Biomolecule–Mercury Interactions: Modalities of DNA Base–Mercury Binding Mechanisms. Remediation Strategies

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1. Introduction

The evident ubiquity of heterocyclic residues in nature as key constituents of many biologically important molecules such as proteins, enzymes, vitamins, nucleic acids, etc. and the demonstrated requirement for metal ions for a variety of physiological processes in plants and animals[1] provide the impetus for investigations into metal ion–biomolecule interactions. As well, metal ions have been shown to have far-reaching biological and environmental consequences.[1,2] These investigations are diverse in nature, involving a variety of metal ions and a range of simple to complex heterocyclic residues of key biological molecules. Binding of metal ions to the heteroatomic sites of biomolecules is without doubt fundamental to their observed physiological effects.[1,3] Considerable attention has therefore been attached to understanding the structural, kinetic, and thermodynamic details of these interactions in a number of laboratories, as a key requirement for unraveling and discussing the mechanisms of action and physiological roles of metal ions in living systems.

Research in this domain in our laboratory spanning over two decades has focused largely on two aspects. The interactions of the heavy metal mercury, mainly as CH_3Hg^{II}, with DNA bases and other model systems have been studied as a probe of the molecular basis for its toxicity and environmental effects. Structural features of these complexes as revealed through spectroscopic studies bear relevance to the biological chemistry of Hg^{II}. Novel complexes have been isolated in a number of cases, and these provide insight into additional pathways for the physiological action of metals and clues for remedial intervention. The second aspect of our work concerns isotopic hydrogen exchange in heterocycles such as imidazole, histidine, and thiazole complexed to metal ions, deriving from the fact that isotopic hydrogen exchange of biomolecules in different environments has been a practical tool for probing biological function.[4] These studies have been expanded to include metal ions other than CH_3Hg^{II}, to investigate *substitution-inert* complexes[5] and to explore the chemistry of Pt^{II}-based complexes as anticancer agents.[6] These studies[7–9] have yielded significant information on the relative catalytic abilities of metal ions as surrogates for the proton,[10a] and emphasize the importance of the electronic structures of metal ions in determining reactivities in the ligand portion of the metal complexes. An account of our studies on metal ion effects on isotopic hydrogen exchange in imidazoles and related substrates has been published recently.[10b] Sigel and co-workers[11] have recently quantified the relative acidifying effects of protonation and metalation in a number of DNA bases and related substrates.

The above two aspects of our research have a functional linkage, to the extent that structure–reactivity relationships and electronic effects highlighted by the dynamics of isotopic hydrogen exchange enable an evaluation of factors responsible

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Ikenna Onyido graduated from the University of Ibadan, Nigeria, with a B.Sc. First Class Honors (1974) and Ph.D. (1979) with a thesis on mechanisms of aromatic nucleophilic substitution under the supervision of Jack Hirst. After a year of postdoctoral work with Per Ahlberg at the University of Uppsala, Sweden, he returned to Ibadan to take up a lectureship position in chemistry. In 1989, he was appointed Professor and Head of the Department of Chemistry in the new University of Agriculture, Makurdi, where he also served as the foundation Dean of Science and, later, became Deputy Vice-Chancellor for four years and Acting Vice-Chancellor for one year. He is currently the Director of the Center for Agrochemical Technology in Makurdi, which is concerned with the applications of chemical science and technology for the realization of sustainable agriculture. Since 1983, Ikenna has maintained an active collaboration with Erwin Buncel, with whom he shares common research interests in different aspects of chemistry.

Albert R. Norris, born in Meadow Lake, Saskatchewan, Canada, in 1937, earned B.E. (chemical engineering) and M.Sc. (chemistry) degrees at the University of Saskatchewan in 1958 and 1959, respectively. He obtained his Ph.D. in chemistry at the University of Chicago in 1962 for research carried out under the supervision of Professor Weldon G. Brown. From 1962 to 1964, an NRC Fellowship enabled him to do postdoctoral research at University College, London, under the direction of Sir Ronald Nyholm and Dr. Martin Tobe. He was appointed an Assistant Professor of Chemistry at Queen's University in 1964 and became a full Professor in 1976. He was made a Fellow of the Chemical Institute of Canada in 1977. Over the years he has maintained a keen interest in the unique Engineering Chemistry program at Queen's and served as Undergraduate Chair of the program from 1990 to 1998. He was designated a Professional Engineer in the province of Ontario in 1989. His main research interests have been in the areas of σ and π complexes of nitroaromatic compounds, the oxidation and reduction reactions of coordinated ligands in inert transition-metal-ion-containing complexes, and metal ion–biomolecule interactions. He has been an Emeritus Professor at Queen's since 1999.

for binding site preferences and selectivities noted in the structural studies of metal ion−biomolecule interactions.

A number of metals such as Pb, As, Bi, etc. have proven toxicity and deleterious environmental con-

Erwin Buncel, Professor Emeritus of Chemistry at Queen's University, is a physical organic chemist with strong interests in bioorganic/bioinorganic chemistry (for a previous biographical account, see *Chem. Rev.* **1995**, *95*, 2261). Recent research in Buncel's group in this area has focused on metal ion–biomolecule interactions with an environmental emphasis. Other major interests include photochromic materials, molecular switches, and carbanion chemistry. Buncel is the author/coauthor of 300 research papers in these areas as well as a number of chapters/review articles and monographs. He was the recipient of the SYNTAX Award in Physical Organic Chemistry in 1985 and the R. U. Lemieux Award in Organic Chemistry in 1999, both from the Canadian Society of Chemistry. He was an editor for the *Canadian Journal of Chemistry* (1981–1993) and for the *Journal of Labelled Compounds and Pharmaceuticals* (1995–2002). As an Emeritus Professor, Erwin is continuing an active research program with graduate students, postdoctoral fellows, and colleagues in different countries, and looks to continuing challenges that the world of chemistry holds.

sequences. We have focused mainly on Hg because of its wide distribution in the environment as organomercurials, mainly CH_3Hg^{II}, through an elaborate number of chemical and biological pathways.[12,13] Over time, the anthropogenic activities of our industrial society have resulted in a significant load of mercury in the biosphere, leading to widespread contamination of water and soils with attendant environmental and health concerns.

This review elucidates the various modes of mercury interaction with DNA bases and other biologically important molecules, identifying, in each case, the binding sites of the substrates of interest. The relevance of competition and exchange reactions between Hg^{II}/CH_3Hg^{II} and nucleobases to the mechanism of DNA binding with mercury are explored. In addition to discussing the diversity of structural types encountered in CH_3Hg^{II}/Hg^{II} complexes of biomolecules and the mechanism of Hg^{II} binding to DNA, this review also highlights recent work on mercury detoxification strategies, encompassing anthropogenic and nature-based methods. The necessity for exploring chemical mimics of nature in addressing the issues of environmental remediation of mercury pollution and mercury intoxication in humans is emphasized.

2. Modalities for Binding of Mercury to Biomolecules

The binding of heavy metals to DNA results in potential toxic effects.[14] The pattern of Hg^{II} binding has been used to probe DNA structures in viruses and nucleosomes, as well as to separate DNAs of

different base compositions.[14,15] CH_3Hg^{II} is one of the most toxic forms of Hg^{II}; its environmental significance has been underscored by studies which reveal the chemical and microbiological transformations of Hg^{II} into CH_3Hg^{II} and its bioaccumulation.[16] Being a prototype soft acid which shows strong preference for unifunctionality and minimum steric effects, it has been used as a selective probe for unpaired bases in superhelical DNA[14,17] and has shown greater tendency than Hg^{II} to partition into lipids or hydrophobic regions of the cell.[18] Chronic intake of CH_3-Hg^{II} at subtoxic levels results in chromosomonal damage in humans, presumably due to its direct interaction with DNA; the mutagenic nature of organomercurials in general has been demonstrated[19] and is now well recognized.

Inasmuch as the interaction of metal ions with the sulfur atoms of nucleosides and amino acids bearing the thiol group has provided the dominant mechanism for explaining the deleterious biological effects of heavy metals,[20] certain aspects of metal ion toxicity, e.g., mutagenic effects, may not be satisfactorily explained by solely invoking binding to DNA or protein sulfur functions.[21] Ribose/ribophosphate groups and purine/pyrimidine bases which abound in biomolecules present several N and O donor atoms as potential binding sites.[22] Binding has been demonstrated for copper and uranyl ions to the ribose moiety in nucleobases, in addition to coordination of metal ions to endocyclic and exocyclic N sites of DNA bases.[3b] Metal ion ligation to nucleobases is thought to give rise to nucleobase mispairing; this phenomenon has been suggested as being relevant to the mutagenic potential of metal ions[6a] and the altered sequence of amino acids in proteins resulting from interference with the transmission of genetic information in protein synthesis.[2a]

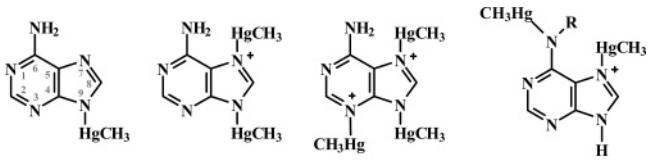
Our studies sought to provide an insight into the modes of heavy metal binding to nucleic acids and other biomolecules, with a view to assessing the importance of N and O donor atoms in contributing to the overall effects of heavy metal ions. Structural evidence for the isolated complexes was obtained primarily from $^1H/^{13}C$ as well as ^{199}Hg NMR studies, X-ray, and IR spectroscopy. It is important to note that although O atoms in the sugar portion and/or the carbonyl function(s) of the substrates investigated are possible binding sites, no definitive evidence was found for binding of Hg^{II}/CH_3Hg^{II} at these sites in nucleobases. The variety of structures and bonding patterns realized in these studies enrich our understanding at the molecular level of metal ion effects in biological systems and provide additional pathways for discussing DNA base–Hg^{II}/CH_3Hg^{II} binding mechanisms and the effects of Hg^{II}/CH_3Hg^{II} coordination on the secondary structures of DNA.

As shown in the sections following, Hg^{II}/CH_3Hg^{II} complexes of DNA bases with metal:ligand ratios of 1:1, 2:1, and 3:1 have been reported. Coordination to N1, N3, N7, N9, and exocyclic NH_2 in these nucleobases has been demonstrated. In addition, binding of metal ions to C8 has been realized under H^+ or metal ion activation at N7. Bridged species of the types Nuc_1–Hg–Nuc_1, Nuc_1–Hg–Nuc_2, and Nuc_2–Hg–Nuc_2 have been characterized. S-ligation of Hg^{II}/CH_3Hg^{II} is a facile process with these biomolecules whenever the thiol group is part of the ligand structure. Amino acids such as cysteine, penicillamine, glutathione, etc. exhibit NH_3^+ coordination; amino acid COO^--electrophile binding has been shown to occur in the solid state, but not in solution. Thus, there is an inherent diversity of coordination patterns in these interactions, whose binding modalities have been elucidated with spectroscopic methods and in which X-ray diffraction has played a significant role.

