Methylation of Iron−Sulfur Complexes by Trimethyl Phosphate

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Received July 28, 1998

Reaction of $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5, C_2H_5) with $(CH_3O)_3PO$ in DMSO-d_6 afforded $[(C_4H_9)N]_2\{Fe_4S_4(SR)_3[(CH_3O)_2PO_2]\}$ and CH_3SR as revealed by ^1H and $^{31}P\{^1H\}$ NMR spectroscopy. The more reduced species $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ gave uncoordinated $(CH_3O)_2PO_2^-$ and $CH_3SC_2H_5$ in addition to an unidentified iron thiolate species. Stoichiometric methylation of mononuclear $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ by $(CH_3O)_3PO$ afforded $[Fe_2(SC_2H_5)_6]^{2-}$ as well as free $(CH_3O)_2PO_2^-$ and $CH_3SC_2H_5$. Kinetic studies revealed the rate constant for methylation of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ to be more than 200-fold higher than that of the oxidized analogues $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5, C_2H_5). The compound $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ had the highest rate constant, $\geq 5 \times 10^{-3}$ s^{-1} at concentrations of 5.0 mM in complex and 1.0 mM in $(CH_3O)_3PO$. Attempts to prepare site-differentiated tetranuclear iron−sulfur complexes by removing one thiolate via methylation and addition of second, capping ligands are described. These results are discussed in the context of protein metal thiolate moieties that transfer methyl cations for substrate synthesis, such as carbon monoxide dehydrogenase/acetyl coenzyme A synthase, and repair of DNA alkylation damage.

Introduction

Tetranuclear iron−sulfur clusters having cuboidal structures are well-known in bioinorganic chemistry. They occur in four distinct oxidation states, mediating electron transfer reactions in a variety of protein environments that afford a wide range of oxidation potentials.[1−5] The $\{Fe_4S_4\}^{2+}$ cluster of aconitase, by contrast, catalyzes the interconversions of citrate, *cis*-aconitate, and isocitrate without changing oxidation state.[6−9] This hydratase/dehydratase activity depends upon the ability of an iron atom at a cube corner to alternate between four- and six-coordinate geometries, differentiating this iron−sulfur-cluster-dependent transformation from similar reactions catalyzed by zinc enzymes.

More recently, three examples of iron−sulfur clusters have been found in DNA repair proteins. Endonuclease III (endo III)[10−13] and MutY[14−17] from *Escherichia coli* and ultraviolet endonuclease (UV endonuclease)[18] of *Micrococcus luteus* all have glycolysis activity and $\{Fe_4S_4\}^{2+}$ units that seemingly do not participate directly in catalysis. Endo III removes the products of purine reduction, cleavage, and hydration.[19,20] MutY initiates repair of spontaneous guanine−adenine (G−A) and 7,8-dihydro-8-oxoguanine−adenine (8-oxoG−A) mismatches by eliminating the offending adenine base and creating an apurinic site.[19,21−23] UV endonuclease removes light-induced thymine dimers.[18] The postulated structural role of the $\{Fe_4S_4\}^{2+}$ cluster in these repair enzymes is similar to that of the prototypical zinc finger motif [Zn(*S*-cysteine)$_2$(*N*-histidine)$_2$], in which a metal center is employed to stabilize protein secondary structure.[24−27]

Previously, we investigated the reactions of zinc(II) and related metal thiolate complexes with $(CH_3O)_3PO$ as models for the zinc-containing *E. coli* Ada protein.[28−30] In Ada, a [Zn-

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10.1021/ic9808899 CCC: $18.00
Published on Web 07/08/1999

$(S\text{-cys})_4]^{2-}$ unit repairs DNA alkylation damage by stoichiometric transfer of the alkyl group to a thiolate ligand.[31−34] Reaction of $[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$ with $(CH_3O)_3PO$ afforded chemistry which paralleled DNA methylphosphotriester repair.

