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Metal Ion–Biomolecule Interactions. Part 13.* NMR Evidence for the Formation of the Mixed Ligand Thymidine–Mercury–Guanosine Complex. A Model for a Putative Hg(II) Interstrand Cross-linking Structure of DNA

E. BUNCEL**, C. BOONE and H. JOLY

Department of Chemistry, Queen's University, Kingston, Ont., K7L 3N6, Canada

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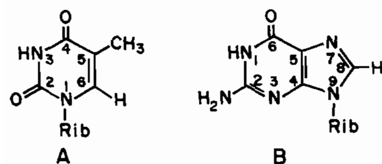
Abstract

^1H and ^{13}C NMR evidence is presented for the formation of the mixed ligand complex, [Thy–Hg–Guo] (**E**). This was obtained through equilibration, in dimethyl sulfoxide solution, of 1 equivalent of the symmetrical complex [Thy–Hg–Thy] (**C**) with 2 equivalents of free guanosine, or similarly 1 equivalent of [Guo–Hg–Guo] (**D**) with 2 equivalents of free thymidine. The relative stabilities of the nucleoside–mercury–nucleoside complexes involved in the equilibration process is **C** > **D** > **E**. The mixed ligand complex **E** appears to contain a $ThyN_3$–Hg–$GuoN_1$ bond and thus supports an interstrand structure previously proposed for Hg(II) binding to DNA. The relative stability **C** > **D** > **E** is consistent with the postulate that the [Thy–Hg–Thy] interstrand complex represents the thermodynamically most stable mode of Hg(II)–DNA interaction under physiological conditions.

Introduction

Mercury(II) binds specifically to the heterocyclic bases of DNA rather than the ribose moiety or phosphate oxygens of the phosphodiester linkages [2–4]. The preferred interactions at 1:1 $Hg(OH)_2$ (or CH_3HgOH): mononucleoside ratios are through proton displacement at N_3–H of thymidine (ThyH, **A**) > N_1–H of guanosine (GuoH, **B**), with several weaker sites available in adenosine and cytosine [4, 5]. The binding of Hg(II) is favoured by adenosine–thymidine rich DNAs [4].

It has been postulated by Katz [6] that the most stable complex of Hg(II) with DNA is achieved when

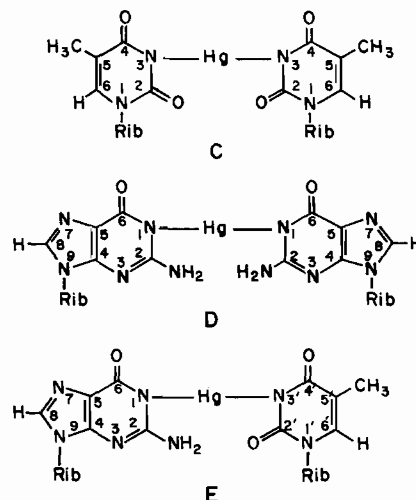


*Part 12, ref. 1.

**Author to whom correspondence should be addressed.

thymidine nucleoside pairs on opposite strands of a DNA molecule chelate Hg(II) through proton substitution. The elucidation of the crystal structure of the [methylthymine–Hg–methylthymine] complex provided a model of this type of structure and presented geometrical constraints for such a Hg(II) interstrand bridge of DNA [7]. Furthermore, ^1H NMR analysis of the mercuration of poly (dA–dT) at $r = 0.25$ (r = Hg(II)/nucleotide) confirmed that binding occurred at thymidine N_3–H through proton substitution, and supported the model that Hg(II) cross-linked opposite strands of a DNA polymer [8]. Yet, in general, the binding of Hg(II) to DNA is still poorly understood at the molecular level. Little information is available on the significance of the guanosine-N_1 binding site, and thus experimental evidence for interstrand structures of the type [Nuc_1–Hg–Nuc_1] and [Nuc_1–Hg–Nuc_2], where Nuc_1 (Nuc_2) = Guo(Thy) have not been documented.