3. Complexes with Nucleosides and Related Substrates

3.1. Adenine Derivatives

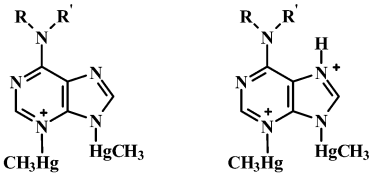
Beauchamp and co-workers[23] showed that adenine reacts with CH_3Hg^{II} at pH 9 and $r = 1$ (r is defined here and in all cases as the ratio [metal ion]/[ligand]) to yield **1**. With $r = 2$ under neutral conditions or in the presence of 1 equiv of NaOH relative to adenine, the product of the interaction is **2**.[23b] With $r = 3$ and 1 molar equiv of NaOH or $r = 4$ without added NaOH, a 3:1 metal–base complex with structure **3** is obtained. Complex **4a** is formed at $r = 4$ when the pH is adjusted to 7 with NaOH.[23b] The structure of **4a** was initially deduced from IR spectra[23b,d] and subsequently solved by X-ray crystallography.[23e] In H_2O–EtOH mixtures,[24] the amino group is doubly metalated to yield **4b**. Significantly, no binding of CH_3Hg^{II} to N1 was observed with adenine in these systems, contrasting the behavior of adenosine and 9-methyladenine (vide infra).



1 **2** **3** **4**

(a): R = H
(b): R = CH_3Hg

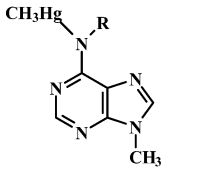
*N*6,*N*6-Dialkylaminopurines[25] yield complexes analogous to **1** at $r = 1$; in the presence of 1 M CH_3HgOH, a 2:1 complex of type **5** with metal binding to N3 and N9 resulted. Using high metal-to-ligand ratios gives a new complex, **6**, in which N7 is now protonated. These results highlight the steric influence of the alkyl groups at N6 on the complexation process.



5 **6**

(a): R = R' = CH_3 (a): R = R' = CH_3
(b): R = R' = CH_3CH_2 (b): R = R' = CH_3CH_2

The reaction of a slight excess of 9-methyladenine (9-MeAd) with ethanolic trismethylmercurioxonium gave **7a**, as a mixture of *syn* and *anti* isomers,[26] favoring the former presumably due to steric factors. A ribose analogue of **7a** was obtained when adenosine (AdoH) was reacted with a slight excess of the organomercurial hydroxide.[26] With 2 equiv of CH_3-HgOH and 1 equiv of 9-MeAd in CH_3CN or DMF, a neutral compound, **7b**, in which both NH_2 protons have been replaced by linearly coordinated CH_3Hg^{II}, results.[27] In the presence of 1 M NaOH and with r = 2, 9-MeAd yields a 2:1 complex in which CH_3Hg^{II} is attached to N1 and N6, i.e., **8**, existing mainly in its *anti* conformation.[23a] On the other hand, N7 is the primary binding site for 3-methyladenine (3-MeAd),[28a] which has been shown[29] by semiempirical calculations and NMR spectroscopy to exhibit thermodynamic preference for its amino form. 3-MeAd gave, respectively, **9** at r = 1 and pH 4, **10** at r = 1 and pH 2, and **11** at r = 3 and pH 4. NMR evidence suggests[28a] that complexes **12** (r = 1) and **13** (r = 3) were also obtained at pH 8. For the structures of **9**−**13**, NMR data indicate significant reduction in electron density in the imidazole ring, in accord with the structures shown.



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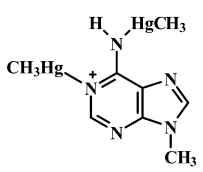
(a): R = H
(b): R = CH_3Hg



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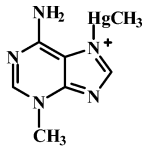

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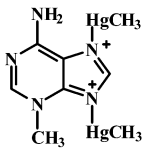

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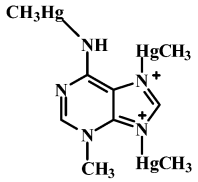

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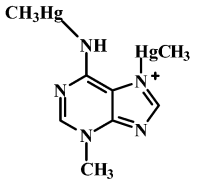

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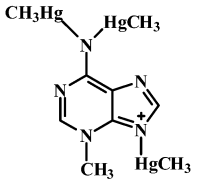

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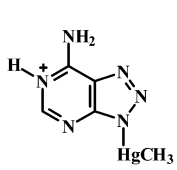
8-Azaadenine[28b] forms a 1:1 complex analogous to **1** at r = 1 and pH 4; at pH 2, N1 is protonated to yield **14**. With r = 2 and pH 5, a complex in which CH_3Hg^{II} coordinates to N3 and N9 results, while at r = 3 and pH 6.5 methylmercuration of N1, N6, and N9 occurs to give **15**. Blocking N9 in 8-azazadenine through methyl substitution alters[28c] its binding pattern to give, at r = 1 and pH 2−3, a complex which has structure **16** in the solid state; in DMSO-d_6 solution, NMR evidence suggests structure **17** as the product of this interaction. With r = 2 and pH ≈ 5, coordination occurs at N1 and N6 of 9-methyl-8-azaadenine to give **18**, believed to exist in two isomeric forms. With r = 5 and pH ≥ 7, a complex analogous to **7b** was obtained. It is important to note that, with these aza-modified adenines, no complex was obtained with CH_3Hg^{II} binding at N7 or N8.



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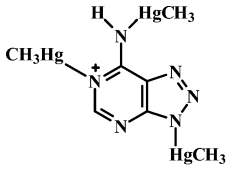

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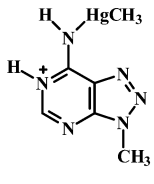

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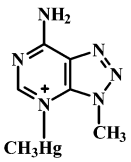

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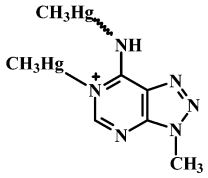

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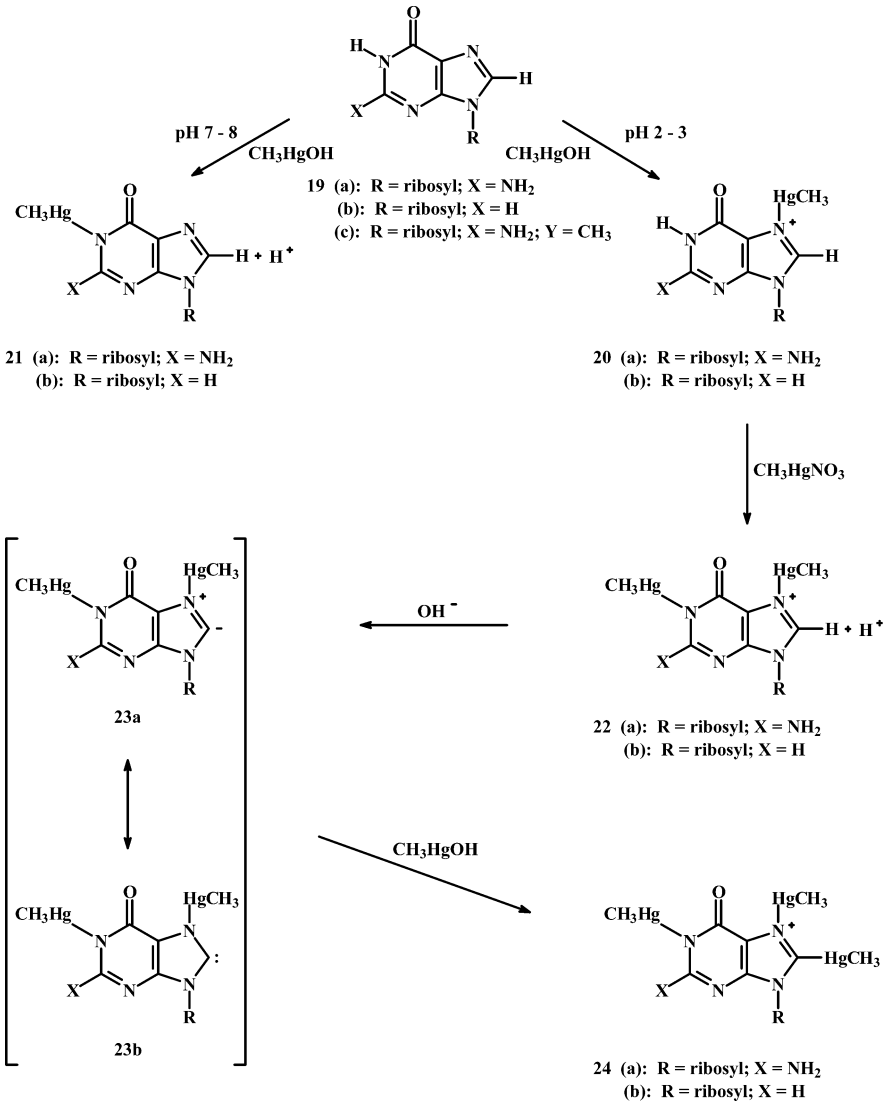
3.2. Guanosine and Inosine Nucleosides and Related Compounds

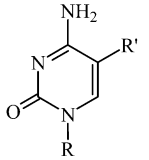
A number of complexes of guanosine (GuoH), **19a**, and inosine (InoH), **19b**, with Hg^{II} and CH_3Hg^{II} in solution were postulated by Simpson[30] on the basis of UV studies, and confirmed through Raman spectroscopy by Tobias and co-workers.[31] With r = 1 at low pH, N7-coordination of the electrophile to these nucleobases results in **20a** and **20b**, while reaction at high pH affords the N1-bound products **21a** and **21b**, isolated as solids.[32] In addition, **22a** and **22b** were reportedly obtained at high and low pH, with r = 2. Our work[33,34] not only reproduced the isolation and characterization of **20a**, **20b**, **21a**, and **21b** but also revealed additional features of the interaction, to include the formation of 3:1 CH_3Hg^{II}−nucleoside complexes. With GuoH and InoH, at pH 3 and r = 2, **22a** and **22b** were obtained, respectively; at pH 7, an additional product characterized as **24** was obtained in each case in low yield, on leaving the reaction mixture for 48 h. Complex **24b** was also obtained in reasonable yield at pH 7 and r = 3 by heating the reaction solution to 50 °C; a similar situation obtains with GuoH, although incomplete substitution of C8−H by CH_3Hg^{II} results due to an additional interaction of the electrophile with the exocyclic NH_2.[33] The highlight of this study was the first reported isolation of a purine nucleoside C8−Hg^{II} bonded complex, **24**; the greater stability of **24** relative to its N-bound counterpart was unambiguously demonstrated by the $^2J(^1H-^{199}Hg)$ values: 159.5 and 215.8 Hz for C−$Hg^{II}CH_3$ and N−$Hg^{II}CH_3$, respectively. Formation of **24** results from deprotonation of **22** to generate the resonance-stabilized ylide intermediate **23** at pH 7. Proton abstraction from **22** by HO^- is rendered facile by the activating influence of the N7-coordinated electrophile. The irreversible addition of CH_3Hg^+ to **23** essentially precludes its reprotonation by H_2O as occurs in hydrogen exchange processes.[7−9]

Scheme 1



Scheme 1 summarizes the range of structures obtained with GuoH and InoH under the conditions of pH and stoichiometries indicated. The essentially irreversible formation of **24** and the thermodynamic preference of C8-bound CH_3Hg^{II} complexes over their N1- and N7-bound counterparts suggest an alternative and plausible mechanism for the observed mutagenicity of organomercurials.

