Methyl Transfer to Mercury Thiolates: Effects of Coordination Number and Ligand Dissociation

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The complexes $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ demethylate $(CH_3O)_3PO$ as revealed by ^1H, $^{31}P\{^1H\}$, and $^{199}Hg\{^1H\}$ NMR spectroscopy in DMSO-d_6 solution. The products of the $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ reaction are $CH_3SC_6H_5$, $(CH_3O)_2PO_2^-$, and $[Hg(SC_6H_5)_3]^-$, whereas $[Hg(SC_6H_5)_3]^-$ demethylates $(CH_3O)_3PO$ to yield $CH_3SC_6H_5$ and $\{Hg(SC_6H_5)_2[(CH_3O)_2PO_2]\}^-$. Kinetic and solution studies of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ reveal a rapid equilibrium between bound and free thiolate. The dissociated thiolate is the nucleophile active toward $(CH_3O)_3PO$. These results imply that the metal center of the inactive mercury derivative of the *Escherichia coli* Ada DNA alkylation repair protein may comprise a three-coordinate $[Hg(S\text{-cysteine})_3]^-$ moiety and an unbound, protonated cysteine (HS-Cys69).

Introduction

DNA is continually assaulted by nonenzymatic alkylating agents in the environment.[1−5] Some of the resulting lesions, such as O^6-methylguanine and O^4-methylthymine, disrupt traditional Watson−Crick base pairing and lead to mismatched DNA base pairs.[6−12] To combat these and related processes, most organisms have protein systems responsible for repairing alkylated and mismatched DNA.[2,6,13]

One widely studied example is the Ada protein of *Escherichia coli*. By direct transfer of the offending alkyl groups to cysteine residues in the protein,[14−21] Ada repairs O^6-alkylguanine, O^4-alkylthymine, and the S_p diastereomer of alkylphosphotriesters.[20−22] Two different active sites are responsible for these repair capabilities.[17,18,20] The alkylated base lesions are repaired by Cys321 in the C-terminal domain of the protein,[14,18,19] which is part of an Asn-X_6-Pro-Cys-His-Arg-Val-X_9-Tyr-$X_{13/14}$-Glu consensus sequence common to all known O^6-methylguanine transferases.[23] Alkylphosphotriesters are repaired by Cys69 in the N-terminal domain of the protein,[17,18] one of four cysteine residues which are bound to a zinc ion.[15,24−26] In addition to reversing phosphate damage, alkylation of Cys69 effects a structural change in the protein that enhances genome affinity.[15,16,18,27−29] Binding of the Cys69-alkylated Ada to DNA induces gene products that are part of the cellular response to alkylation damage.[29−33] The induced proteins include both Ada and other alkylation repair proteins.[20,22,30−38]

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Both direct biochemical[15,16,24−27,39,40] and inorganic modeling studies[41,42] of the Ada $[Zn(S\text{-}Cys)_4]^{2-}$ site have elucidated the role of the metal center in DNA repair and cellular regulation. Functional model studies in our laboratory used $[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$ to mimic the $[Zn(S\text{-}Cys)_4]^{2-}$ protein site and $(CH_3O)_3PO$ to represent a methylphosphotriester lesion.[41,42] Both $[Zn(SR)_4]^{2-}$ and RS^- were active in methyl transfer, whereas RSH was not. Benzenethiolate ligand readily dissociated from $[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$ in dimethyl sulfoxide (DMSO) solution, and the nucleophile active in methyl transfer was determined to be the dissociated thiolate.

These studies coupled with protein work distinguishing the nature of Cys69 from the three other zinc ligands[15,16,24,39] formed the basis for a proposed mechanism of alkylphosphotriester repair by Ada. In this mechanism, zinc coordination of Cys69 prevents protonation by accessible solvent to preserve thiolate nucleophilicity. Transient dissociation of Cys69 from zinc enables a thiolate nucleophile to react with the alkylphosphotriester lesion. Cys69 is in equilibrium between zinc-bound and free states.

