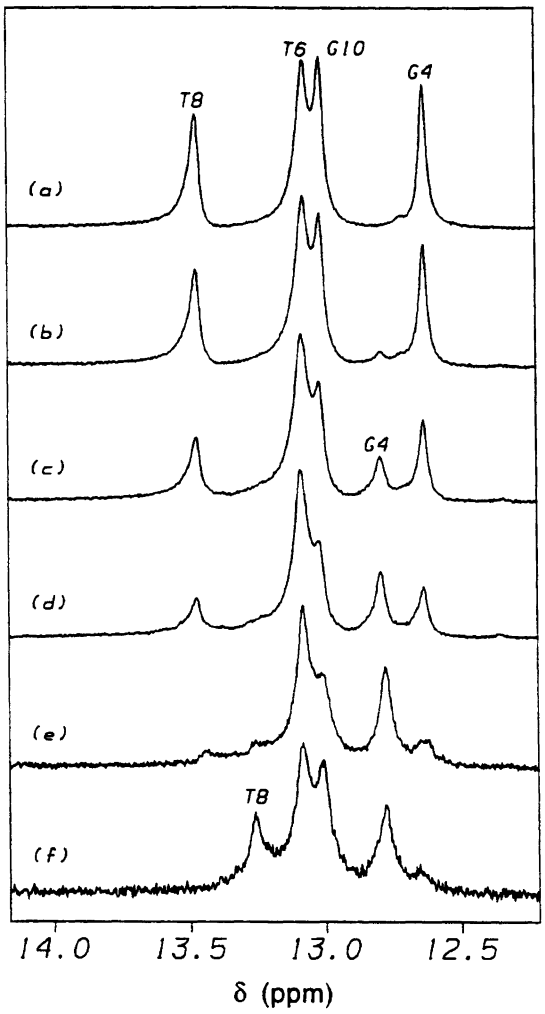


Fig. 2. 400 MHz ^{1}H NMR spectra of the imino region of the dodecamer [d(GCCGATATCGGC)]$_2$ containing 200 mM NaH_2PO_4 buffer (pH 7) and 200 mM $Na(ClO_4)_2$ salt in 90% H_2O/10% D_2O and with successive amounts of $Hg(ClO_4)_2$ added at 303 K. The Hg^{II}/nucleotide ratios were as follows: (a) $r=0$, (b) $r=0.020$, (c) $r=0.038$, (d) $r=0.057$, (e) $r=0.095$ and (f) $r=0.168$. The spectra are obtained by means of a time-shared Redfield '2-1-4' pulse to avoid the excitation of the H_2O resonances.

that motional averaging ('breathing') eliminate the signal. The neighbouring base and sugar residues undergo appreciable structural modifications which leads to changes in ring current shifts.

Young *et al.*[10] have proposed a model for mercurated poly(dA-dT) based on the chemical shift pattern and an analysis of the effects of differences in ring current shifts between purine and pyrimidine. We find it difficult to carry out a detailed quantitative chemical shift analysis for the heteropolynucleotides. However, the spectroscopic data clearly show that there is no major alterations in the secondary structure, e.g. B→Z-form or duplex→hair pin transition. The sequential walk is completed except for the T6···A7 break. This result is in complete agreement with the previous NMR study on

Table 2. ^{1}H Resonances assignement for the observed exchangeable protons of the DNA dodecamer [d(GCCGATATCGGC)]$_2$, Sample B in H_2O: (i) without Hg^{II} and (ii) in the presence of Hg^{II}.

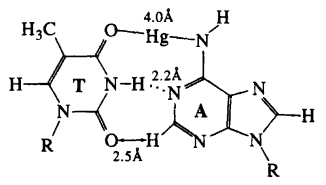
	^{1}H chemical shifts (ppm)		
Base pair[a]	G NH1/T NH3	C NH4[b]	
G4:C9′	12.65	8.37	6.62
	0.15	−0.10	0.00
T6:A7′	13.09		
	0.01		
T8:A5′	13.48		
	−0.12		
G10:C3′	13.02	8.50	6.98
	0.01	0.14	0.01

[a] The numbering of the residues are the same on both strands; the two symmetrically related strands are distinguished by a prime (′) on the residue number of the second nucleotide of each base pair. [b] The cytosine amino protons which participate in the Watson–Crick hydrogen bonds resonate at lowest field.

the -AATT- sequence in which a 'bulged' structure was suggested.[15]

The hydrogen ion release experiments of Yamane and Davidson[7] on natural DNA and poly (dA-dT) showed that the number of protons released per Hg^{II} ion in the DNA sample is close to 2 in the initial phase of titration and gradually decreases to 1 as $r\rightarrow 0.5$, except for the A-T polymer where the decrease is rather markedly around $r=0.25$. If we assume that: (i) on the average one proton is released per Hg^{II} ion in the reaction with the EcoRV oligonucleotide; (ii) the thymines are not deprotonated in the reaction; and (iii) interstrand bridging is required to allow for reversible renaturation, we propose a tentative model where the amino group on adenine is deprotonated and cross-linked by Hg^{II} to thymine O4 (Scheme 3).