Because iron−sulfur clusters are functionally more diverse in biology than originally realized, sharing several properties in common with zinc,[35−37] we became interested in examining their ability to serve as receptors in alkyl transfer chemistry. The present study was therefore undertaken to explore the possibility that iron−sulfur clusters might similarly accept a methyl group from $(CH_3O)_3PO$ and, if so, to determine whether alkylation would occur at the sulfide or terminal thiolate group. An earlier report described briefly the reaction of $[(C_2H_5)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ with 1 equiv of $CF_3SO_3CH_3$ in *N*-methyl-2-pyrrolidinone (NMP) to yield $CH_3SC_6H_5$ and an uncharacterized iron-containing species, presumably $[Fe_4S_4(SC_6H_5)_3(CF_3SO_3)]^{2-}$.[38] In our investigations, several specific questions were posed. How will the rate constants for demethylation of $(CH_3O)_3PO$ by the clusters compare with those of the zinc thiolates? Will the reaction rate depend on the cluster charge? Will the methylated thiolate remain coordinated? If a thiolate were released from an $[Fe_4S_4(SR)_4]^{2-}$ complex by methylation, could another ligand be added to capture a site-differentiated cluster? Could dealkylation be used as a general probe for the nucleophilicity of iron−sulfur complexes? And, is there potential for iron−sulfur centers to have such a functional role in DNA repair?

To address these questions, we have investigated the alkylation of tetranuclear iron−sulfur clusters having two oxidation states and two different terminal thiolate ligand types. The mononuclear complex $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ was also examined. To permit comparisons with zinc complexes investigated previously, $(CH_3O)_3PO$ was used as the alkylating agent and dimethyl sulfoxide (DMSO) as solvent.[28−30] Reaction products were characterized in solution by ^1H and $^{31}P\{^1H\}$ nuclear magnetic resonance (NMR) spectroscopy. The kinetics of the methylation reactions were examined, and attempts were made to prepare site-differentiated $[Fe_4S_4(SC_6H_5)_3(L)]^{2-}$ clusters.

Experimental Section

General Procedures. All procedures were carried out under an argon or nitrogen atmosphere by using standard Schlenk and glovebox techniques. Solvents were dried, degassed, and distilled according to standard methods.[39,40] NMR spectra were collected in DMSO-d_6 at 25 ± 1 °C on Varian Unity 300 and JEOL JNM-GX400 instruments. All analytical NMR spectra were recorded on samples with complex concentrations of 50 mM with the exception of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_2H_5)_4]$, which was at 10 mM owing to solubility limitations. The compounds $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$,[41] $[(C_4H_9)_4N]_2[Fe_4S_4(SC_2H_5)_4]$,[41] $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$,[42] and $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$[43] were prepared according to literature methods and characterized by ^1H NMR spectroscopy and elemental analysis.

Kinetics. Kinetics experiments were performed under pseudo-first-order conditions with a metal complex concentration of 5.0 mM and $(CH_3O)_3PO$ at 1.0 mM. This relatively low iron complex concentration was chosen to minimize the effects of ion pairing.[28] Reactions were monitored by ^1H NMR spectroscopy in DMSO-d_6 at 26 (±1) °C. Typical ^1H NMR parameters for kinetic studies included 4 scans per spectrum, 40 s relaxation delays between scans, and 60 spectra per experiment. The total time of data collection was 10 h. Solution volumes were standardized by using calibrated 1 mL volumetric flasks. Concentrations of reactants and products were determined by referencing peak integrals to the resonances of R_4N^+ counterions, the concentrations of which were determined from starting material quantities and known solution volumes. For the reactions of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ and $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$, rate constants were determined by curve fitting $(CH_3O)_3PO$ concentration-versus-time plots with a standard, integrated expression for first-order decay.[44] Pseudo-first-order rate constants for $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ were determined in triplicate. The rate constant provided is an average of the three kinetic runs, and the error shown reflects 1 standard deviation. The slow reactions of $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5) permitted only an upper limit of the pseudo-first-order rate constants to be determined. The initial-rate method was used in these cases.[44]

Results

Reaction of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ with $(CH_3O)_3PO$. After 111 days at room temperature, this reaction was still progressing. ^1H NMR spectroscopy indicates the formation of $CH_3SC_6H_5$ (Figure 1). This thioether product was uncoordinated since its ^1H NMR resonances are identical to those of a genuine sample. Both ^1H and $^{31}P\{^1H\}$ NMR spectra display peaks attributable to unreacted $(CH_3O)_3PO$. In neither spectrum, however, are resonances observed for the expected[28] phosphate product $(CH_3O)_2PO_2^-$. Resonances of the benzenethiolate cluster ligands show small changes relative to the $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ starting material, at 5.15 ppm (para), 5.69 ppm (ortho), and 8.15 ppm (meta). New peaks have grown in at 4.1 ppm (ortho), 5.10 ppm (para), and 8.24 ppm (meta) (Figure 1). Such small cluster resonance changes were not observable after 33 days reaction time. From these data, as well as the lack of observable ^1H and $^{31}P\{^1H\}$ NMR resonances for $(CH_3O)_2PO_2^-$, we conclude that the $\{Fe_4S_4\}^{2+}$ cluster core remains intact with a ligand environment slightly perturbed from that of the starting material. The iron-containing reaction product is assigned as $\{Fe_4S_4(SC_6H_5)_3[(CH_3O)_2PO_2]\}^{2-}$, depicted in Scheme 1.