As part of a continuing investigation of the interactions of mercury(II) and methylmercury(II) with nucleic acid constituents [1, 9–14], we previously reported [1] the preparation of the mercury(II)-bridged nucleoside complexes [Thy–Hg–Thy] (**C**) and [Guo–Hg–Guo] (**D**):



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The complexes **C** and **D** were used as reference compounds in studies to determine the preferential binding of Hg(II) towards guanosine and thymidine. From competition studies involving 2 equivalents of GuoH, ThyH and 1 equivalent of HgO, it was concluded that Hg(II) binds, with proton displacement, preferentially to N_3 of ThyH as compared to N_1 of GuoH; yet reaction with both nucleosides was significant. Since the reactions appeared to be thermodynamically controlled, the preferential formation of the bridged species **C** provided evidence that the stability of the Hg(II) binding sites, at 2:1 Hg(II):mononucleoside ratio, is N_3 of thymidine > N_1 of guanosine.

Interestingly, no evidence was found in the previous work [1] for formation of the mixed species [Thy–Hg–Guo] (**E**). Moreover, there appears to be no report in the literature on the characterization of mixed nucleoside–mercury–nucleoside complexes. Mixed mercury compounds of the type [A–Hg–A′] are known [15–18], but the factors which determine the formation of any particular mixed ligand complex are still incompletely understood [17, 18].

As an extension of the previous study [1], it was desirable to carry out equilibration experiments by a modified procedure. In the earlier work the nucleosides ThyH and GuoH were allowed to equilibrate with HgO in aqueous solution, following which the reaction mixture was lyophilized and the products dissolved in $(CD_3)_2SO$ for NMR analysis. This raises the question whether equilibrium redistribution could have occurred in the DMSO medium. In the present work the symmetrical species **C** and **D** were allowed to separately equilibrate with the free nucleosides GuoH and ThyH, respectively, directly in $(CD_3)_2SO$ solution, and the reaction mixtures analyzed by ^1H and ^{13}C NMR *in situ*. Moreover, the sensitivity of the NMR method was considerably improved through use of 400 MHz instrumentation as compared to the 60 MHz and 200 MHz instruments used in the earlier study [1].

The series of experiments reported here have in fact provided ^1H and ^{13}C NMR evidence for the formation of the unsymmetrical species **E**. The results show that the relative stabilities of the mercury bridged complexes follow the order [Thy–Hg–Thy] > [Guo–Hg–Guo] > [Thy–Hg–Guo]. In a broader sense, these findings support the chain slippage mechanism proposed by Katz [6] for the binding of Hg(II) to DNA.

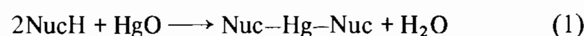
Experimental

^1H and ^{13}C NMR spectra were obtained on a Bruker AM 400 instrument operating in the Fourier transform mode (400 MHz for ^1H and 100.6 MHz for ^{13}C). Chemical shifts are referenced with respect to internal tetramethylsilane (TMS) for ^1H and $(CD_3)_2SO$ for ^{13}C. All spectra were run at room temperature (25 ± 2 °C).

Guanosine (Sigma), thymidine (Sigma) and HgO (Chemalog) were used as received. The complexes [Thy–Hg–Thy] and [Guo–Hg–Guo] were prepared as described previously [1]. The equilibrations were performed by mixing 1 equivalent [Thy–Hg–Thy] and 2 equivalents GuoH, or 1 equivalent [Guo–Hg–Guo] and 2 equivalents ThyH, in $(CD_3)_2SO$ solution at room temperature, and NMR spectra were recorded within a few minutes of mixing.

Results and Discussion

The complexes [Thy–Hg–Thy] (C) and [Guo–Hg–Guo] (**D**) were prepared by reaction of 2 equivalents of ThyH or GuoH with 1 equivalent of HgO in aqueous solution (eqn. (1)) as described previously [1].



$$2NucH + HgO \longrightarrow Nuc{-}Hg{-}Nuc + H_2O \qquad (1)$$

Equilibrations were performed in dimethyl sulfoxide solution by mixing 1 equivalent of **C** with 2 equivalents of free GuoH, or using 1 equivalent of **D** with 2 equivalents of free thymidine. The ^1H and ^{13}C NMR spectra of the resulting reaction mixtures were identical in all respects for the two types of experiments.