Binding at N1 and the exocyclic NH_2 group could have implications for the disruption of base-pairing capabilities of GuoH and InoH, with far reaching consequences, especially for the secondary structures of polymeric biomolecules. Methylmercuration of the exocyclic NH_2 group has been demonstrated[26] in GuoH, **19a**, 1-methylguanosine (1-MeGuo), **19c**, and cytidine (Cyt), **25a**, as well as in 9-MeAd and AdoH



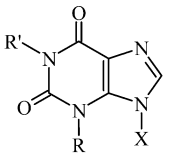
25 (a): R = ribosyl; R' = H
(b): R = ribosyl; R' = CH_3

described above. Clarke[35] had earlier adduced spectroscopic and chemical evidence for binding of Ru^{III} to deprotonated NH_2 groups in adenosine, cytidine, and tubercidine. A 2:1 CH_3Hg^{II}–Guo adduct was obtained[26] with **19a**, consistent with CH_3Hg^+ binding at N1 and deprotonated NH_2. With a slight excess of CH_3HgOH over substrate, ^1H NMR spectra of isolated complexes revealed the NH_2 group also as the primary target in **19c** and **25a**. Since $N-Hg^{II}CH_3$ bonds of exocyclic nitrogens are stronger than those of their endocyclic counterparts,[36] Hg^{II}/CH_3Hg^{II} binding to exocyclic NH_2 groups is potentially capable of causing greater disruption to base pairing than the labile methylmercuration of endocyclic thymine N3 or guanine N1 positions, from a thermodynamic perspective.

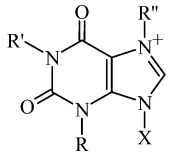
3.3. Theophylline and Xanthosine Nucleosides

Theophylline (ThH), **26**, and xanthosine ($XanthH_2$), **27**, exhibit a number of potential binding sites and are considered valid models for certain DNA bases and their derivatives.[36] Metal binding to **26** occurs through deprotonation of N7–H, since methyl substitution precludes reaction at N1 and N3; a neutral

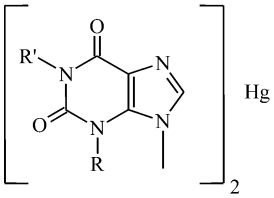
N7-bound metal complex, realized through the displacement of N7−H by the electrophile, has been described for this compound.[37] Our study[38] shows that initial CH_3Hg^{II} binding in **26** is pH-dependent, giving a range of products in which coordination occurs also at N7 and N9 at $r > 1$. At pH 8−9 and $r = 1$, **28** is the product which has been unambiguously shown by X-ray analysis[39] to bind the electrophile at N7. The crystal structure of the monohydrate of **28** shows the roughly linear N7-coordination to CH_3Hg^{II} with the expected values of bond lengths for Hg−N7 = 2.06 Å and Hg−CH_3 = 2.04 Å. Hg···O(6) bonding in **28** is probably very weak or absent, judging from its distance (3.18 Å); an intermolecular contact of 2.98 Å is established between N9 and Hg. The H_2O molecule forms a moderately strong Hg−O bond (2.94 Å) to Hg and is simultaneously H-bonded with carbonyl C2−O2 and C6−O6 of two different molecules.



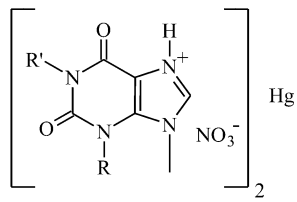
26: R = R' = CH_3; X = H
27: R = R' = H, X = ribosyl
28: R = R' = CH_3; X = CH_3Hg
33 (a): R = CH_3Hg; R' = H; X = ribosyl
(b): R = R' = CH_3Hg; X = ribosyl



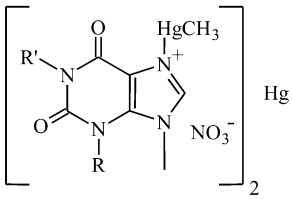
29 (a): R = R' = CH_3; R" = H; X = CH_3Hg
(b): R = R' = CH_3; R" = X = CH_3Hg
34 (a): R = R' = H; R" = CH_3Hg; X = ribosyl
(b): R = R' = R" = CH_3Hg; X = ribosyl



30: R = R' = CH_3



31: R = R' = CH_3

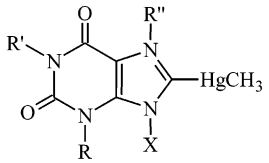


32: R = R' = CH_3

Treatment of **28** with 1 M HNO_3 (pH 2−3) yields **29a**, which bears H^+ at N9; **29a** can also be accessed directly from equimolar mixtures of **26** and CH_3Hg^{II} at pH 2−3. Reaction of **28** under neutral conditions or **29a** under basic conditions with another equivalent of CH_3Hg^{II} affords **29b** in which CH_3Hg^{II} is coordinated to N7 and N9; NMR data indicate rapid exchange of the CH_3Hg^{II} moieties between these positions. Reaction of **26** with $Hg(OAc)_2$ leads to the formation of **30**, which demonstrates Hg^{II} bridging between two deprotonated N7 sites; protonation of **30** in acidic aqueous solution yields **31**. Treatment of **30** with an additional molar equivalent of CH_3Hg^{II} at pH 2−3 affords **32**, which can also be accessed directly from **31** by reacting the latter with another equivalent of the electrophile under basic conditions. Significantly, no C8-bound Hg^{II}/CH_3Hg^{II} complexes are realized with **26**, showing clearly that the two electrophilic groups at N7 and N9 in **29b** have not provided sufficient activation for C8−H abstraction, contrasting the behavior of inosine[33,34] discussed above and xanthosine (see below).

Xanthosine, **27**, has two ionizable protons in the pyrimidine moiety, making it a good model for uracil and thymine as pyrimidine-type nucleic acid constituents. Treatment of **27** with CH_3HgOAc at pH 5 and $r = 1$ afforded **33a** in good yield,[40] consistent with the order of acidity N3−H > N1−H.[41] At pH 8, **33a** was formed along with small quantities of **33b** as shown by ^1H and ^{13}C NMR, due to the disproportionation reaction depicted in eq 1. Similar disproportionation reactions have been observed in the CH_3Hg^{II}/imidazole (see below) and CH_3Hg^{II}/adenine[23a] systems. Pure **33b** was obtained at pH 8−9 with $r = 2$; further treatment of **33b** with 1 equiv of CH_3Hg^{II} yields **34b**, which at pH 5−6 undergoes C8-methylmercuration with a further equivalent of the electrophile to form **35**. Under acidic conditions (pH 2−3)

$$\mathbf{33a} + \mathbf{33a} \rightleftharpoons \mathbf{33b} + \mathbf{27} \qquad (1)$$



35: R = R' = R" = CH_3Hg; X = ribosyl

and with $r = 1$, **27** yields **34a** in which CH_3Hg^{II} coordinates to N7. Thus, depending on the pH and reactant stoichiometry, methylmercuration of xanthosine is possible at all four potential sites: N1, N3, N7, and C8. The dichotomy between xanthosine and theophylline in terms of the occurrence of C8-methylmercuration in the former but not in the latter is worthy of note.

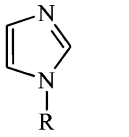
3.4. Imidazole, 1-Methylimidazole, and 1,3-Dimethylimidazolium Ion

Potential binding sites decrease progressively in the series[42] imidazole, **36a**, 1-methylimidazole, **36b**, and 1,3-dimethylimidazolium ion, **37**. A 1:1 molar mixture of **36a** and CH_3HgNO_3 in H_2O yields **38a**; an ethanolic solution of **36a** reacts with aqueous CH_3HgNO_3 to form **36c**, while **38b**, the product of methylmercuration at both N1 and N3 according to the disproportionation reaction of eq 2, is obtained

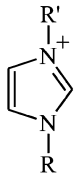
$$\mathbf{38a} + \mathbf{36c} \rightleftharpoons \mathbf{38b} + \mathbf{36a} \qquad (2)$$

under neutral or basic conditions.[42a] Similar results

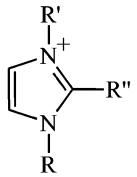
for **36a** were reported by Rabenstein and coworkers.[42b,43] Precedents for eq 2 exist in the adenine/CH_3Hg^{II} [23a] and xanthosine/CH_3Hg^{II} [38] systems (vide supra). The symmetrical Hg-bridged products **39a** and **39b** are obtained when **36a** and its 4-nitro derivative are treated with ethanolic HgO. Structures of complexes of 4-nitroimidazole with CH_3Hg^{II} and Ag^{I} have been described.[42c]

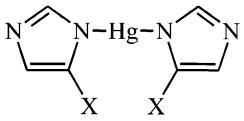


36 (a): R = H
(b): R = CH_3
(c): R = CH_3Hg



37: R = R' = CH_3





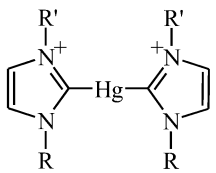
38 (a): R = R" = H; R' = CH_3Hg
(b): R = R' = CH_3Hg; R" = H
(c): R = CH_3; R' = CH_3Hg; R" = H
(d): R = CH_3; R' = R" = CH_3Hg
(e): R = R' = CH_3; R" = CH_3Hg

39 (a): X = H
(b): X = NO_2

1-Methylimidazole, **36b**, bears close resemblance to the five-membered ring portion of the purine nucleosides **19a**, **19b**, and **27**, already studied.[33,34] An equimolar mixture of **36b** and CH_3HgNO_3 affords the N3-bonded species **38c**. At pH 7 and $r = 2$, the crude product realized was shown by NMR to be a mixture of **38d** and **40a**. Subsequent recrystallization of the crude product from hot water yields **40a** only, suggesting a symmetrization process according to eq 3.

$$\mathbf{38d} + \mathbf{38d} \rightleftharpoons \mathbf{40a} \qquad (3)$$

No methylmercuration occurs with **37** at low pH; at high pH **38e** was observed in solution, consistent with C2−H/C8−H bond activation being prerequisite for C2/C8 methylmercuration[33,34] (see Scheme 1). Attempted recrystallization of **38e** from H_2O leads to isolation of the symmetrized product **40b** (cf. eq 3 for formation of **40a**).