To understand further the chemistry of the $[Zn(S\text{-}Cys)_4]^{2-}$ center in Ada, many metal substitution studies have been carried out.[15,16,24,27,40] The cadmium form of N-terminal Ada fragments exhibited both a structure similar to the zinc form and methylphosphotriester repair capability.[15,16,24,27] The second-order rate constant of methylphosphotriester repair by Cd-*N*-Ada10 was one-quarter that of Zn-*N*-Ada10.[27] A preliminary report of a cobalt-substituted Ada fragment (Co-*N*-Ada16) is available.[16] Most recently, a mercury form of *N*-Ada17 was prepared to examine the potential effects of mercury exposure on cellular ability to repair alkylated nucleic acids.[40] Although the structure of Hg-*N*-Ada17 appears to be similar to that of Zn-*N*-Ada17, no methylphosphotriester repair activity was observed.

We envision three possible explanations for the native structure and inactivity of Hg-*N*-Ada17. First, the mercury cysteine thiolate center could be three-coordinate, with Cys38, -42, and -72 acting as ligands. In such a situation, Cys69 might be protonated and deactivated for methyl transfer. Second, Cys69 could be coordinated to mercury in a three-coordinate center, resulting in diminished nucleophilicity and the observed lack of reactivity. Third, all four cysteine residues (Cys38, -42, -69, and -72) could bind mercury, which would also decrease the nucleophilicity of Cys69.

In the present investigation, we have extended our functional model system for methylphosphotriester repair by Ada to mercury thiolates. The solution behavior and reactivity of the mercury thiolate complexes $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ with $(CH_3O)_3PO$ were investigated. The results afford insight into the nature of the mercury cysteine site of Hg-*N*-Ada17.

Experimental Section

General. Due to the photosensitivity of mercury compounds, all procedures were performed in a reduced light environment. Solutions were manipulated in a darkened hood, and reaction flasks were covered with aluminum foil. All manipulations were carried out under argon by using standard Schlenk techniques. Solvents were degassed with argon. DMSO-d_6 was dried, degassed, and distilled according to standard procedures.[43] Analytical NMR spectra were recorded at 25 ± 1 °C on Varian Unity 300, Unity Plus 300, and VXR-500 instruments.

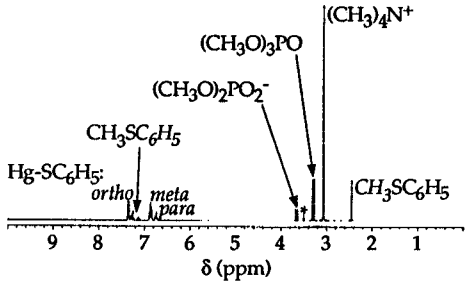

Figure 1. ^1H NMR spectrum in DMSO-d_6 of an equimolar reaction of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3O)_3PO$ in progress. The asterisk denotes residual H_2O. Starting reagent concentrations were 211 mM each. This spectrum was taken 2 days after mixing of the reagents.

$^{31}P\{^1H\}$ and $^{199}Hg\{^1H\}$ (at 53.707 MHz) NMR spectra were recorded on samples of 211 mM concentration. The compound $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ was synthesized according to a literature procedure[44] and characterized by elemental analysis and ^1H NMR spectroscopy.

Kinetic Studies. Kinetic runs were performed under pseudo-first-order conditions with 5.0 mM metal thiolate and 1.0 mM $(CH_3O)_3PO$. Reactions were monitored by ^1H NMR spectroscopy in DMSO-d_6 at 26.9 ± 0.7 °C. Typical ^1H NMR parameters for kinetic studies were 4 scans/spectrum, 40 s relaxation delay between scans, 60 spectra/experiment, and a data collection time of 8 h. Concentrations of reactants and products were determined by referencing peak integrals to the resonances of $(CH_3)_4N^+$ and $(C_4H_9)_4N^+$ counterions, the concentrations of which were determined from known starting material quantities and known solution volumes. Rate constants were determined by curve-fitting $(CH_3O)_3PO$ concentration versus time plots with a standard, integrated expression for first-order decay.[45] The pseudo-first-order rate constant for the $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ reaction was determined in triplicate. The rate constant provided is an average of the three kinetic runs, with the error reflecting one standard deviation. The slow reaction of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ permitted only an upper limit of the pseudo-first-order rate constant to be determined. The initial rate method was used in this case.[45]