The ^1H and ^{13}C NMR data for authentic **C**, **D**, free ThyH (A) and GuoH (**B**), as well as for the equilibrium mixtures containing **E**, are given in Tables I and II respectively. Representative NMR spectra following the equilibrations are presented in Figs. 1 and 2.

The binding of Hg(II) in reaction (1) results in the displacement of the nucleoside imino proton (e.g. guanosine N_1–H, thymidine N_3–H) and leads to minor but significant perturbations of the intrinsic nucleoside proton resonance chemical shifts [1, 8]. A comparison of the proton decoupled ^{13}C NMR spectra of complexes of the type (R–Hg–Nuc) (where R = CH_3 or Nuc, *i.e.* Guo or Thy) with the spectra of the unreacted nucleoside, has demonstrated that changes occur in the chemical shift of the carbon atoms in the vicinity of mercuration [1, 9, 20]. A brief description of the ^1H and ^{13}C NMR spectra of [Thy–Hg–Thy] and [Guo–Hg–Guo] is presented below (see also ref. 1).

The ^1H NMR spectrum of [Thy–Hg–Thy] (**C**) exhibits a downfield shift of the C_5–CH_3 and $H_{1\text{-}Rib}$ resonances relative to thymidine (A), the N_3–H signal being absent (see Table I). Similar relative shifts have been observed for the reaction of $HgCl_2$ with thymidine at a ratio of 1:2 in aqueous solution [8]. The proton decoupled ^{13}C NMR spectrum of **C**

TABLE I. ^1H NMR Chemical Shifts for ThyH, GuoH, [Thy–Hg–Thy], [Guo–Hg–Guo] and the Equilibration Reaction of 1 Equivalent of [Thy–Hg–Thy] and 2 Equivalents of GuoH, or 1 Equivalent of [Guo–Hg–Guo] and 2 Equivalents of ThyH

Compounds	Chemical shifts[a, b] (ppm)						
	N_3–H	N_1–H	C_8–H	C_6–H	NH_2	$H_{(1\text{-rib})}$[c]	C_5–CH_3
[ThyH] (**A**)	11.28	–	–	7.70	–	6.17(t)	1.77(d)
[Thy–Hg–Thy] (**C**)	–	–	–	7.70	–	6.21(t)	1.82
[GuoH] (**B**)	–	10.64	7.94	–	6.46	5.70(d)	–
[Guo–Hg–Guo] (**D**)	–	–	7.92	–	6.78	5.71(d)	–
Equilibration[d]	11.30	10.65	7.94	7.70	6.46	6.17(t)	1.77
Reaction			7.92	7.75	6.79	6.21(t)	1.82
			7.91[e]		6.54[e]	6.25(t)[f]	1.85[f]

[a]In $(CD_3)_2SO$; chemical shifts are measured from $(CH_3)_4Si$ internal standard. [b]All resonances are singlets unless otherwise indicated; d = doublet, t = triplet. [c]$H_{(1\text{-rib})}$ signals resulting from the thymidine moiety could only be resolved under the equilibration reaction condition. [d]Equilibration reaction contains ThyH, GuoH, [Thy–Hg–Thy], [Guo–Hg–Guo] and [Thy–Hg-Guo]. [e]Signals assigned to the guanosine moiety of [Thy–Hg–Guo]. [f]Signals assigned to the thymidine moiety of [Thy–Hg–Guo].

TABLE II. ^{13}C NMR Chemical Shifts for ThyH, GuoH, [Thy–Hg–Thy], [Guo–Hg–Guo] and the Equilibration Reaction of 1 Equivalent [Thy–Hg–Thy] and 2 Equivalents of GuoH, or 1 Equivalent of [Guo–Hg–Guo] and 2 Equivalents of ThyH

Compounds	Chemical shifts[a, b] (ppm)					
	C_6	C_2	C_4	C_8	C_5	C_5–CH_3
[ThyH] (**A**)	136.2	150.5	163.8	–	109.4	12.3
[Thy–Hg–Thy] (**C**)	136.3	153.2	166.3	–	109.5	13.1
[GuoH] (**B**)	156.9	153.7	151.4	135.8	116.7	–
[Guo–Hg–Guo] (**D**)	161.0	157.2	152.0	135.7	116.8	–
Equilibration[c]	136.1	150.4	163.7		109.3	12.3
Reaction	136.3	153.2	166.3		109.4	13.1
	156.7	153.6	151.3	135.5	116.7	
	161.0	157.2	151.9	135.7		
		153.3[d]	166.5[d]			13.2[d]