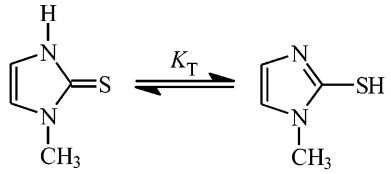


40 (a): R = CH_3; R' = CH_3Hg
(b): R = R' = CH_3

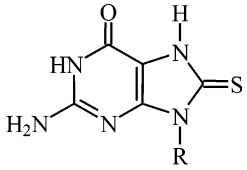
C2-binding by Hg^{II}/CH_3Hg^{II} was established for **36b** under conditions for N3 protonation and for **37**, but not for imidazole, **36a**. The formal difference is methyl substitution at N1 in **36b** and **37** but not in **36a**. Similarly, C8-methylmercuration occurs in **19a**, **19b**, and **27**, all of which bear the ribosyl group at N9, but not in **26**, where there is a proton on N9.[33,34,38] This difference has been explained on the basis of the concept of the "minimum degree of activation" requirement.[42a]

3.5. Sulfur-Modified Nucleosides and Related Substrates

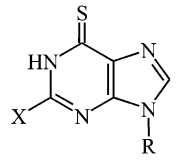
Complex formation has been investigated between CH_3Hg^{II} and the following S-modified substrates: 1-methylimidazoline-2-thione, also called methimazole (MeImSH), **41/42**,[44] 8-thioguanine (8-thioGuoH_2), **43**,[45] and 6-mercaptopurine riboside (6-MPurH_2), **44a**.[46] MeImSH exists in tautomeric equilibrium[47] as



41 **42**



43: R = ribosyl

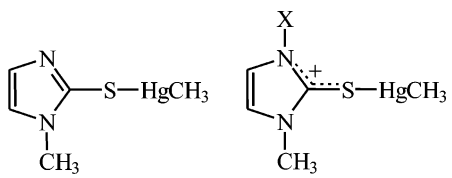


44 (a): R = ribosyl; X = H
(b): R = ribosyl; X = NH_2

shown in eq 4, whereas **43** exists predominantly as the thione form in solution and in the solid state. The biological importance and practical utility of these substrates, which incorporate the soft S atom in their structure, have been variously described.[48]

$$\mathbf{41} \overset{K_T}{\rightleftharpoons} \mathbf{42} \qquad (4)$$

Reaction of **41/42** with CH_3Hg^{II} affords[44] the S-bound complexes **45** and **46a** at high and low pH, respectively, with $r = 1$, contrasting a literature report[49] of binding of Pd^{II} and Pt^{II} to this substrate via N3 at low pH and via both N3 and S at high pH. With $r = 2$, further replacement of N3−H occurs in **46a** to give **46b**. Thus, the introduction of the S atom to the parent compound **36b** shifts the primary reaction center from N3 to S, in accord with the documented preference of Hg^{II} for soft donor atoms.[50] The ability of this ligand to compete for Hg^{II} with S-containing bioligands makes it a potential protective agent against Hg^{II} intoxication. Thiazolidine-2-thione complexes, analogous to **45**, **46a**, and **46b**, have been reported.[51]



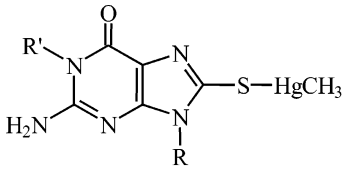
45

46 (a): X = H
(b): X = CH_3Hg

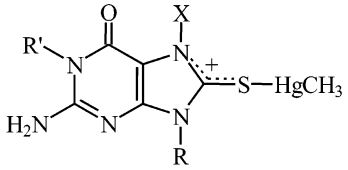
Unambiguous information regarding binding sites in **45** and **46a** was obtained by X-ray structure

analysis,[52] which confirmed conclusions on structural features reached on the basis of chemical and spectroscopic data[44] and revealed the following details: H^+ is bound to N3 while CH_3Hg^{II} is attached to sulfur. Mercury in the complexes exhibits linear coordination, with S−Hg−C6 = 176.1°; the Hg−C bond is a normal one (2.09 Å). The exocyclic Hg−S bond (2.382 Å) is typical of 2-coordinated Hg, forming a number of secondary bonds with NO_3^- but not coplanar with the ring (Hg−S−C2−N1 = −134°, with the angle at S ~100°). H-bonds are formed between NO_3^- oxygens and the acidic proton on N3. The Hg−S bond in **45** (2.338 Å) is significantly shorter than in **46a** (2.382 Å), probably due to the absence of intermolecular contact between Hg and donor atoms on adjacent molecules. In both **45** and **46a**, the C2=S bond in the parent compound (1.691 Å) is lengthened upon coordination with CH_3Hg^{II}, suggesting that it approximates a single bond in the complexes.

Site preferences in CH_3Hg^{II} binding with **43** are both pH- and stoichiometry- dependent.[45] At pH 8−9 and $r = 1$, the S-bonded complex **47a** results; also at this pH and when $r = 2$, **47b**, which manifests N1-binding in addition to S-methylmercuration, is obtained. Under acidic conditions (pH 1−2), the ionic S-bonded product **48a** is obtained at $r = 1$. Reacting **47a** with a further mole equivalent of CH_3Hg^{II} yields the cationic product **48b** in which methylmercuration has occurred at N9. A 3:1 cationic complex, **48c**, is realized by treating **47b** with an additional equivalent of CH_3Hg^{II} at pH 2−3.

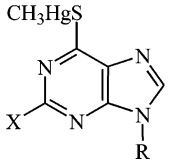


47 (a): R = ribosyl; R' = H
(b): R = ribosyl; R' = CH_3Hg

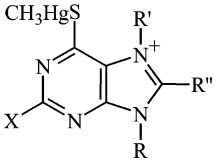


48 (a): R = ribosyl; R' = X = H
(b): R = ribosyl; R' = H; X = CH_3Hg
(c): R = ribosyl; R' = X = CH_3Hg

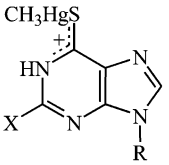
In relation to the parent substrates guanosine (**19a**) and inosine (**19b**), already investigated,[33,34] in which N1, N7, and C8 were found to be binding sites, the mercapto analogues **44a** and **44b** contain the S atom as a likely additional coordination site.[46] A range of products is possible depending on the pH of the medium, reactant stoichiometry, and relative affinities of N, S, and C centers toward CH_3Hg^{II}.[47] At pH 7−8, reaction occurs at the exocyclic S of the pyrimidine moiety in both **44a** and **44b**[46] to give **49a** and **49b**, respectively; further reaction with 1 molar equiv of CH_3Hg^{II} at pH 2−3 affords **50a** and **50b**, respectively, in which N7-methylmercuration is realized. These products are converted to the 3:1 CH_3Hg^{II}−nucleoside complexes **50c** and **50d**, in which there is C8−H displacement at pH 7−8. Reaction of **44a** and **44b** under acidic conditions (pH 2−3) and with $r = 1$ affords the cationic products **51a** and **51b**. Thus, S-methymercuration is achieved first, before N- and/or C-methylmercuration occurs in all cases, formation of the latter species being dependent on the pH and reactant stoichiometry. Furthermore, the occurrence of C-methylmercuration accords with our finding on C8−H/C2−H activation in inosine, xanthosine, and imidazole derivatives, resulting from CH_3Hg^{II} coordination to N7/N3 in these systems, which facilitates proton abstraction and ylide formation.[26,33,42a]



49 (a): R = ribosyl; X = H
(b): R = ribosyl; X = NH_2



50 (a): R = ribosyl; R' = CH_3Hg; R" = X = H
(b): R = ribosyl; R' = CH_3Hg; R" = H; X = NH_2
(c): R = ribosyl; R' = R" = CH_3Hg; X = H
(d): R = ribosyl; R' = R" = CH_3Hg; X = NH_2



51 (a): R = ribosyl; X = H
(b): R = ribosyl; X = NH_2

3.6. 7-Methylguanine, a Minor t-RNA Base

Binding of CH_3Hg^{II} to the minor t-RNA base 7-methylguanine (7-MeGua) was studied by Sheldrick.[53] Two different 1:1 complexes with CH_3Hg^{II} coordination at N1 and N9 were obtained at $r = 1$ in the pH ranges of 9−12 and 1−4, respectively. With $r = 3$, a 2:1 complex in which the metal is attached to N1 and N9 was isolated in the pH range 4−6; a change of the pH range to 1−3 gave a 3:1 complex with metal bonding to N1, N3, and N9. No evidence was found for metal bonding to N7 and the exocyclic NH_2. 9-Methylguanine (9-MeGua), on the other hand, gave solid complexes analogous to **20a**, **21a**, and **22a**; binding to N3 was not observed.[54]

3.7. Nucleotides and Related Substrates

The results presented above do not reveal any interactions of Hg^{II} and CH_3Hg^{II} with the O donor atoms of the sugar and phosphate moieties in nucleosides and their analogues. This observation is relevant in the following discussion of recent literature on the interaction of these electrophiles with nucle-

otides and related substrates. Section 5 below touches on the structural and conformational consequences of binding interactions involving polynucleotides.

^{1}H NMR spectroscopic study of the interaction of d[CGCGAATTCGCG]$_2$ with Hg^{II} reveals decreasing intensities of the thymine imino protons with increasing metal ion concentration and a concurrent downfield shift of the guanine G4 imino hydrogens.[14,55] Decreasing thymine proton signals are consistent with N3−Hg^{II} binding, following deprotonation of imino hydrogen. Alternatively, this could be a result of fast exchange of the thymine imino protons with the aqueous environment, consequent upon Hg^{II} binding to other adenine and thymine sites.

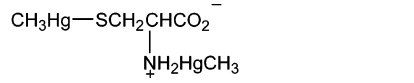
Earlier studies with nucleases demonstrate preferential binding of Hg^{II} to thymine whereas, Cu^{II} favors guanine.[56] The opposite effects of Hg^{II} and CH_3-Hg^{II} on staphylococcal nuclease digestion of calf thalamus DNA, whereby the DNA cleavage rate is increased irreversibly by CH_3Hg^{II} but decreased reversibly by Hg^{II}, indicate that the secondary structure of DNA is unaffected by Hg^{II}.[57] On the other hand, CH_3Hg^{II} breaks down the secondary structure of DNA to give single strands which are rapidly and irreversibly hydrolyzed by staphylococcal nuclease.[58]

In a recent study,[59] two simple mononucleotide models of DNA, 2′-deoxyguanosine 5′-methylmonophosphate (Me*p*dG) and 2′-deoxyguanosine 3′-methylmonophosphate (dG*p*Me) were used to demonstrate strong binding affinity of Hg^{II} for guanosine N7. High-field multinuclear (^{1}H, ^{13}C, ^{15}N, and ^{31}P) NMR evidence was obtained for the strengthening of the anomeric effect through binding of Hg^{II} to N7, leading to an attenuation of the electron density in the imidazole moiety.

4. Complexes with Amino Acids and Derivatives

4.1. Cysteine

CH_3Hg^{II} coordinates with cysteine over the pH range 0−14 to form a 1:1 complex.[60] Of the three potential binding sites in the molecule, only the sulfhydryl group is involved in the 1:1 interaction, consistent with the observation that no binding is observed with S-methylcysteine. X-ray crystallographic studies[60b,c] have shown that the electrophile is bound to the deprotonated sulfhydryl group, and that a weak intramolecular Hg···O interaction (2.85 Å) exists with the carboxylate group. Replacement of the S with Se in cysteine gives a complex with a stronger metal−ligand bond;[61] this may account for the protective effects against CH_3Hg^{II} poisoning ascribed to selenium-based compounds.[14] In vivo formation of a CH_3Hg^{II}−cysteinylglycine complex has been reported.[14] By contrast, methionine is coordinated to CH_3Hg^{II} via the amino nitrogen.[62] The formation of the 2:1 complex **52** at high pH and in the presence of excess metal ion has been reported;[63] **52** manifests CH_3Hg^{II} coordination to S and N sites. Potentiometric evidence has been presented[63] for the formation at low pH of a 2:1 complex analogous to **53** (see below) in which the two CH_3Hg^{II} moieties are bound to sulfur.