Reaction of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_2H_5)_4]$ with $(CH_3O)_3PO$. This compound reacts similarly after 115 days to transfer a methyl group. The ^1H NMR spectrum revealed peaks of the uncoordinated, methylated thiolate $CH_3SC_2H_5$ (data not shown). Evidence that the reaction is still in progress was provided by the observation of $(CH_3O)_3PO$ resonances in both the ^1H and $^{31}P\{^1H\}$ NMR spectra. As is the case with the previous reaction, $(CH_3O)_2PO_2^-$ is observed in neither the ^1H nor the $^{31}P\{^1H\}$ NMR spectrum. The ethanethiolate cluster ligand resonances of the starting material remain visible at 2.3 and 12.4 ppm. In

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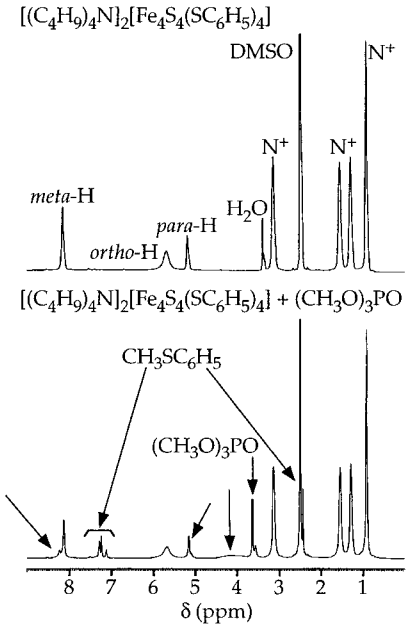

Figure 1. ^{1}H NMR spectra in DMSO-d_6 of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ (top) and $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ after reaction with $(CH_3O)_3PO$ for 111 days at room temperature (bottom). Peaks labeled N^+ indicate $(C_4H_9)_4N^+$ counterion resonances. The starting concentration of each species was 50 mM. The unlabeled arrows denote new cluster resonances discussed in the text.

addition, a new peak began to appear at 13.5 ppm ($\Delta\nu_{1/2} = 72$ Hz), similar in width to the thiolate methylene resonance of the starting cluster ($\Delta\nu_{1/2} = 68$ Hz). Since the concentrations of reactants (10 mM) are lower than in the previous reaction (50 mM), the spectroscopic changes were less dramatic.

Reaction of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ with $(CH_3O)_3PO$. Methylation of this reduced cluster proceeded much more rapidly than the previous reactions. Methyl transfer from $(CH_3O)_3PO$ to $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ was >50% complete ~4 h after the reaction began, judging by $^{31}P\{^1H\}$ NMR spectroscopy. In this case, a narrow ($\Delta\nu_{1/2} = 11.7$ Hz) peak at 3.21 ppm was observed in the $^{31}P\{^1H\}$ NMR spectrum for the product $(CH_3O)_2PO_2^-$. For comparison, $(CH_3O)_3PO$ also appeared as a narrow ($\Delta\nu_{1/2} = 11.7$ Hz) peak at 4.54 ppm. These data indicate that the $(CH_3O)_2PO_2^-$ product is not coordinated to the $\{Fe_4S_4\}^+$ core. Changes in the ^{1}H NMR spectrum were more difficult to assign for this reaction owing to significant paramagnetic line broadening of all peaks present. After ~4 h, the thiomethyl resonance of uncoordinated $CH_3SC_2H_5$ product appeared at 2.04 ppm (Figure 2). A shoulder on the $(C_2H_5)_4N^+$ resonance at 3.13 ppm may indicate $(CH_3O)_2PO_2^-$ (see unlabeled arrow in Figure 2). At this stage in the reaction, resonances for the ethanethiolate ligands of the starting cluster were unobservable (4.7 ppm) or significantly diminished (35 ppm). A prominent peak grew into the ^{1}H NMR spectrum at 48.5 ppm ($\Delta\nu_{1/2} = 360$ Hz) as did a shoulder on this peak at ~50.3 ppm and a very broad ($\Delta\nu_{1/2} =$ ~2600 Hz) resonance at 21 ppm.