[a]In $(CD_3)_2SO$; chemical shifts are measured from $(CD_3)_2SO$ internal standard. [b]Ribose resonances omitted. [c]Equilibration reaction contains ThyH, GuoH, [Thy–Hg–Thy], [Guo–Hg–Guo] and [Thy–Hg–Guo]. [d]Signals assigned to the thymidine moiety of [Thy–Hg–Guo].

indicates a prominent downfield shift of the C_2 and C_4 signals with a minor shift of the C_5–CH_3 signal, consistent with the binding of Hg(II) at N_3 of thymidine (see Table II).

The ^1H NMR spectrum of [Guo–Hg–Guo] (**D**) exhibits an upfield shift of the C_8–H resonance and a downfield shift of the NH_2 resonance relative to guanosine (**B**), with the N_1–H signal absent (see Table I). It has been postulated that this somewhat large downfield shift of the amino resonance results from an intramolecular H-bonding interaction analogous to that observed in the crystal structure of the platinated [G–G$^-$] pair [1, 21]. The binding of Hg(II) leads to a downfield shift of the guanosine C_2 and C_6 ^{13}C NMR resonance signals implicating guanosine N_1 as the site of reaction (Fig. 2, Table II).

When [Guo–Hg–Guo] (**D**) and thymidine (**A**), or [Thy–Hg–Thy] (**C**) and guanosine (**B**), are dissolved in $(CD_3)_2SO$ with a molar ratio of 1:2 ([Nuc–Hg–Nuc]/nucleoside), an identical, rapid, equilibrium redistribution results from each system. The ^1H NMR spectrum of this exchange reaction is consistent with the presence of **C**, **D**, the uncomplexed nucleosides **A** and **B**, as well as the mixed ligand complex [Thy–Hg–Guo] (**E**). This is shown by the individual characteristic resonance signals appearing at 1.77 ppm (C_5–CH_3 of **A**), 1.82 ppm (C_5–CH_3 of **C**), 1.85 ppm (assigned to C_5–CH_3 of **E**), 6.46 ppm (NH_2 of **B**), 6.79 ppm (NH of **D**), 6.54 ppm (assigned to NH_2 of **E**), 10.65 ppm (N_1–H of **B**) and 11.30 ppm (N_3–H of **A**) (see Fig. 1, Table I). Minor signal shifts which also allow the resolution of three forms of each