$[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$. Benzenethiol (16.6 g, 151 mmol), $(C_2H_5)_3N$ (15.3 g, 151 mmol), and $(CH_3)_4NCl$ (5.94 g, 54.2 mmol) were combined in methanol (140 mL). To this solution was added $Hg(NO_3)_2 \cdot H_2O$ (6.91 g, 20.2 mmol) in methanol (60 mL) over 30 min. During addition of the mercury solution, a white precipitate formed which dissolved upon further stirring. 1-Propanol (140 mL) was added over a 10 min period, and the reaction mixture was stored at −20 °C overnight. The resulting off-white crystals were collected, washed with 1-propanol, and dried in vacuo. Yield: 10.9 g, 69%. ^1H NMR (DMSO-d_6): δ 3.06 (s, 24 H, $(CH_3)N^+$), 6.71 (t, 4 H, *p*-H), 6.86 (t, 8 H, *m*-H), 7.28 (d, 8 H, *o*-H). Anal. Calcd for $C_{32}H_{44}N_2S_4Hg$: C, 48.93; H, 5.65; N, 3.57. Found: C, 49.14; H, 5.75; N, 3.57.

Results

Reaction of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ with $(CH_3O)_3PO$. An equimolar DMSO-d_6 solution of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3O)_3PO$ reacted quantitatively to form $[Hg(SC_6H_5)_3]^-$, $CH_3SC_6H_5$, and $(CH_3O)_2PO_2^-$ products as revealed by ^1H NMR spectroscopy (Figure 1). The methylated thiolate $CH_3SC_6H_5$ was not coordinated to the mercury ion since its ^1H NMR resonances were identical to those of a genuine sample. The $^{31}P\{^1H\}$ NMR spectrum of the reaction mixture exhibited one peak at 2.54 ppm, $\Delta\nu_{1/2} = 3.1$ Hz, the sharpness of which contrasted with the broad ($\Delta\nu_{1/2} = 60$ Hz) peak at 1.70 ppm in the analogous zinc reaction. This peak in the zinc reaction was assigned to $(CH_3O)_2PO_2^-$ in equilibrium between free and zinc-

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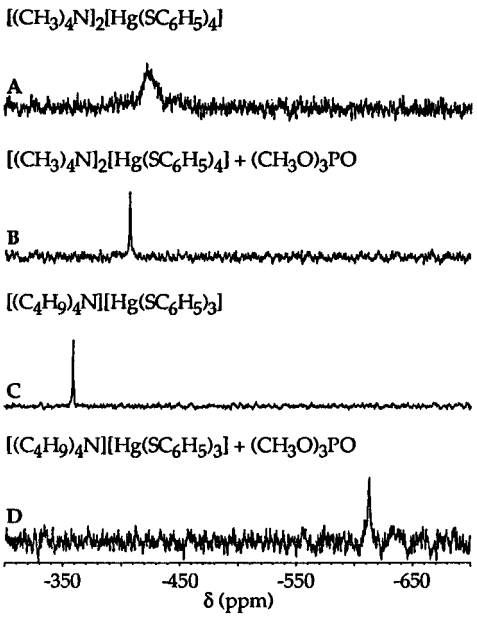

Figure 2. ^{199}Hg{^{1}H} NMR spectra in DMSO-d_6 of (A) $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$, (B) the reaction product of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3O)_3PO$, (C) $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$, and (D) the reaction of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ with $(CH_3O)_3PO$ at 23% completion 2 months after the reaction began. Starting concentrations were 211 mM in all cases. Chemical shifts are referenced to neat $(CH_3)_2Hg$.

bound states.[41,42] The sharp ^{31}P{^{1}H} NMR resonance in the mercury reaction indicated the absence of any such equilibrium process. Following the reaction, the ^{1}H NMR resonances of the product $[Hg(SC_6H_5)_3]^-$ (ortho = 7.27 ppm, meta = 7.01 ppm, and para = 6.88 ppm) were slightly shifted from those of the starting $[Hg(SC_6H_5)_4]^{2-}$ complex (ortho = 7.28 ppm, meta = 6.86 ppm, and para = 6.71 ppm) but were similar to those of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ (ortho = 7.24 ppm, meta = 6.99 ppm, and para = 6.87 ppm), reflecting the lower charge.