Reaction of $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ with $(CH_3O)_3PO$. This mononuclear complex also reacts rapidly to form $CH_3SC_2H_5$ and $(CH_3O)_2PO_2^-$, both of which are uncoordinated judging by their ^{1}H NMR resonances. The observed $^{31}P\{^1H\}$ resonance of $(CH_3O)_2PO_2^-$ is sharp ($\Delta\nu_{1/2} = 6.0$ Hz), confirming the conclusion that this product is not coordinated to iron. The remaining three thiolates continue to bind to the iron center, with paramagnetically shifted $^-SC_2H_5$ resonances at 6.32 ppm ($\Delta\nu_{1/2} = 61.5$ Hz) and 70.5 ppm ($\Delta\nu_{1/2} = 152$ Hz). These values differ greatly from those of the starting $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ complex, 9.97 ppm ($\Delta\nu_{1/2} = 156$ Hz) and 196 ppm ($\Delta\nu_{1/2} = 483$ Hz), no resonances of which were observed following the reaction. The reaction product may be $[Fe_2(SC_2H_5)_6]^{2-}$, which has a methylene ^{1}H NMR resonance in CD_3CN at 69 ppm.[45]

Kinetics Studies. Reactions of $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5) were slow and provided similar rate constants. Upper limits of $\leq 1 \times 10^{-7}$ s^{-1} and $\leq 4 \times 10^{-7}$ s^{-1} were obtained. The more reduced complex $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ reacted with a significantly higher rate constant of $(7.8 \pm 0.7) \times 10^{-5}$ s^{-1}. The mononuclear tetrathiolate $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ exhibited the highest pseudo-first-order rate constant, the value of which could only be estimated as $\geq 5 \times 10^{-3}$ s^{-1} under the conditions employed here. Table 1 summarizes these results.

Attempts To Prepare a Site-Differentiated $\{Fe_4S_4\}^{2+}$ Cluster. In an attempt to isolate site-specifically modified tetranuclear iron−sulfur clusters,[46−48] a thiolate ligand was removed by methylation and a second, capping ligand was added. In these trials, $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ was first allowed to react with 1 equiv of CH_3I in DMSO-d_6. Subsequently, an equimolar quantity of $Na[HB(pz)_3]$ (pz = pyrazolyl), $(CH_3)_4N(OOCCH_3)$, or $NaSC_2H_5$ was added. The ^{1}H NMR spectra of these reaction solutions all displayed the resonances of uncoordinated $CH_3SC_6H_5$. The thiolate $^-SC_6H_5$ resonances indicated persistence of the $\{Fe_4S_4\}^{2+}$ core. In none of the reactions, however, were distinct resonances for $Na[HB(pz)_3]$, $(CH_3)_4N(OOCCH_3)$, or $NaSC_2H_5$ observed. Paramagnetic broadening of these ligands had occurred, rendering them unobservable and suggesting formation of the desired complexes. Efforts were made to isolate these site-differentiated tetranuclear complexes with bound capping ligands. In CH_3CN, solutions of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ and CH_3I were treated with one of the ligands $Na[HB(pz)_3]$, $(CH_3)_4N(OOCCH_3)$, and $NaSC_2H_5$. Vapor diffusion of diethyl ether into the resulting reaction mixtures yielded black, needlelike crystals in each case. An X-ray crystallographic unit cell determination at 188 K revealed the presence of the starting material $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ (orthorhombic unit cell with $a = 11.888(5)$ Å, $b = 23.21(1)$ Å, $c = 22.22(1)$ Å).[49]