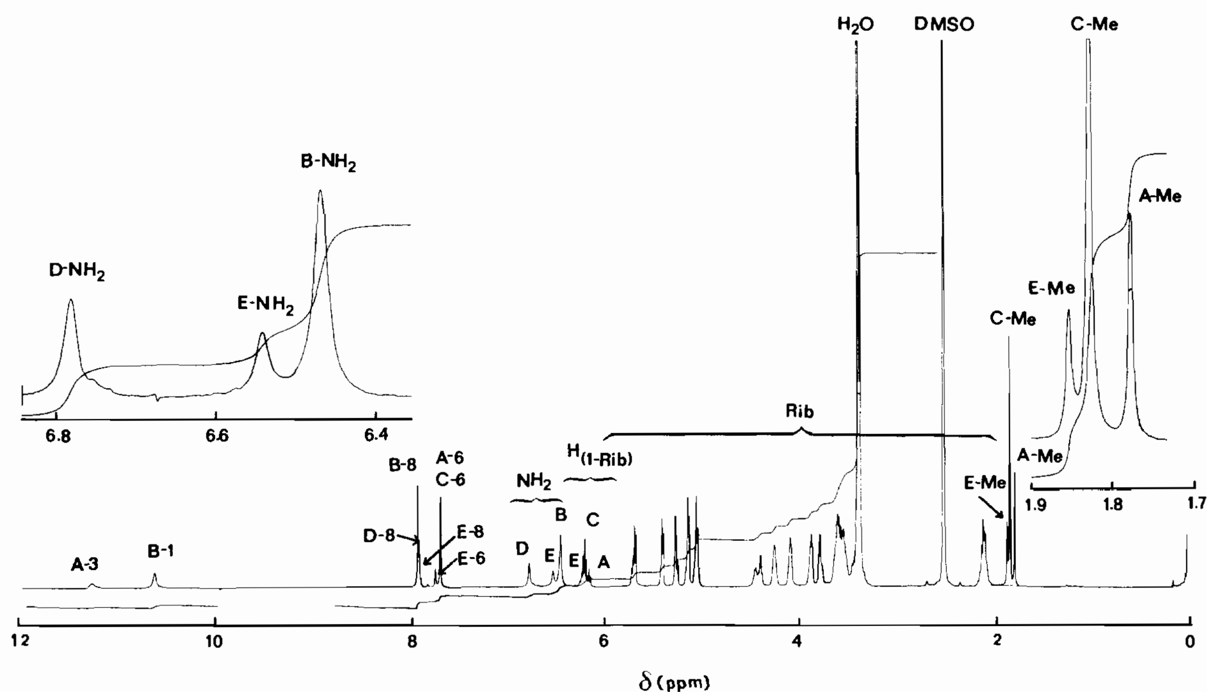


Fig. 1. ^1H NMR spectrum showing the partial formation of [Thy–Hg–Guo] (**E**), [Guo–Hg–Guo] (**D**) and thymidine (**A**) in the equilibration of guanosine (**B**) and the bridged complex [Thy–Hg–Thy] (**C**).

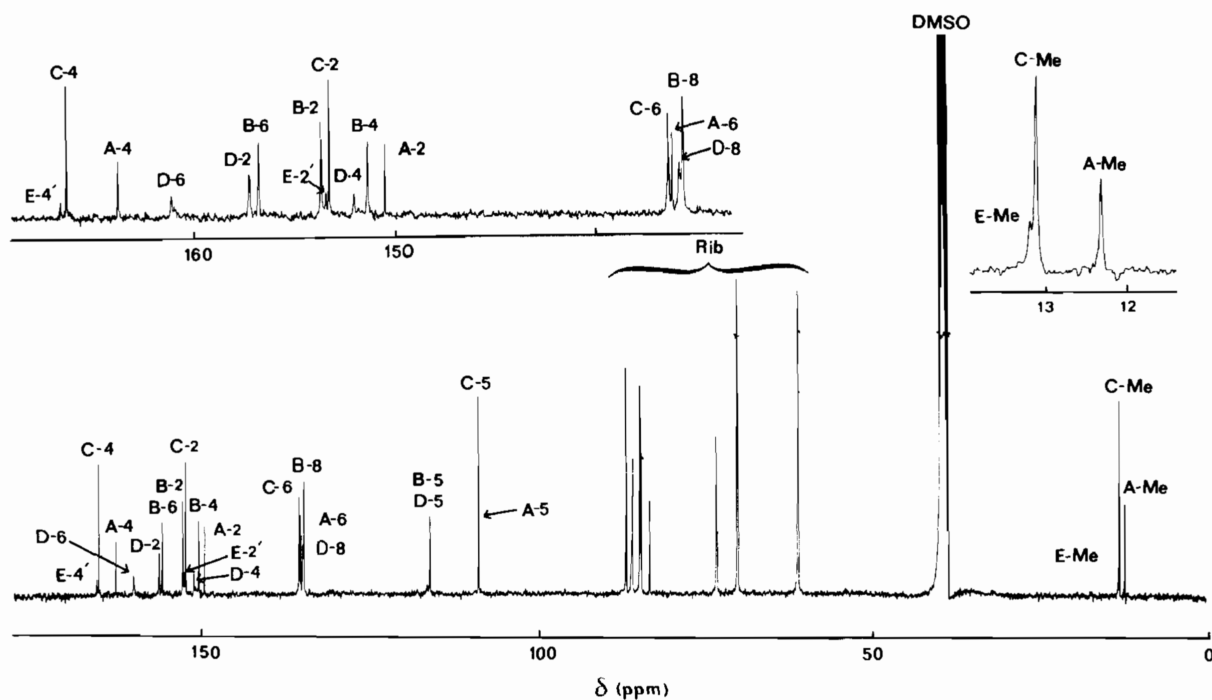


Fig. 2. ^{13}C NMR spectrum showing partial formation of [Thy–Hg–Guo] (**E**), [Guo–Hg–Guo] (**D**) and thymidine (**A**) in the equilibration of **B** and **C**.