The ^{199}Hg{^{1}H} NMR spectrum of the reaction product displayed one peak at −407 ppm ($\Delta\nu_{1/2}$ = 57.4 Hz; Figure 2). This chemical shift value is very similar to that of the starting $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$, −421 ppm, which falls in the −300 to −500 ppm range reported for mercury tetrathiolates in solution.[46] The ^{199}Hg{^{1}H} NMR shift of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ is −359 ppm (Figure 2), similar to a literature value of −354 ppm.[47] The small difference in ^{199}Hg{^{1}H} chemical shifts between $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ and the reaction product $[Hg(SC_6H_5)_3]^-$ is probably the result of ion pairing at the high concentrations employed here.[42] The reaction solution contains 2 equiv of $(CH_3)_4N^+$ compared to 1 equiv of $(C_4H_9)_4N^+$ in the $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ standard. ^{199}Hg NMR shifts are generally very sensitive to medium effects.[48] The ^{1}H NMR shifts of the $[Hg(SC_6H_5)_3]^-$ reaction product and $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ alone also differ slightly. Previous ^{199}Hg NMR studies have established the viability of three-coordinate $[Hg(SR)_3]^-$ (SR = -SC(CH$_3$)$_3$,[49] -SC$_6$H$_5$[47]) species in acetonitrile and DMSO solutions, and most mononuclear, homoleptic mercury thiolate complexes characterized crystallographically are three-coordinate.[50,51]

Table 1. Pseudo-First-Order Rate Constants for Reactions of Benzenethiolate and Its Metal Complexes with $(CH_3O)_3PO$[a]

compound	k (s^{-1})
$[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$	$(1.1 \pm 0.1) \times 10^{-4}$
$[(C_4H_9)_4N][Hg(SC_6H_5)_3]$	$\leq 3 \times 10^{-7}$
$[(CH_3)_4N]_2[Cd(SC_6H_5)_4]$[b]	$(3 \pm 1) \times 10^{-5}$
$[(CH_3)_4N]_2[Co(SC_6H_5)_4]$[b]	$(4 \pm 1) \times 10^{-5}$
$[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$[b]	$(8.2 \pm 0.6) \times 10^{-5}$
$(CH_3)_4N(SC_6H_5)$[b]	$(1.1 \pm 0.3) \times 10^{-4}$

[a] Reactions were carried out with 5.0 mM thiolate or metal complex and 1.0 mM $(CH_3O)_3PO$ in DMSO-d_6. Error estimates reflect one standard deviation from the average of three runs. [b] Reference 42.

Reaction of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ with $(CH_3O)_3PO$. Methyl transfer from $(CH_3O)_3PO$ to $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ occurred quite slowly, forming products similar to those found for $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$. As indicated by ^{1}H NMR spectroscopy, $(CH_3O)_2PO_2^-$, $\{Hg(SC_6H_5)_2\}$, and free $CH_3SC_6H_5$ were formed. The ^{31}P{^{1}H} NMR spectrum displayed one sharp peak (2.50 ppm, $\Delta\nu_{1/2}$ = 3.8 Hz) attributable to $(CH_3O)_2PO_2^-$. The ^{199}Hg{^{1}H} NMR spectrum (Figure 2) of a stoichiometric reaction mixture of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ and $(CH_3O)_3PO$ displayed a single peak at −613 ppm when the reaction was 23% complete as determined by ^{1}H NMR spectroscopy 2 months after mixing. By comparison, the starting material $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ had a resonance at −359 ppm ($\Delta\nu_{1/2}$ = 35.5 Hz) (Figure 2). Oxygen donors shift ^{199}Hg NMR resonances upfield.[48] Thus, we attribute these spectral results to tight binding of the demethylated phosphate to the $\{Hg(SC_6H_5)_2\}$ moiety. The appearance of only one resonance in the ^{199}Hg{^{1}H} NMR spectrum of this reaction solution indicated that the $\{Hg(SC_6H_5)_2[(CH_3O)_2PO_2]\}^-$ product is in equilibrium with the remaining $[Hg(SC_6H_5)_3]^-$ starting material. The ^{199}Hg{^{1}H} NMR chemical shift of $\{Hg(SC_6H_5)_2[(CH_3O)_2PO_2]\}^-$ can be calculated from eq 1, in which the observed chemical shift