Discussion

Reaction Stoichiometry. Tetranuclear iron−sulfur complexes react readily with $(CH_3O)_3PO$ transferring the methyl group to a terminal thiolate ligand. Although free sulfide ion may be a superior nucleophile, coordination of this moiety to three iron atoms in the cuboidal structure substantially diminishes its nucleophilicity. The thiolate ion, in contrast, binds only one metal ion, leaving two lone pairs available for electrophilic attack by trimethyl phosphate. These results and the presence of intact $\{Fe_4S_4\}^{2+}$ core units after the reaction are in agreement with an earlier account describing reactions of $[Fe_4S_4(SR)_4]^{2-}$ (R = C_6H_5, $CH_2C_6H_5$, $C(CH_3)_3$) complexes with the electrophiles CH_3COCl, $(CH_3CO)_2O$, and $HOCOCH_3$.[38]

The reaction of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ with $(CH_3O)_3PO$ appears to have yielded the novel cluster $\{Fe_4S_4(SC_6H_5)_3$-

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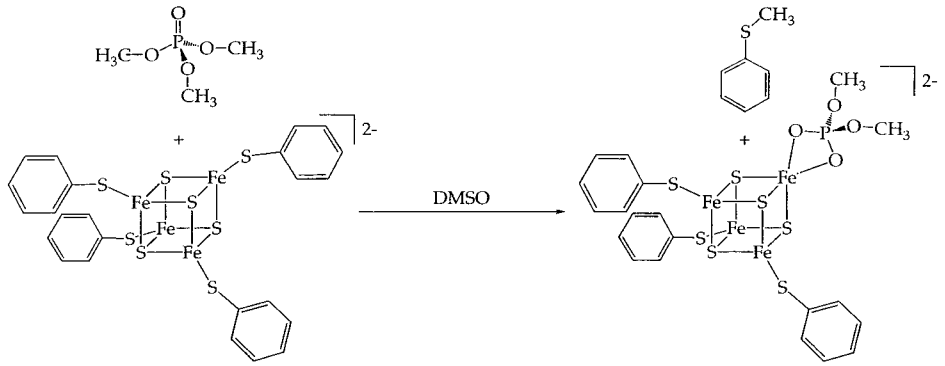
Scheme 1



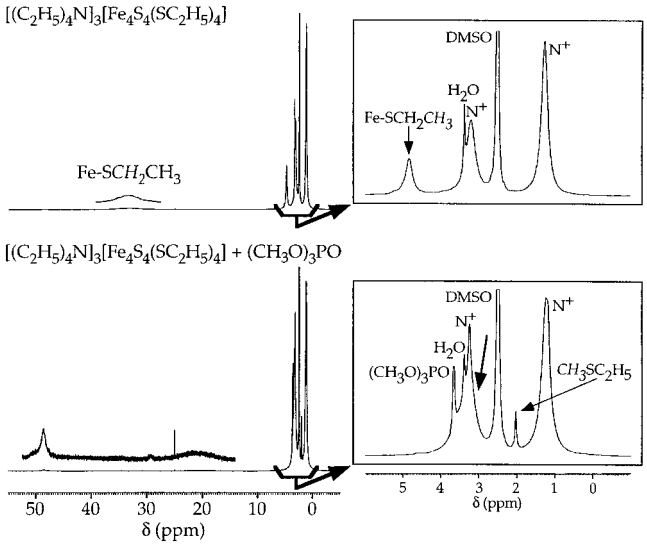


Figure 2. ^{1}H NMR spectra in DMSO-d_6 of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ (top) and $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ after reaction with $(CH_3O)_3PO$ for 4 h at room temperature (bottom). Peaks labeled N^+ indicate $(C_2H_5)_4N^+$ counterion resonances. The starting concentration of each species was 50 mM. The unlabeled arrow denotes a shoulder resonance discussed in the text.

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

compd	k (s^{-1})
$[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$	$(7.8 \pm 0.7) \times 10^{-5}$
$[(C_4H_9)_4N]_2[Fe_4S_4(SC_2H_5)_4]$	$\leq 4 \times 10^{-7}$
$[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$	$\leq 1 \times 10^{-7}$
$[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$	$\geq 5 \times 10^{-3}$
$[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$[b]	$(8.2 \pm 0.6) \times 10^{-5}$
$[(CH_3)_4N]_2[Co(SC_6H_5)_4]$[c]	$(4 \pm 1) \times 10^{-5}$
$[(CH_3)_4N]_2[Cd(SC_6H_5)_4]$[c]	$(3 \pm 1) \times 10^{-5}$
$[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$[d]	$(1.1 \pm 0.1) \times 10^{-4}$
$[(CH_3)_4N][Zn(SC_6H_5)_3(MeIm)]$[b]	$(6 \pm 1) \times 10^{-6}$
$[Zn(SC_6H_5)_2(MeIm)_2]$[b]	$\leq 3 \times 10^{-8}$
$(CH_3)_4N(SC_6H_5)$[b]	$(1.1 \pm 0.3) \times 10^{-4}$

[a] Reactions were carried out with 5.0 mM thiolate complex and 1.0 mM $(CH_3O)_3PO$ in DMSO-d_6. [b] From ref 30. [c] From ref 28. [d] From ref 29.