This model is constructed by opening one side of the AT base pair through an in-plane 26° rotation to accommodate the O4···Hg···N6 bond. The distance of 4 Å used for the TO4–Hg–N6A bridge is roughly equal to the sum of covalent Hg–N and Hg–O distances as determined in linear O–Hg–O and N–Hg–N complexes by X-ray crystallography.[23] The rotation leads to a weakening of the central T–N3–H···N1–A hydrogen bond and shortening of the non-bonded inter-atomic distance A–H2···O2–T. If a certain degree of propeller twist, which often is observed for Watson–Crick base pairs, is invoked for the model, this will reduce the strain further. A similar model is less favourable for Hg binding



Scheme 3.

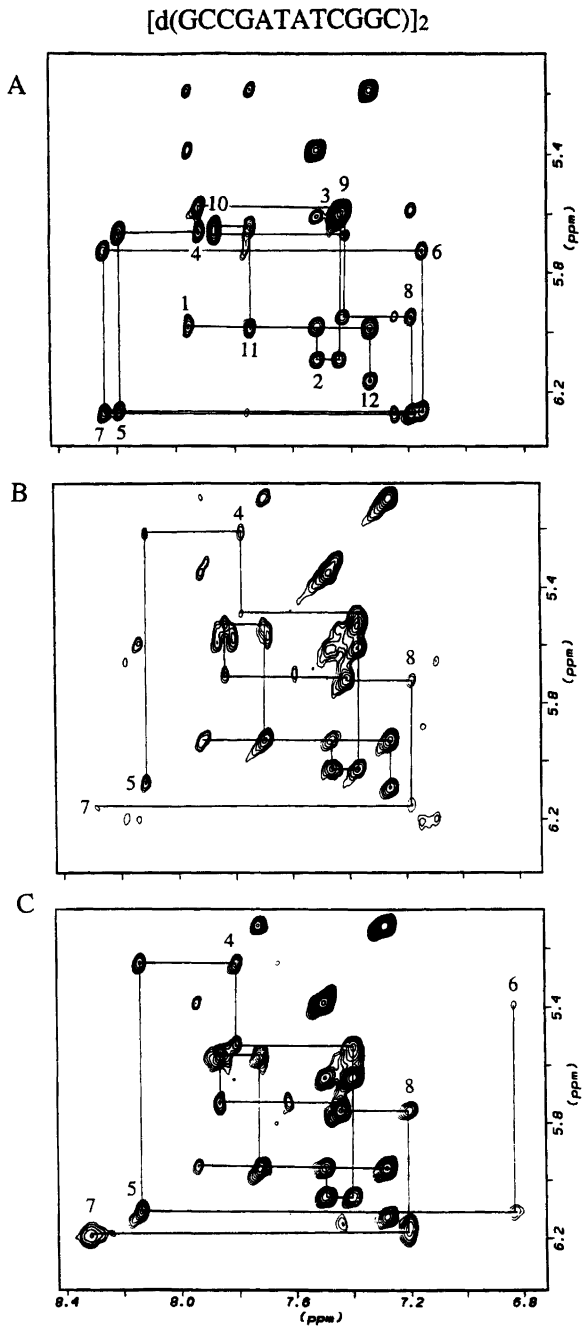


Fig. 3. 400 MHz ^{1}H NOESY (300 ms mixing time) contour plots of the H8/H6 vs. H1′/H5 region of the dodecamer [d(GCCGATATCGGC)]$_2$ (sample B, see caption of Fig. 1) in D_2O with different amounts of $Hg(ClO_4)_2$ added: (A) $r=0$, (B) $r=0.057$ and (C) $r=0.095$. The sequential walks are marked with a solid line.

to a GC base pair in which case the third hydrogen bond in the base pair will have to be disrupted in order to accommodate the mercury ion. It is tempting to speculate that this may be one of the factors which explain why Hg^{II} favours AT-rich regions rather than GC-rich regions.[7]

The proposed model does indeed explain the first part of the titration experiment ($r<0.09$). In this phase of the

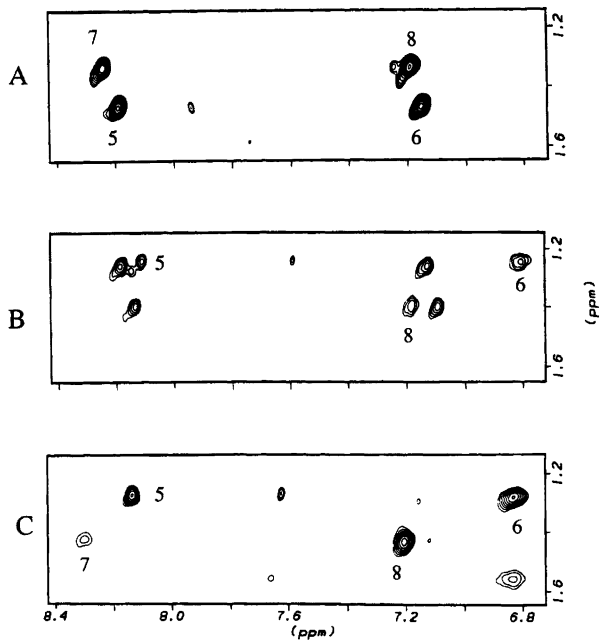


Fig. 4. 400 MHz ^{1}H NOESY contour plots of the H6/H8 vs. methyl region of the dodecamer duplex [d(GCCGATATCGGC)]$_2$ (see caption of Fig. 3).