$$\delta_{obs} = (\delta_{HgS_3})(\chi_{HgS_3}) + (\delta_{HgS_2OP})(\chi_{HgS_2OP}) \qquad (1)$$

(δ_{obs}) is a function of the chemical shifts of $[Hg(SC_6H_5)_3]^-$ (δ_{HgS_3}) and $\{Hg(SC_6H_5)_2[(CH_3O)_2PO_2]\}^-$ (δ_{HgS_2OP}) and their respective mole fractions (χ_{HgS_3} and χ_{HgS_2OP}). From the reaction solution at 23% completion and the data presented above, we calculated a ^{199}Hg{^{1}H} NMR shift for $\{Hg(SC_6H_5)_2[(CH_3O)_2PO_2]\}^-$ of −1465 ppm. We are unaware of any published ^{199}Hg chemical shift data for complexes of mixed sulfur and oxygen donors with which to compare this calculated value.

Kinetic Studies. To compare the mercury complex reactions with those of its zinc, cobalt, and cadmium analogs, kinetic data were obtained. The tetrathiolate $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ reacted with $(CH_3O)_3PO$ with a pseudo-first-order rate constant of $(1.1 \pm 0.1) \times 10^{-4}$ s^{-1}, essentially the same rate constant as for free benzenethiolate (Table 1). In contrast, the trithiolate $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ reacted significantly more slowly than any of the tetrathiolate metal complexes (Table 1). An upper limit of $k = 3 \times 10^{-7}$ s^{-1} was estimated for the reaction of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ with $(CH_3O)_3PO$.

Solution Behavior. To address the question of whether the active nucleophiles of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ are dissociated or metal-bound thiolates, a series of DMSO-d_6 solutions were prepared containing 50 mM mercury, with or without 50 mM added benzenethiolate. Figure 3 displays the ^{1}H NMR spectra of these solutions in the aromatic region. In all cases, distinct ortho (most downfield), meta, and

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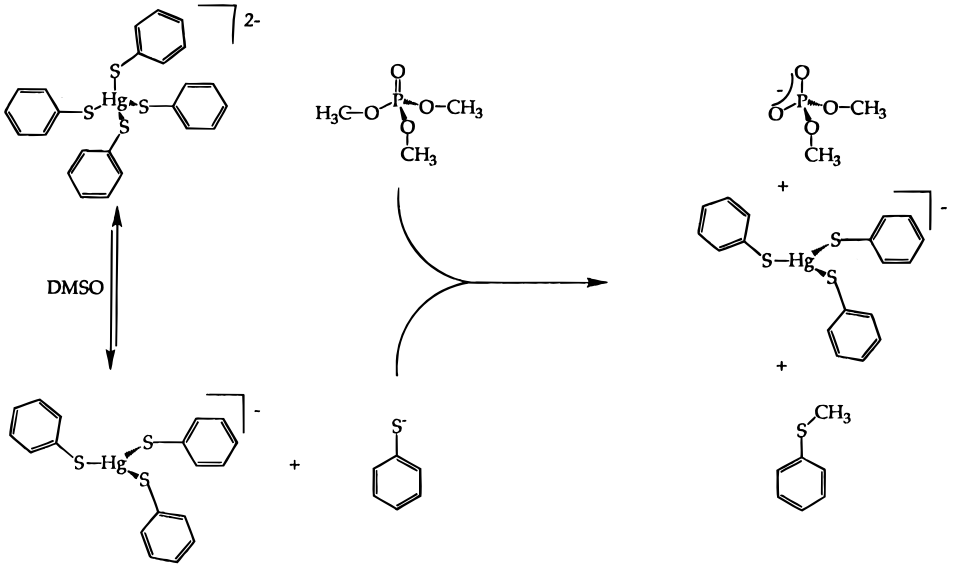
Scheme 1