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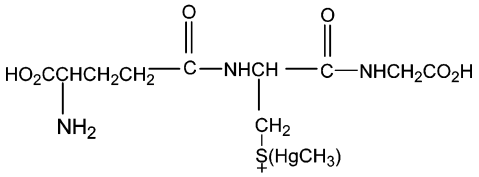


53

4.2. Gluthathione

Gluthathione (GSH) is a physiologically important sulfhydryl-containing tripeptide. As the predominant low molecular thiol in all living organisms, it has been studied as a model compound for the binding of mercury to S-amino acid residues of proteins. In the reduced form, this peptide has been shown to attenuate the cytotoxic effects of mercury.[14] GSH has been implicated in a host of cellular biochemical processes,[64] including the protection of the living cell against free radicals,[65] oxidation damage,[66] thermosensitivity,[67] and sulfhydryl-reactive agents.[68] The roles of GSH in the protection of bacterial cells and higher life forms against heavy metals and xenobiotic toxicology have been discussed.[69,70] GSH has been identified by ^{1}H NMR as a major binding site for Hg^{II} in intact human erythrocytes[71] and has been implicated in several other studies of Hg^{II} toxicology.[72]

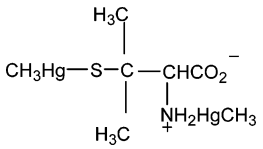
CH_3Hg^{II} coordinates to GSH over the pH range 0.5−13 in a manner that is pH-dependent.[60] At pH < 4, a 2:1 complex is formed in which two CH_3Hg^{II} cations are attached to the sulfhydryl group, resulting in the charged complex **53**; between pH 4 and pH 8, there is a shift of one CH_3Hg^{II} to the amino group. Dissociation of the second CH_3Hg^{II} occurs at pH 10 and above, presumably to form CH_3HgOH. A Raman spectroscopic study[73] of the CH_3Hg^{II}/GSH system revealed that, at $r = 1$, a 1:1 complex was formed via coordination of the eletrophile to S; a value of log $K = 15.9$ has been measured for the formation constant of the 1:1 complex.[43] Methylmercuration of the sulfhydryl group is the favored step in CH_3Hg^{II}−GSH interaction.

Complexes of the types Hg(glutathione)$_2$[74] and Hg(glutathione)$_3$[75] with formation constants log K(25 °C) = 40.95[74] and 3.28,[75a] respectively, were obtained at physiological pH and $r < 0.5$. Although binding of the third ligand to form Hg(glutathione)$_3$ is much weaker than binding of the two ligands in Hg(glutathione)$_2$, it is sufficiently strong to ensure that a significant fraction of Hg^{II} is present as Hg(glutathione)$_3$ at physiological pH and excess metal ion (i.e., $r < 0.5$).[75] The multiplicity of potential GSH−Hg^{II} complexes and the favorable thermodynamics for their formation are indicative of the possible role of glutathione in mercury remediation (see below).

4.3. Penicillamine

A 1:1 S-bound complex with CH_3Hg^{II} was reported by Rabenstein and Fairhurst[60a] for the sulfur amino

acid penicillamine. The amino acid moiety was shown to be present in the complex in its zwitterionic form and coordinated to the metal via a deprotonated sulfhydryl group.[76] The 2:1 complex $CH_3HgSC(CH_3)_2CH(NH_2CH_3Hg)COO^-$ (**54**), isolated by Carty and co-

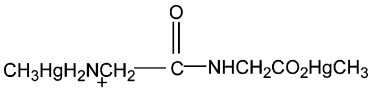


54

workers[76] from an aqueous solution, exhibits bonding of CH_3Hg^{II} to the deprotonated S and N centers, as shown by X-ray analysis. C−Hg−S and C−Hg−N bonds deviate from linearity possibly due to Hg interactions with neighboring S and O atoms. Phenylmercury(II) complexes of penicillamine analogous to those of CH_3Hg^{II} have also been reported;[77] these decompose to form diphenylmercury when stirred as suspensions in benzene at ambient temperature.

4.4. Other Amino Acids

The interaction of CH_3Hg^{II} with the amino acids glycine and alanine was studied by vibrational and X-ray spectroscopy.[78] Complexes of 1:1 stoichiometry in which the metal is complexed via the amino function were obtained. Two different crystalline complexes were realized from aqueous mixtures of the dipeptide glycylglycine (GlyGly) and CH_3Hg^{II}.[79] In the 1:1 complex, a proton of the charged amino group is substituted by the metal ion. A 2:1 complex, **55**, in which the second CH_3Hg^{II} moiety is coordi-

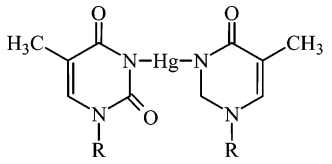


55

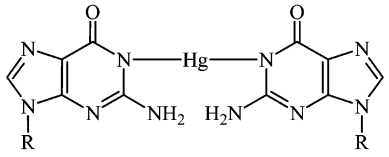
nated to the carboxylate, is formed on prolonged treatment of GlyGly with a mixture of CH_3HgOH and CH_3HgClO_4 in ethanol. A Raman spectroscopic investigation of several amino acids[80] established the following general principles in the interaction of these biomolecules with CH_3Hg^{II}: (i) All amino acids coordinate to CH_3Hg^{II} via their terminal NH_3^+; facile coordination also occurs with $-NH^+$ (histidine) and −NH (tryptophan). (ii) While terminal COO^- coordination with the electrophile may be observed in the solid state, no binding with this function was detected in solution. (iii) Side-chain reactivities of NH_3^+ and COO^- functions are lower than those of their terminal counterparts. (iv) Groups such as −OH (serine, threonine, and tyrosine), $-NHC(NH_2)_2^+$ (arginine), and $-C(=O)NH_2$ (asparagine and glutamine) show no reactivity toward CH_3Hg^{II} coordination in aqueous solution. (v) The −SH group is the most reactive site in amino acids, being preferred to NH_3^+; substitution of H with CH_3 renders the sulfur site unreactive toward CH_3Hg^{II} in methionine, although some coordination may occur at very low pH (<2).

5. Competition and Exchange Reactions: Probes for the DNA Binding Mechanism

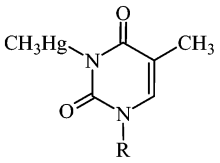
Katz's model[81] for binding of Hg^{II} to DNA emphasizes coordination of the electrophile to thymine,-thymine (ThyH,ThyH) pairs to form Thy−Hg−Thy species with N3 proton displacement, in preference to other nucleoside pairs. CH_3Hg^{II} has been shown[17] to denature Ado,ThyH-rich DNAs in preference to GuoH,Cyd-rich ones. However, binding site preferences in these interactions are not clearly delineated because binding of Hg^{II}/CH_3Hg^{II} to DNA and synthetic polynucleotides has not been understood with sufficient clarity at the molecular level. Competition and exchange reactions of Hg^{II} and CH_3Hg^{II} with nucleic acid constituents, following the preparation of Hg-bridged complexes (Thy−Hg−Thy, **56**, and Guo−Hg−Guo, **57**) and CH_3Hg^{II} complexes **21a** and **58**, have been investigated with the objective of streamlining information on the binding modalities of these Hg-based electrophiles.[82,83]



56: R = ribosyl

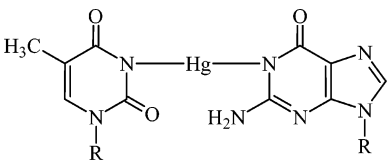


57: R = ribosyl



58: R = ribosyl

In the competition reactions, the two nucleosides GuoH and ThyH were reacted with deficit amounts of HgO or CH_3Hg^{II} in aqueous solution. For the exchange reactions, ThyH and **57** were reacted in the molar ratio 2:1, or as an the alternative, ThyH was allowed to react with 1 equiv of [CH_3Hg(Guo)], **21a**, in water. ^1H and ^{13}C NMR analyses of the reaction products were performed in DMSO. Results[82] for the competition reaction involving Hg^{II} showed the presence of **56** and **57** in the reaction mixture in the ratio 3:1; no evidence was obtained in this study for the mixed product Thy−Hg−Guo, **59**. Equations 5−8 give



59: R = ribosyl

the equilibria leading to the symmetrical products

with Nuc_1-H = GuoH or ThyH. Formation of the mixed bridged species can be envisaged to arise from the reaction of eq 9,

$$Nuc_1-H + H_2O \rightleftharpoons Nuc_1^- + H_3O^+ \quad (5)$$

$$H_3O^+ + HgO \rightleftharpoons HgOH^+ + H_2O \quad (6)$$

$$Nuc_1^- + HgOH^+ \rightleftharpoons Nuc_1-HgOH \quad (7)$$

$$Nuc_1-H + Nuc_1-HgOH \rightleftharpoons Nuc_1-Hg-Nuc_1 + H_2O \quad (8)$$

$$Nuc_2-H + Nuc_1-HgOH \rightleftharpoons Nuc_1-Hg-Nuc_2 + H_2O \quad (9)$$

where Nuc_2-H is different in identity from Nuc_1-H. Similar results were obtained[82] in the exchange reactions involving ThyH and Guo−Hg−Guo in the ratio 2:1; the reaction sequence can be formulated as eqs 10−14.

$$ThyH + H_2O \rightleftharpoons Thy^- + H_3O^+ \quad (10)$$

$$[Guo-Hg-Guo] + H_3O^+ \rightleftharpoons GuoH + Guo-Hg^+ + H_2O \quad (11)$$

$$Guo-Hg^+ + Thy^- \rightleftharpoons [Guo-Hg-Thy] \quad (12)$$

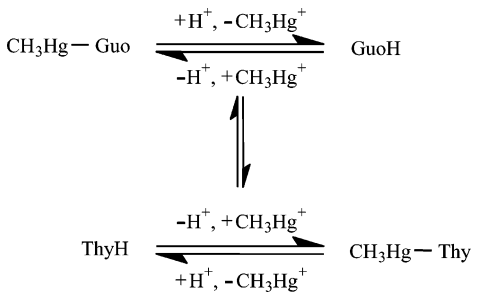
$$[Guo-Hg-Thy] + H_3O^+ \rightleftharpoons Thy-Hg^+ + GuoH + H_2O \quad (13)$$

$$Thy\text{-}Hg^+ + Thy^- \rightleftharpoons [Thy-Hg-Thy] \quad (14)$$

Of particular interest is the absence of the mixed bridged species Guo−Hg−Thy, **59**. Since **59** is a plausible intermediate in the formation of **56** from the reaction of ThyH with **57** according to eqs 10−12, it was concluded[82] that **59** is a metastable species[84] (see below for evidence for its formation).