nucleoside are observed for the resonances appearing at 6.17 ppm ($H_{1\text{-Rib}}$ of **A**), 6.21 ppm ($H_{1\text{-Rib}}$ of **C**), 6.25 ppm (assigned to $H_{1\text{-Rib}}$ of the thymidine ribose moiety of **E**), 7.94 ppm (C_8–H of **B**), 7.92 ppm (C_8–H of **D**) and 7.91 ppm (assigned to C_8–H of **E**) (see Table I).

The relative shift of the signals assigned to [Thy–Hg–Guo], as compared to the uncomplexed nucleo-

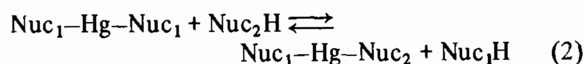
sides, preserves the trend established by the symmetrical bridged complexes. For example, [Thy–Hg–Guo] exhibits a downfield shift for the thymidine moiety C_5–CH_3 and $H_{1\text{-}Rib}$ resonances relative to thymidine, while an upfield shift of the C_8–H resonance and a downfield shift of the NH_2 resonance is observed relative to guanosine. As postulated for [Guo–Hg–Guo], an intramolecular H-bond, albeit weaker, could contribute to the [Thy–Hg–Guo] amino resonance downfield shift. Integration of the signals assigned to [Thy–Hg–Guo] is consistent with an equal ratio of the thymidine and guanosine moieties in the mixed ligand complex. Moreover, the resultant state of the equilibration reaction contains an equal ratio of ThyH/[Guo–Hg–Guo] and GuoH/[Thy–Hg–Thy] (see Fig. 1). Integration of the thymidine moiety C_5–CH_3 resonance signals, or the guanosine moiety NH_2 resonance signals, indicates that the relative abundance of the mercury bridged complexes resulting from the equilibration process is in the order: [Thy–Hg–Thy] > [Guo–Hg–Guo] > [Thy–Hg–Guo] (*ca.* 3.2:1.5:1.0 respectively).

The presence of [Thy–Hg–Thy] (**C**), [Guo–Hg–Guo] (**D**), guanosine (**B**), thymidine (A), and [Thy–Hg–Guo] (**E**) in the exchange reaction system, is also evidenced from the ^{13}C NMR data in Fig. 2, Table II: C_2, C_4, C_5–CH_3 resonances due to **C** at 153.2, 166.3 and 13.1 ppm; C_2, C_4, C_5–CH_3 resonances due to **A** at 150.4, 163.7 and 12.3 ppm; C_2, C_6 resonances due to **D** at 157.2 and 161.0 ppm; C_2, C_6 resonances due to **B** at 153.6 and 156.7 ppm; C_2, C_4, C_5–CH_3 resonances assigned to the thymidine moiety of **E** at 153.3, 166.5 and 13.2 ppm.

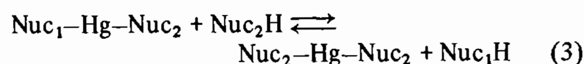
The significant downfield shift of the C_2 and C_4 carbon resonances assigned to the thymidine moiety of [Thy–Hg–Guo] is indicative of metallation of N_3 of thymidine. Direct ^{13}C NMR evidence for the mercuration of the guanosine moiety in the putative [Thy–Hg–Guo] complex was not observed, and probably reflects the limit of resolution of the system employed.

The above observations indicate that ligand redistribution of complexes of the type [Nuc–Hg–Nuc] with free nucleosides, will occur in DMSO. Therefore, the resultant equilibrium of the exchange reaction described here is equivalent to the competition and exchange reaction of the previous study, where initial equilibration was achieved in aqueous solution with the analysis of the reaction products ultimately observed in DMSO [1]. Hence the NMR instrumentation of the previous work precluded resolution of the [Thy–Hg–Guo] complex, which at the time suggested that this structure was metastable with respect to the symmetrically bridged species. The ^{1}H NMR resonance signals diagnostic for the formation of [Thy–Hg–Guo], *i.e.* guanosine NH_2 and thymidine C_5–CH_3, were previously assigned to guanosine and [Thy–Hg–Thy], respectively, and thus led to an underestimation of the total mercuration of guanosine.