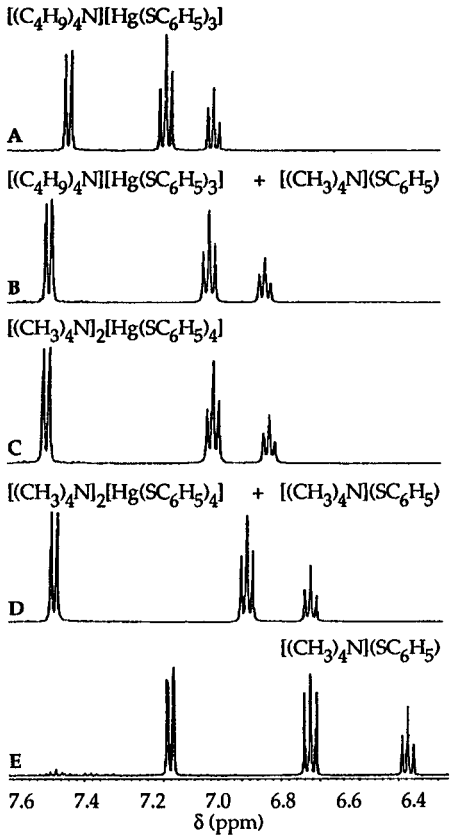


Figure 3. Aromatic region ^{1}H NMR spectra in DMSO-d_6 of (A) $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$, (B) $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ and $(CH_3)_4N(SC_6H_5)$, (C) $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$, (D) $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3)_4N(SC_6H_5)$, and (E) $(CH_3)_4N(SC_6H_5)$. The concentration of each species is 50 mM.

para (most upfield) resonances are visible. The solution containing both $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3)_4N(SC_6H_5)$ displayed only one set of benzenethiolate resonances, indicating rapid exchange between bound and free states. The chemical shifts of the resonances for the solution containing $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and those for the solution containing $[(C_4H_9)_4N][Hg(SC_6H_5)_3] + (CH_3)_4N(SC_6H_5)$ were almost identical. The peaks of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ were broader and displayed diminished splitting relative to those of each $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ and $(CH_3)_4N(SC_6H_5)$, indicating slower exchange between bound and free benzenethiolate. The sharper peaks of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$, by contrast, suggested little or no ligand dissociation.

Reaction of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3O)_3PO$ with a Protic Acid. To prove experimentally that protonation of a thiolate can inactivate its methyl transfer capability, we added HBF_4 etherate to a mercury thiolate methyl transfer reaction. Addition of $(CH_3O)_3PO$ to a DMSO-d_6 solution containing $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $HBF_4{\cdot}O(C_2H_5)_2$, each at 211 mM concentration, blocked methyl transfer. No $CH_3SC_6H_5$ formed, even after 3 weeks, as monitored by ^{1}H NMR spectroscopy. For comparison, a reaction of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3O)_3PO$ without added acid at similar concentrations formed products quantitatively after 1 month. An analogous reaction of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ with $(CH_3O)_3PO$ requires 1 month to detect products by ^{1}H NMR spectroscopy.

Discussion

Solution Behavior and Reactivity. The pseudo-first-order rate constant for methyl transfer from $(CH_3O)_3PO$ to solutions containing $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ is identical to that of benzenethiolate (Table 1). Rate constants for the complexes $[(CH_3)_4N]_2[M(SC_6H_5)_4]$ (M = Zn(II), Co(II), and Cd(II)) are below or close to that of $(CH_3)_4N(SC_6H_5)$ (Table 1). Since metal-bound thiolates generally have decreased nucleophilicity relative to that of free thiolates,[42] these results indicate that the solution reactivity of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ can be attributed to dissociated thiolate. The very similar ^{199}Hg{^{1}H} NMR chemical shifts of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ before and after reaction with $(CH_3O)_3PO$, together with the ^{1}H NMR data showing rapid equilibrium between bound and free $C_6H_5S^-$ for the $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ complex, support this conclusion.