The exchange reaction involving CH_3Hg^{II} was conducted by reacting molar equivalents of CH_3Hg−Guo, **21a**, and ThyH in water.[82] Instantaneous exchange occurred; ^1H and ^{13}C NMR analyses of the reaction products in DMSO revealed extensive methylmercuration of ThyH and partial methylmercuration of GuoH. The equilibrium between the methylmercuriated and free nucleosides can be visualized as shown in Scheme 2 , for which eqs 5, 15, and 16

Scheme 2[a]



[a] Reprinted with permission from ref 82. Copyright 1985 Elsevier.

are the constituent equilibria with Nuc_1-H and Nuc_2-H as the two nucleosides.

$$Nuc_1-H + H_2O \rightleftharpoons Nuc_1^- + H_3O^+ \quad (5)$$

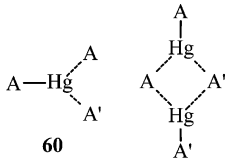
$$Nuc_2-HgCH_3 + H_3O^+ \rightleftharpoons Nuc_2-H + CH_3Hg^+ + H_2O \quad (15)$$

$$Nuc_1^- + CH_3Hg^+ \rightleftharpoons Nuc_1-HgCH_3 \quad (16)$$

In the competition experiments, ThyH and GuoH were made to compete for a limited amount of the electrophile (molar ratio 1:1:1). The results of the competition reaction involving CH_3Hg^{II} follow closely those of the exchange reactions, and similar conclusions can be reached. The same set of equations (5, 15, and 16) for the exchange reaction are applicable to the competition reaction.

These results demonstrate unequivocally the relative order of the preferred binding site by Hg^{II}/CH_3Hg^{II} via H^+ displacement as N3−H (ThyH) > N1−H (GuoH), although binding to both bases is significant. This conclusion provides direct support for Katz's model[81] for Hg^{II} binding to DNA, postulating initial reaction at appropriate ThyH,ThyH pairs which leads to the formation of Thy−Hg−Thy dimers. This initial process is followed by other cross-linking reactions, after all readily available ThyH,ThyH sites have been exhausted. It should however be pointed out that the mononucleoside models employed in this study are incapable of mirroring any stereochemical factors inherent in polymeric structures. This limitation was explored further in subsequent studies, as described below.

Equilibration of GuoH with **56** or ThyH with **57** in pure DMSO[83] in the ratio 2:1 results in identical, rapid, equilibrium redistribution of the species present in each system as revealed by in situ ^1H and ^{13}C NMR spectroscopic analyses. From each reaction system, the bridged complexes **56** and **57**, uncomplexed nucleosides GuoH and ThyH, and the mixed bridged complex **59** were demonstrated[85] to be present. Starting with GuoH + **56**, or ThyH + **57**, as reactants yielded the identical equilibrium composition of **56**:**57**:**59**, i.e., 3.2:1.5:1.0. Thus, the redistribution process establishes the relative thermodynamic stability order of **56** > **57** > **59**. In a broader sense, these results support Katz's chain slippage mechanism for Hg^{II} binding to DNA.[81] The redistribution in DMSO could, in principle, occur via a three-centered exchange process or, in a secondary process, via a four-centered transition state depicted as **60** and **61**,



respectively.[83] Precedents for such associative mechanisms have been proposed in other systems.[43,86] A dissociative process (eqs 5, 15a, and 17) can also

Table 1. $^2J(^1H-^{199}Hg)$ Coupling Constants for Representative CH_3Hg^{II} Complexes

complex	$^2J(^1H-^{199}Hg)$/Hz N-bound	S-bound	C-bound	ref
20b $[(CH_3Hg)(InoH_2)]NO_3$	233.3			34
21b $[(CH_3Hg)(InoH)]$	207.5			34
	(211)[c]			31c
22b $[(CH_3Hg)_2(InoH)]NO_3$	221.2			34
25b $[(CH_3Hg)_3(Ino)]NO_3$	215.8		159.5	34
30b $[(CH_3Hg)_2(Th)]NO_3$	227.4			37a
33 $[\{(CH_3Hg)(Th)\}_2Hg](NO_3)_2$	232.2			37a
34a $[(CH_3Hg)(XanthH)]$	211.8			40
35b $[(CH_3Hg)_3(Xanth)]NO_3$	219.1			40
36 $[(CH_3Hg)_4(Xanth)]NO_3$	213.4		156.9	40
37c $[(CH_3Hg)(ImH)]$	196.0			42a
39a $[(CH_3Hg)(MeImH)]NO_3$	222.1			42a
39d $[(CH_3Hg)_2(MeIm)]NO_3$	214.2		151.4	42a
39e $[(CH_3Hg)(Me_2Im)]NO_3$			165.8	42a
46 $[(CH_3Hg)(MeImS)]$		184.6		44
47a $[(CH_3Hg)(MeImSH)]NO_3$		204.2[d]		44
47b $[(CH_3Hg)_2(MeImS)]NO_3$		207.8[d]		44
48a $[(CH_3Hg)(8\text{-thioGuoH})]$		187.2		45
48b $[(CH_3Hg)_2(8\text{-thioGuo})]$		197.8[d]		45
49a $[(CH_3Hg)(8\text{-thioGuoH}_2)]$		211.9[d]		45
50a $[(CH_3Hg)(6\text{-MPurH})]$		185.5		46
50b $[(CH_3Hg)(2\text{-A-6-MPurH})]$		184.9		46
51c $[(CH_3Hg)_3(6\text{-MPur})]NO_3$		195.3[d]	146.7	46
51d $[(CH_3Hg)_3(2\text{-A-6- MPur})]NO_3$		193.1[d]	156.7	46

[a] The number of H atoms shown in each complex is equal to the number of potentially ionizable protons still present in the complex. [b] Abbreviations shown in the complexes are as follows for the basic skeletons of the ligands: Ino = inosine; Th = theophylline; Xanth = xanthosine; Im = imidazole; MeIm = 1-methylimidazole; Me_2Im = 1,3-dimethylimidazolium ion; MeImS = methimazole; 8-thioGuo = 8-thioguanosine; 6-MPur = 6-mercaptopurine riboside; 2-A-6-MPur = 2-amino-6-mecaptopurine riboside. [c] Measured in D_2O solution (see ref 31d). [d] Values are averaged coupling constants due to rapid CH_3Hg^{II} exchange between N and S sites (see the text).

account for the results,[43,83] the proton acceptor in this case being DMSO or adventitious H_2O present in DMSO.

$$Nuc_1-H + H_2O \rightleftharpoons Nuc_1^- + H_3O^+ \quad (5)$$

$$Nuc_2-Hg-Nuc_2 + H_3O^+ \rightleftharpoons Nuc_2-Hg^+ + Nuc_2-H + H_2O \quad (15a)$$

$$Nuc_2-Hg^+ + Nuc_1^- \rightleftharpoons Nuc_2-Hg-Nuc_1 \quad (17)$$

Circular dichroism (CD) studies of Hg^{II}-induced transitions in a series of polynucleotides[87] support initial binding to thymine, in broad agreement with Katz's model; this initial process, in turn, triggers duplex strand separation with concomitant cooperative binding to an adenine amino group of poly(dA) strands. Such studies on Hg^{II}–nucleic acid interactions also provide evidence for conformational changes,[88] typically involving transitions from right-handed to left-handed structures.[89] Recent NMR investigations of Hg^{II} binding to oligonucleotides,[14,85] on the other hand, suggest that Hg^{II} binding to DNA is largely determined by the requirement for linear geometry at adenine N6 and thymine O4; thus, a cross-link is indeed formed with no consequence on the adenine–thymine N3–H···N1 hydrogen bond or the H2···O2 distance. Clearly, more work is needed to construct a model that fully correlates Hg^{II} effects with biomolecule complexity. In this regard, it is pertinent to point to a number of recent high-level quantum chemical studies[90–92] which demonstrate qualitative differences between adenine-containing and guanine-containing base pairs, with the consequence that the stabilities of adenine–thymine and guanine–cytosine base pairs are affected differently by interactions involving the hydrated metal ion.[90,91] Thus, while the stability of adenine–thymine base pairing is enhanced through electrostatic effects, that of the guanine–cytosine pair is increased through a polarization mechanism.[91]

6. Diagnostic and Practical Utility of NMR Features of CH_3Hg^{II} Complexes

Certain ^1H and ^{13}C NMR features of some of the CH_3Hg^{II} complexes presented above deserve some comment. $^2J(^1H-^{199}Hg)$ coupling constants show significant sensitivity to the strength of the Hg^{II}–ligand bond. In general, ^{13}C chemical shifts are inherently more sensitive to the environment of the metal ion than are ^1H chemical shifts; hence, the former provide a useful indicator of the nature of the donor ligand. For example, the conclusion that the ribose moiety of the nucleosides investigated is not involved in binding is reached on the basis that the sugar C atoms show only very slight changes in their ^{13}C chemical shifts upon complexation of the nucleobase with Hg^{II}/CH_3Hg^{II}.[33,34]

One can generalize, from the $^2J(^1H-^{199}Hg)$ values presented in Table 1 for representative examples, that coupling constants follow the order N- → S- → C-bound complexes. This order reflects the thermodynamic stability of the $X-Hg^{II}$ bond (X = N, S, or C donor atom) in which the $C-Hg^{II}$ bond is the most stable and the $N-Hg^{II}$ bond most labile; the $S-Hg^{II}$ bond is of intermediate stability relative to the

Table 2. pK_a Values at 25 °C for NH_2 Groups of Several Nucleosides and Related Compounds Derived by Application of Eq 19[a]

compound	pK_a[b,c]	compound	pK_a[b,c]
adenosine	17.0 (18−19)[d]	1-methylguanosine	14.9
9-methyladenine	17.0 (16.7)[e]	cytidine	15.5
1-methyladenosine	− (8.55)[f]	1-methylcytosine	− (16.7)[e]
guanosine	15.1	2,3-*O*-benzylidene-5-*O*-tritylcytidine	− (14.8)[e]
1,9-dimethylguanine	− (14.6)[e]		

[a] Reprinted with permission from ref 26. Copyright 1981 Elsevier. [b] Estimated uncertainties ±0.3 pK_a unit. [c] Data in parentheses refer to literature values. [d] See: McConnell, B. *Biochemistry* **1974**, *13*, 4516. [e] See ref 94. [f] See: Hoo, D.-L.; McConnell, B. *J. Am. Chem. Soc*. **1979**, *101*, 7470.

C−Hg^{II} and N−Hg^{II} bonds. This thermodynamic order of stability differs from the observed ease of mercuration which manifests the hierarchy S- → N- → C-mercuration; C2/C8-mercuration has been shown above to be crucially dependent on protonation or metal coordination at N3/N7. ^{13}C chemical shifts are considerably larger for S-bound complexes than for N-bound ones. In complexes containing S- and N-bound CH_3Hg^{II}, the ^{13}C signal is intermediate in value compared to those of the respective S-bound/N-bound complexes, indicating rapid exchange of CH_3Hg^{II} between the two centers (N and S) on the NMR time scale.