The equilibration of (Nuc_1–Hg–Nuc_1) with Nuc_2H can most readily be accounted for as resulting through successive overall equilibria of the type:

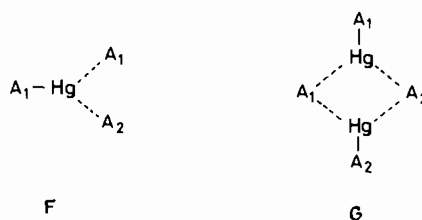


$$Nuc_1\text{–Hg–}Nuc_1 + Nuc_2H \rightleftarrows Nuc_1\text{–Hg–}Nuc_2 + Nuc_1H \quad (2)$$

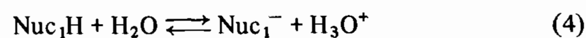


$$Nuc_1\text{–Hg–}Nuc_2 + Nuc_2H \rightleftarrows Nuc_2\text{–Hg–}Nuc_2 + Nuc_1H \quad (3)$$

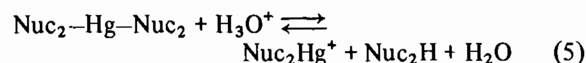
The redistribution could occur in principle via a 3-centre ligand exchange process as depicted by **F** or, in a secondary process, via a 4-centred transition state as shown in G, where A_1 and A_2 are the two nucleoside bases; precedents for such mechanisms have been proposed in other systems [21–23].



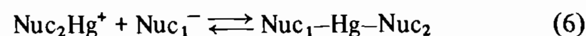
Alternatively, the equilibration could occur through dissociation processes such as given in eqns. (4)–(6), by means of the trace of H_2O which is present in the DMSO, or possibly with CH_3SOCH_3 acting as the proton acceptor in place of H_2O.



$$Nuc_1H + H_2O \rightleftarrows Nuc_1^- + H_3O^+ \quad (4)$$



$$Nuc_2\text{–Hg–}Nuc_2 + H_3O^+ \rightleftarrows Nuc_2Hg^+ + Nuc_2H + H_2O \quad (5)$$



$$Nuc_2Hg^+ + Nuc_1^- \rightleftarrows Nuc_1\text{–Hg–}Nuc_2 \quad (6)$$

The present results do not enable one to differentiate between the multi-stage ionization mechanisms such as given by the reactions (4)–(6), or the concerted mechanisms as represented by **F** and **G**.

It is noteworthy that the redistribution processes observed in the present systems occur rapidly and that the results are independent of whether the reactants are **C** + GuoH, or **D** + ThyH. Therefore, the resulting equilibrium species composition provides a measure of the relative thermodynamic stabilities of the species involved, *i.e.* [Thy–Hg–Thy] > [Guo–Hg–Guo] > [Thy–Hg–Guo].

Conclusions

The ^{1}H and ^{13}C NMR results for the redistribution process presented here provide evidence for the for-

mation of the mixed nucleoside mercury complex [Thy–Hg–Guo], and determine the relative thermodynamic stabilities of the species involved, *i.e.* [Thy–Hg–Thy] > [Guo–Hg–Guo] > [Thy–Hg–Guo].

The characterization of [Thy–Hg–Guo] is pertinent to the molecular analysis of the binding of Hg(II) to DNA. Katz [6] proposed the chain slippage mechanism for the complexation of Hg(II) by DNA, which attributes the most stable mode of binding to thymidine, thymidine pairs capable of forming the interstrand [Thy–Hg–Thy] structure. Furthermore, this mechanism predicts the formation of [Guo–Hg–Guo] and [Thy–Hg–Guo] interstrand complexes as significant but secondary modes of complexation. The stability of a mixed ligand complex is a function of the equilibrating species and experimental conditions employed [17]. The redistribution process observed in the present work provides a mononucleoside model for the binding of Hg(II) to DNA. The model system includes mercury(II) nucleoside complexes as predicted for the analogous interstrand structures of DNA and conserves the relative stability predicted for these structures.

Acknowledgements

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