reaction, the T8–N3H···N1–A′5 hydrogen bond in the new mercurated form is weakened with N3H in rapid exchange with bulk water. The T8 signal in the native form is gradually reduced in intensity until the transition to a new conformer is completed at around 2.3 Hg/duplex. Since one proton per Hg^{II} ion is assumed to be released in this process deprotonation of the amino group of adenine is invoked. Unfortunately, the amino resonances of adenine and guanine could not be assigned even at 277 K owing to motional broadening and general deterioration of signal-to-noise during the titration. Thus we are not able at this stage to prove unambiguously, that the amino group is indeed deprotonated.

In the second part of the titration the hydrogen bond involving T8–N3H is re-established, indicating that some kind of cooperative bridging by additional Hg^{II} ions has stabilized the duplex through structural alteration. Williams and Crothers[24] investigated the binding kinetics of poly(A)·poly(U) and showed that the reaction occurs in two phases which differ in timescale by a factor of about 100. The fast phase is second order, whereas the slow phase is first order and exhibits cooperative behaviour. They proposed a working hypothesis in which Hg^{II} serves to crossbridge the adenine amino group to N3 of uridine leaving the double helix intact in the fast reaction phase. The involvement of adenine amino groups in this type of reaction is also implied in a CD study of Hg^{II}-poly(dA) interaction where the authors suggest that Hg^{II} cross-links the adjacent amino groups of the stacked adenine bases in the same strand.[25]

The selection of metal binding sites in DNA is in general related to two important features: (i) the molecular electrostatic potential and (ii) the steric accessibility

of the reactive sites.[26] For monodentate binding of metal ions to DNA the nucleophilicity of the binding site has been shown to be the determining factor. The binding of Mn^{II} and Zn^{II} to oligonucleotides was found to be localized at the most basic sites.[4] Hg^{II}, on the other hand, prefers linear coordination, and thus the steric factors may be decisive in order to accommodate a linear interstrand bridge in the DNA matrix. However, we cannot rule out guanine N7 as a secondary monodentate binding site for Hg^{II} ions in the present study.

The final point to be discussed is the apparent sequence-selective binding of mercury to AT base pairs. In the EcoRI sequence the four central base pairs, -AATT-, were influenced in an identical manner by mercury ions.[15] The imino signals T7 and T8 disappeared at exactly the same rate due to rapid exchange with solvent water. Furthermore, the two thymine methyl signals gradually moved to new chemical shift positions at the same rate. At about five Hg^{II} ions added per dodecamer duplex, all original ^1H NMR signals vanish, i.e. the conformational transition to a fully mercurated form is completed. In contrast to EcoRI, the mercuration of EcoRV appears, initially, to be sequence dependent as judged from the behaviour of the thymine imino signals (Fig. 2). While T8–N3H shows dramatic Hg^{II} induced shift variation T6–N3H is unperturbed. The two sequences do differ in one important sequential aspect; in EcoRI both thymines appear in the context thymine-pyrimidine (T-Py) while in EcoRV, T6 and T8 are found in the context thymine-purine (T-Pu) and thymine-pyrimidine (T-Py), respectively. It appears that there is a difference in Hg selectivity for thymines between (T-Py) and (T-Pu) sequences. Indications of sequence-dependent Hg^{II}-polynucleotide binding have also been observed by CD spectroscopy by Gruenwedel and Cruikshank.[13] They report preliminary CD results which shows that while polynucleotides with (Pu-Pu) sequences undergo Hg^{II}-induced CD inversions, those with Pu-Py or Py-Py sequences do not. Molecular models containing -AATT- and -ATAT- sequences in idealized B-form DNA indicate that of the two T-Pu and T-Py contexts, the latter has a more favourable electrostatic 'pocket' around T-O4.

Conclusion

A comparison between the results from our previous study on mercurated $[d(CGCGAATTCGCG)]_2$ (EcoRI) and the present study on mercurated $[d(GCCGATATCGGC)]_2$ (EcoRV), shows that Hg^{II} ions induce a transition to a new conformer of the dodecamers. In the former case five Hg^{II} ions are needed to complete the transition, in the latter case a two-stage transition occurs, at two and four Hg^{II} ions per duplex, respectively. The apparent sequence-selectivity may be related to differences in affinity between thymines in the context (T-purine) and (T-pyrimidine). The presence of the imino signals at a mercuration level of 4 Hg/duplex clearly reveals that thymine N3 is not the Hg^{II} binding site in this short heteropolynucleotide. For longer homopolymers of the type poly(dA-dT) the chain slippage mechanism where T-N3 is deprotonated cannot be completely ruled out based on the present data. In order to account for reversible Hg^{II} binding and the reported proton release in these reactions a model is proposed where the amino group of adenine is deprotonated and cross-links thymine O4 via Hg^{II}.

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