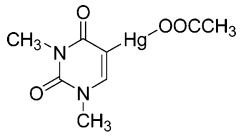
^{13}C NMR chemical shifts also elicit $^1J(^{13}C-^{199}Hg)$ couplings which are significantly larger in magnitude than $^2J(^1H-^{199}Hg)$ couplings and can be correlated with the latter according to eq 18. The origin of the relationship in eq 18 has been discussed.[83]

$$^1J = 8.460(^2J) - 155.6 \qquad (18)$$

The importance of the acidity of NH_2 groups in defining complementarity of adenine−thymine and guanine−cytosine base pairing through H-bonding interactions underscores the need for reliable methods for the accurate measurement of their pK_a values in the different nucleosides in which they occur. Data from our work[33,34,42a,c] and other laboratories[43,60a,61,93] suggest that, for a given ligand donor atom, the magnitude of the coupling constant $^2J(^1H-^{199}Hg)$, or simply J, of the CH_3Hg^{II} complex is related to the basicity of the donor atom. We have found[26,42a] that eq 19 holds for sites that can undergo protonation and methylmercuration, insofar as there is no 3-coordinate Hg in the CH_3Hg^{II} complex. Application of eq 19 to J values measured for exocyclic NH_2-bound CH_3Hg^{II} complexes provides the pK_a values of the NH_2 groups of several nucleosides and related compounds (Table 2), which compare well with literature values. The accessibility of NH_2 pK_a values, hitherto derived by indirect methods,[94] through this simple NMR method enhances the prospects of quantifying the contributions of H-bonding[95] and electronic complementarity[96] to the specificity of base pairing.

$$J = -3.88\text{p}K_a + 248.5 \qquad (19)$$

A number of interesting NMR studies, which reveal important structural details in the solution interactions of Hg^{II} with biomolecules, have appeared in the literature recently. ^1H and ^{199}Hg NMR spectroscopy was applied to the exchange reactions of the complex 1,3-dimeU-*C*5-Hg−$OOCCH_3$ (1,3-DimeU = 1,3-dimethyluracil), **62**, with a variety of anions and



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model nucleobases.[97] ^3J coupling of the ^{199}Hg isotope with H6 of the 1,3-dimeU ligand was found to be diagnostic of the donor atoms *trans* to C5 of the uracil ring. ^{199}Hg NMR spectra of mixed nucleobase complexes of the type 1,3-DimeU-*C*5-HgL (L = second nucleobase) demonstrate a C−Hg−N arrangement, with strong binding of the electrophile to the second nucleobase at N3 of uracil, N3 of thymine, N4 of cytosine, or N1 of guanine. Binding to N7 of guanine was found to be weak.

^{199}Hg NMR studies and Biograf energy-minimum calculations of Hg_2Cl_2(*cys*,*his*-peptide) [*cys*,*his* = Cys and His residues coordinated to Hg^{II}] show that the ^{199}Hg NMR chemical shift of the complexes Hg_2Cl_2-(Z-*cys*-X-Y-*his*-OMe) (Z = benzyloxycarbonyl; X-Y = Ala-Ala, Ala-Pro, or Pro-Val) correlate with the coordinating ability of the Cys-X-Y-His moiety, which is a function of the interposed amino acid residues.[98] The coordination modes of the cysteinethiolate groups are similar in the complexes. On the other hand, weak interactions occur between the histidine imidazole group and Hg^{II}; these interactions depend on the number of amino acid residues intervening between the Cys and His residues. Sensitivity of the ^{199}Hg NMR chemical shifts in the complexes to the steric effects of the amino acid residues was also demonstrated.

The novel work of O'Halloran and co-workers[99−101] provides direct structural data for MerR and MerR−DNA adducts, and demonstrates the application of ^{199}Hg NMR spectroscopy to the study of the active sites of metalloproteins. Specifically, homonuclear and heteronuclear proton-detected ^{199}Hg NMR data were employed to delineate the metal ion receptor environment of MerR and to characterize the allostery of MerR−DNA interactions by locating the ligands coordinating to Hg^{II} both in the MerR protein and in the protein−DNA complex. Comparison of the ^{199}Hg NMR chemical shifts of MerR and MerR−DNA adducts with those of model complexes and metalloproteins provides definitive information regarding the modes of Hg^{II} coordination.

7. Neurotoxicity of CH_3Hg^{II}

CH_3Hg^{II} is a widespread and highly toxic environmental pollutant and has been long recognized as a neurotoxic hazard. Neuorological degeneration in animals and humans and the Minamata disease have been ascribed by medical scientists to high levels of ingested CH_3Hg^{II}.[102] There is, however, no agreement regarding the mechanism of its neurotoxicity[103,104] as well as the relationship between the distribution of the toxicant in the central nervous system and CH_3Hg^{II} neurotoxicity.[105] It has been suggested that both CH_3Hg^{II} accumulation and biotransformation are correlated with neurotoxicity[105b] and that the presence of CH_3Hg^{II} affects the neuron in various ways that interfere with neurotransmission.[106]

In our study,[107,108] the correlation between CH_3Hg^{II} burden and its metabolism to Hg^{II}, and the structural damage in distinct regions of the mouse brain, was assessed following the administration of subchronic CH_3Hg^{II} treatment. No correlation was found to exist between total Hg^{II} and structural damage, whereas CH_3Hg^{II} was evenly distributed in the brain. However, a correlation was found between Hg^{II} concentration and the amount of structural damage observed in the anterior cerebral cortex. These observations suggest different mechanisms of sensitivity to Hg^{II} in the different areas of the brain and emphasize a possible role for the inorganic metabolite of CH_3Hg^{II} in the anterior cerebral cortex.[109]

The distribution of inorganic Hg^{II}, presumably resulting from demethylation of CH_3Hg^{II}, and the disruption of the blood−brain barrier have been demonstrated in the central nervous system of rats using autometallographic techniques.[109] Results from studies on the immunomodulating effects of CH_3Hg^{II} on mice[110] and of Hg^{II} and CH_3Hg^{II} on Ca^{II} fluxes in rat brain microsomes[111] have appeared recently. CH_3Hg^{II} has been reported to increase cystolic Ca^{II} concentration in rat cerebrum synaptosomes[112] as well as inhibit ATP synthesis.[113]

8. Mercury Detoxification Strategies

Mercury bioaccumulates as CH_3Hg^{II} in the food chain. Its dynamic redox chemistry in the atmosphere and condensation via climatic mechanisms lead to widespread contamination of soils and water. It is therefore an important concern that environmental mercury pollution and associated effects be kept to the barest minimum. This calls for the development of effective technologies for reducing the mercury burden of the environment and for treating environments that are already mercury-contaminated. In this section, various emerging methods and technologies for remediation of mercury pollution and intoxication are reviewed.

8.1. Anthropogenic Methods

The conventional methods for removal of mercury from contaminated sites are mainly physical or physicochemical, such as dredging and landfilling of hazardous sediments, precipitation, chemical coagulation, or adsorption.[114] These methods either are costly or generate secondary pollution.[115] This has led to the search for new, convenient, safe, and possibly more cost effective methods of environmental remediation.

A recent study[116] demonstrated that CH_3HgCl is degraded by $HO^{\bullet}$ radicals which are generated via nitrate photolysis in the wavelength range of 285−800 nm with a 450 W xenon lamp. Hg^{II}, Hg^0, $CHCl_3$, and CH_2O were identified as products of the process. It is argued that formation of formaldehyde as one of the reaction products is good evidence that the reaction proceeds by way of C−Hg bond fission. The second-order rate constant ($9.83 \times 10^9\ M^{-1}s^{-1}$) calculated for the process at pH 5 and room temperature with benzoic acid as scavenger enabled an estimation of the CH_3Hg^{II} degradation rate in natural waters, leading to the conclusion that $HO^{\bullet}$ degradation of organomercurials may be one of the important natural pathways in surface water for reducing the levels of this toxicant in the environment (see below).

Electrokinetic means for in situ remediation of contaminated soils, in which an imposed electric field affects the directional migration of the contaminant, have been suggested,[117] and laboratory-scale experiments have been published.[118] The potential for this method was demonstrated recently for the removal of ionic mercury from contaminated soils.[119] It was shown that Hg^{II} is mobilized toward the anode, probably as HgI_4^{2-}. A variant of this principle[120] involves the addition of I_2/I^-, as a lixiviant near the cathode, to contaminated soils. The presence of the lixiviant ensured the oxidation of reduced mercury in the soil and its complexation and subsequent transport as the complex ion HgI_4^{2-}.

A method which utilizes acidic KI to clean up mercury-contaminated soils in the absence of an applied electric field has also been demonstrated on the bench scale.[121] Repeated passage of acidic KI solution (pH 1.5) through a column packed with the contaminated soil (containing 47.1 mg of Hg/g) decreased the mercury content by ca. 76%. The HgI_4^{2-} in the leachate from the column was then treated with activated carbon.

Removal of inorganic mercury contaminant from wastewaters using a crandalitte-type compound with the formula $Ca_{0.5}Sr_{0.5}Al_3(OH)_6(HPO_4)(PO_4)$ has been described.[122] Ca^{II} and Sr^{II} exchanged with Hg^{II} in the wastewater according to eq 20, reducing the mercury content from 90 to <0.1 ppm. The crandallite is recharged by treating it with HCl solution at pH 2.25, during which 75% recovery of mercury is achieved.

$$2Ca_{0.5}Sr_{0.5}Al_3(OH)_6(HPO_4)(PO_4) + 2Hg^{2+} \rightleftharpoons 2HgAl_3(OH)_6(HPO_4)(PO_4) + Ca^{2+} + Sr^{2+} \quad (20)$$

Other recently suggested methods for mercury remediation are the immobilization of mercury(II) in contaminated soils with used tire rubber[123] and the removal of the ionic form of the toxicant from the soil as an inclusion complex of cyclodextrin.[124] In the former case, the material was found to adsorb Hg^{II} in the soil under the optimum condition of acidic to

neutral pH range; the long-term leaching behavior of the rubber-treated soil has so far not been accounted for.

8.2. Nature's Strategies

Considerable interest has been shown in bioremediation mechanisms, implicating bacteria, for dealing with unfavorable heavy metal burdens, since these metals in their organometallic forms tend to bioaccumulate in the food chain. A number of excellent reviews on bacterial detoxification of Hg^{II} and organomercurials have appeared recently,[125−129] in addition to Sigel's important collection of insightful reviews on the biological chemistry of mercury. An overview of the status of research in this area would serve to highlight the potentials and advantages inherent in exploring for practical application chemical mimics of nature's strategies for mercury detoxification.