The aromatic ^{1}H NMR peaks of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ were broader and exhibited diminished splitting relative to those of $C_6H_5S^-$ and $[Hg(SC_6H_5)_3]^-$, indicating the presence of an equilibrium between mercury-bound and free benzenethiolate. The ^{1}H NMR spectrum of an equimolar mixture of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and $(CH_3)_4N(SC_6H_5)$ displayed one set of benzenethiolate peaks, as expected for rapid exchange of bound and free thiolates. Previous work also revealed ligand loss from $[(CH_3)_4N]_2[Hg(S\text{-}p\text{-}C_6H_4Cl)_4]$ in DMSO, as evidenced by a change in ^{199}Hg NMR chemical shift values upon titration with added ligand.[47] Taken together, these results reveal that $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ generates transiently dissociated thi-

olate in DMSO solution. This dissociated thiolate then reacts with $(CH_3O)_3PO$ to yield the products observed (Scheme 1). Such a scheme is similar to that previously postulated for $[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$, in which dissociated benzenethiolate reacts with $(CH_3O)_3PO$.[42]

The slow reaction of $[(C_4H_9)_4N][Hg(SC_6H_5)_3]$ may be a function of diminished ligand dissociation relative to that of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$. Dissociation of an anionic ligand from a monoanionic complex such as $[Hg(SC_6H_5)_3]^-$ is expected to be less facile than that from a dianion such as $[Hg(SC_6H_5)_4]^{2-}$. Sharp, well-defined ^{1}H NMR resonances of $[Hg(SC_6H_5)_3]^-$ and the predominance of $[Hg(SR)_3]^-$ units in the solution[47,49] and solid states[44,49,52−55] are consistent with $[Hg(SC_6H_5)_3]^-$ remaining largely intact in DMSO solution. At this time, we cannot ascribe the reactivity of $[Hg(SC_6H_5)_3]^-$ exclusively to a dissociated or a metal-bound thiolate.

Relevance to Hg-*N*-Ada17. The facile reaction of $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ with $(CH_3O)_3PO$ suggests that the mercury derivative of *N*-Ada17 should be capable of methylphosphotriester repair. Such is not the case, however.[40] The inactivity of Hg-*N*-Ada17 can be explained by the present results in the context of previous studies on Ada protein fragments. Cadmium substitution for zinc in active N-terminal fragments of Ada indicated Cys69 to be different from the three other ligating cysteine residues (Cys38, -42, and -72).[15,16,24] Although ^{1}H−^{113}Cd scalar coupling was observed for Cys38, -42, and -72, none appeared for Cys69. Reaction of the zinc form of a 10 kDa N-terminal fragment (Zn-*N*-Ada10) with CH_3I demonstrated enhanced nucleophilicity of Cys69 relative to that of the remaining three ligand residues.[39]

The dissociation of benzenethiolate from $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ and the predominance of three-coordinate mercury thiolates disfavor a four-coordinate $[Hg(S\text{-}Cys)_4]^{2-}$ environment in Hg-*N*-Ada17. Tight zinc binding of Cys38, -42, and -72 in Zn-*N*-Ada10 implies that a three-coordinate mercury center of Hg-*N*-Ada17 would most likely include these residues. Protonation of Cys69 by accessible water would greatly diminish its nucleophilicity and, consequently, its alkylphosphotriester repair activity. We have shown experimentally that protonation of a dissociated thiolate inactivates the nucleophile with respect to methyl transfer ability. Thus, we attribute the inability of Hg-*N*-Ada17 to repair alkylphosphotriesters to a three-coordinate mercury center involving Cys38, -42, and -72 and a protonated, deactivated Cys69 residue.

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