One of the most studied and understood natural detoxification processes involves plasmid- and/or transposon (Tn)-encoded mercury resistance (*mer*) operons incorporating specific structural genes in bacteria such as *Pseudomonas* sp.,[130] *Thiobacillus* sp.,[131] *Serratia marcescens*,[132] *Staphylococcus aureus*,[133] *Streptomyces lividans*,[134] *Bacillus* sp.,[135] *Escherichia coli*,[136] and *Shigella* sp.[137] Bacterial detoxification results in the conversion of toxic organomercurials to elemental Hg (Hg^0), which is volatile and much less reactive, and can be passively eliminated. The detoxification process for organomercurials involves[14] three distinct steps: (a) mercury uptake, (b) cleavage of organomercurials through protonolysis of the C−Hg bond by organomercurial lyase (MerB) as in eq 21, and (iii) reduction of ionic mercury in an enzyme (mercuric reductase)-mediated electron-transfer process (see eq 22)

$$R{-}CH_2{-}Hg^{II} + H^+ \rightarrow Hg^{II} + R{-}CH_3 \quad (21)$$

$$RS{-}Hg^{II}{-}SR' + NADPH + H^+ \rightarrow Hg^0 + RSH + R'SH + NADP^+ \quad (22)$$

in which the reductant is NADPH.[138−140] $RS{-}Hg^{II}{-}SR'$ may be protein-bound Hg^{II} or glutathione$-Hg^{II}$ adducts, since free Hg^{2+} is unlikely to exist in the bacterial protoplasm which contains 5−10 mM concentrations of glutathione, largely in the reduced form.[129] The effect of intracellular glutathione on sensitivity to Hg^{II} cations has been demonstrated experimentally.[141]

Other structural genes are also involved in this process: the MerP (periplasm) protein binds mercury in the periplasm, conveying it to the MerT (transport) protein which undertakes transport of the mercury through the membrane to the cytoplasm.[125,126,142] The structures of the reduced and mercury-bound forms of MerP have been recently determined in aqueous solution by NMR spectroscopy.[142] The rate-limiting step of the mercury detoxification process is the transport step in which Hg^{II} is brought into the cell.[143−145] The overall result of the process described above is the cleaning of the external environment of the bacterium through an integrated management of Hg^{II} uptake, transport, and detoxification. The pairs of cysteine residues, which form part of all proteins expressed from the *mer* operon, are implicated as essential components of the detoxification pathway, given the thermodynamically favorable ligation of Hg^{II} with bisthiols.[14,125−127] Significantly, gene expression in genes that encode for proteins associated with the detoxification process is sensitive to the Hg^{II} concentration gradient.[14,125−127,145] The reader is referred to recent comprehensive reviews by Barkay et al.[125] and Miller[126] for a detailed discussion on the mechanism of bacterial mercury resistance and detoxification, to include the functional components of the Hg resistance operons, diversity of mercury resistance loci, applied biology of mercury resistance, and applications of the underlying principles of bacterial mercury resistance to the development of environmental mercury remediation technologies. The role of the transport proteins and the mechanism of Hg^{II} transfer between the mercury resistance proteins have also been treated by Brown et al.[129]

Three different mechanisms of mercury detoxification of wastewater have recently been demonstrated by Essa et al.[146] to occur in one organism, *Klebsiella pneumoniae* M426. These are (i) enzymatic reduction and volatilization due to the presence of the mercury-resistance determinant *Tn5073*, (ii) aerobic precipitation of ionic Hg^{II} as insoluble HgS, resulting from H_2S production, and (iii) biomineralization of Hg^{II} as an insoluble $Hg^{II}-S$ complex, other than HgS, achieved through aerobic production of a volatile thiol compound. The high efficiency of mercury removal in the presence of high concentrations of mercury and at different pH values and salinity levels recorded in this study point to its potential for industrial application.

These results and the directions noted above have remarkable implications for the design of chemical strategies for environmental remediation and for countering mercury intoxication in humans. A biochemical model for achieving such targets, involving cloning of genes and the study of gene products of bacterial Hg^{II}-resistant phenotypes, has been proposed.[2c,100,147,148] The genetic potential for mercury detoxification by mercury-resistance bacteria in aquatic environments has been discussed.[149]

Genetic engineering applications of the principles of this natural process have been established; some of the recent demonstrations of these applications will be described here. A model plant, *Arabidopsis thaliana*, has been engineered to express a modified bacterial gene, *merBpe*, encoding the organomercurial lyase MerB.[150] The plant was demonstrated to tolerate a wide range of concentrations of CH_3Hg^{II}-Cl and $PhHg^{II}OAc$, which severely inhibited or killed similar plants lacking the *merBpe* gene. This demonstrates the possibility that native macrophytes thus engineered may be capable of cleaning mercury-polluted soils by degrading CH_3Hg^{II} and sequestering Hg^{II} for subsequent removal. When the same plant referred to above was genetically engineered to coexpress *merA* and *merB* genes,[151] it grew on 50-fold higher concentrations of CH_3Hg^{II} than wild-type

plants and ca. 10-fold higher concentrations than the same species in which only *merB* was expressed.

E. coli cells, which have been genetically engineered to express metallothionein, have been shown[152] to accumulate Hg^{II} effectively at low concentrations (<20 mM) across acidic and basic conditions (pH range ≈ 3−11). This process is highly selective against Na^+, Mg^{2+}, and Cd^{2+} and is unaffected by metal chelates such as EDTA and citrate. These results suggest[152] that the *E. coli* strain used in the study could be applied for selective elimination of Hg^{II} from contaminated water and soils, sediments, or particulates. An example of a long-term working mercury reduction system in a fully automated commercial process has been described by Wagner-Döbler.[153] In this process, elemental mercury, produced by microbial detoxification of mercury-containing wastewater, is retained quantitatively in packed-bed bioreactors in which biofilms of mercury-resistant bacteria are grown on an inert porous carrier material. The technology involved is assessed to be simple, environmentally friendly, and cost-effective. *E. coli* has also been genetically engineered to simultaneously express a Hg^{II} transport system and overexpress metallothionein as a carbonyl terminal fusion to glutathione S-transferase.[154] A number of industrial applications of microbial cleanup technologies have also been reviewed by Wagner-Döbler.[153] Biosorption technologies for remediation of mercury-contaminated matrixes have also been investigated, although no commercially viable process is yet available.[155]

A report has also appeared recently[115] in which the principles involved in microbial detoxification of mercury have been adapted for practical, everyday application. Mercuric reductase, immobilized on a chemically modified earth support, was used to detoxify Hg^{II}-containing solutions. Artificial dyes which are known to be efficient electron donors, e.g., azure A, bromophenol blue, safranin, and neutral red, were substituted for NADPH in the mercuric reductase-mediated reduction of Hg^{II} in batch and fixed-bed operations, to improve the applicability of the immobilized enzyme system. Although the artificial dyes were found to be less efficient than NADPH, the results obtained show that this technique is a feasible one.

It has been postulated[156,157] that photodegradation of organomercurials is a potential sink in surface waters. Recent work on degradation of CH_3Hg^{II} by $HO^{\bullet}$ suggests that the photodegradation process is an indirect one in which sunlit natural waters first generate the $HO^{\bullet}$ radical, which then goes on to degrade the toxicant (vide supra).[116]

A recent publication outlines a novel mechanism for defense against CH_3Hg^{II} toxicity.[158] Clones of yeast cells, in which the *Cdc34* gene was overexpressed, grew in the presence of a normally toxic concentration of CH_3HgCl. Since *Cdc34* encodes a ubiquitin-conjugating enzyme, it is speculated that the ubiquitin−proteasome system, which is strongly conserved from yeast to human cells, might be responsible for protection of yeast and human cells against CH_3Hg^{II} intoxication.

Detection of low levels of mercury is critical to the development of an efficient and integrated strategy for the management of environmental mercury pollution. In this regard, it is noted that Palomares et al.[159] have reported recently the design of a novel chemical sensor for the colorimetric detection of mercuric salts. The sensor is based on a mesoporous nanocrystalline TiO_2 film which is sensitized with a commercially available ruthenium dye. The color of the film changes from red to orange when it is immersed in an aqueous solution containing Hg^{2+}, demonstrating high selectivity and submicromolar sensitivity.

9. Concluding Remarks and Future Outlook

The use of the unidentate $(CH_3Hg^{II})^+$ cation to probe metal ion−biomolecule interactions has made possible the identification of individual binding sites in purine nucleosides and related substrates. The demonstration of the binding of CH_3Hg^{II} to N and C centers of DNA bases clearly point to additional mechanisms for the rationalization of the mutagenicity of organomercurials and other heavy metal ions. In particular, the essentially irreversible formation of C-bound complexes may account as much for the toxicity of CH_3Hg^{II}/Hg^{II} as the binding of these electrophiles to S centers. Coordination of CH_3Hg^{II} to deprotonated NH_2 groups in nucleosides demonstrates an important mechanism for the disruption of DNA base pairing; this phenomenon could be of greater physiological significance in the distortion of secondary structures of biomolecules than methylmercuration of endocyclic N3 and N1 sites in thymine and guanine, respectively.

NMR data demonstrate the thermodynamic stability order C → S → N in CH_3Hg^{II}-bound complexes. Where both N and S centers are involved in binding, exchange of CH_3Hg^{II} on the NMR time scale, with an average value of $^2J(^1H-^{199}Hg)$, is observed. Such rapid ligand exchanges provide a key to understanding the bioavailability of CH_3Hg^{II} as well as the use of CH_3Hg^{II}/Hg^{II} binding agents in therapeutic procedures. ^{13}C chemical shifts and coupling constants provide better information than their ^1H counterparts concerning the metal ion environment; a linear correlation exists between $^2J(^1H-^{199}Hg)$ and $^1J(^{13}C-^{199}Hg)$, the former parameter providing a convenient empirical tool for assessing NH_2 acidity in nucleosides and related substrates.

Activation of C2/C8 toward methylmercuration/mercuration depends on the presence of electrophiles at N3/N7 of these substrates, with most metals acting as poorer activators of C2/C8−H abstraction than H^+ or CH_3^+.

Direct support through competition and exchange reactions has been presented for Katz's[81] chain slippage model for binding of Hg^{II} to DNA. The toxicant shows preference for ThyH,ThyH pairs, although binding to other susceptible pairs is significant. The relative thermodynamic stabilities of the bridged species involved in cross-chain linkage follows the order [Thy−Hg−Thy] > [Guo−Hg−Guo] > [Thy−Hg−Guo]. Characterization of the mixed bridged species was achieved in DMSO.

Recent CD data argue for conformational changes induced by Hg^{II} binding to DNA, involving transitions from right-handed to left-handed structures. NMR studies on oligonucleotides suggest that Hg^{II} binding to DNA is determined by the necessity to maintain linear geometry at adenine N6 and thymine O4. Models capable of correlating the specific nature of CH_3Hg^{II}/Hg^{II}–biomolecule interactions with progressive biomolecule complexity are required for a thorough understanding of the structural consequences of Hg^{II} binding to DNA at the molecular level.

Crystal structures for a number of complexes provide direct evidence for binding sites and demonstrate H-bonding and secondary bonding interactions involving Hg in the solid state. A possible role for the inorganic metabolite of CH_3Hg^{II} in neurotoxicity is suggested.

Nature's strategies for mercury detoxification, as exemplified by some bacteria, underscore the huge potential in deploying these strategies in a rational manner for environmental remediation, reversal of mercury intoxication in humans, and the exploration of chemical mimics of these strategies already optimized by nature. Recent efforts at genetic engineering adaptations of the bacterial methods of mercury detoxification demonstrate promise for everyday application.

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