$[(CH_3O)_2PO_2]\}^{2-}$ (Scheme 1). Thiolates persisting after methylation are clearly bound to iron, as indicated by their paramagnetically shifted ^{1}H NMR resonances. The similarity of shifts observed between the starting material and product indicate a modest electronic alteration. Substitution of one thiolate cluster ligand for a phosphate may be such that the charge on the complex is maintained. Our NMR data cannot distinguish between mono- and bidentate phosphate coordination. To the best of our knowledge, this is the first example of a phosphate bound to an iron−sulfur complex. For the reaction of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_2H_5)_4]$ and $(CH_3O)_3PO$, the absence of observable ^{1}H and ^{31}P{^{1}H} NMR resonances of the expected product $(CH_3O)_2PO_2^-$ implies an iron species analogous to $\{Fe_4S_4(SC_6H_5)_3[(CH_3O)_2PO_2]\}^{2-}$. Consistent with this assignment is formation of a new ethanethiolate methylene resonance at 13.5 ppm in the ^{1}H NMR spectrum.

The products of the more highly reduced compounds $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ and $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$, however, had ethanethiolate ^{1}H NMR resonances that differed considerably from those of the corresponding starting materials. In the case of the product from the reaction of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ with $(CH_3O)_3PO$, the remaining three thiolates appeared to be bound to iron, on the basis of their paramagnetically shifted ^{1}H NMR peaks. The values of the shifts, however, did not match those of any known iron−sulfur complexes, so we are unable to identify them. The predominant product of the reaction of $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ with $(CH_3O)_3PO$ is possibly $[Fe_2(SC_2H_5)_6]^{2-}$, on the basis of the ^{1}H NMR data presented. After stoichiometric methylation, $[Fe(SC_2H_5)_4]^{2-}$ is left with three thiolates. Equilibration to form the stable $[Fe_2(SR)_6]^{2-}$ unit is not surprising.

More surprising, however, is the lack of observable ^{1}H or ^{31}P{^{1}H} NMR resonances for $(CH_3O)_2PO_2^-$ upon methylation of $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5) by $(CH_3O)_3PO$. Phosphate binding to iron separates the Fe and P atoms by only two bonds. One may expect paramagnetic broadening of the ^{31}P{^{1}H} NMR resonance, rendering it unobservable. The protons of $(CH_3O)_2PO_2^-$, by contrast, are five bonds removed from the metal center, yet we did not observe ^{1}H NMR peaks for $(CH_3O)_2PO_2^-$ after methylation of $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5). The expected doublet at 3.3 ppm may be hidden underneath resonances of the $(C_4H_9)_4N^+$ counterions. Similarly, no observable peaks for $Na[HB(pz)_3]$, $(CH_3)_4N(OOCCH_3)$, or $NaSC_2H_5$ appeared when each was added in a stoichiometric ratio to $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ after thiolate release by CH_3I. These results differ from those observed in reactions of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ and $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$, in which sharp ^{1}H and ^{31}P{^{1}H} NMR resonances are observed for $(CH_3O)_2PO_2^-$. The more reduced $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ and $[(C_2H_5)_4N]_2[Fe(SC_2H_5)_4]$ complexes apparently do not bind the anionic $(CH_3O)_2PO_2^-$ ligand, being less electron deficient. Taken together, our results are consistent with unobservable resonances for capping ligands, $(CH_3O)_2PO_2^-$, $[HB(pz)_3]^-$, $^-OOCCH_3$, or $^-SC_2H_5$, when bound to iron and well-resolved peaks when no interaction with the metal occurs.

Kinetics and Mechanism. Rate constants for the reactions of $[(C_4H_9)_4N]_2[Fe_4S_4(SC_2H_5)_4]$ and $[(C_2H_5)_4N]_3[Fe_4S_4(SC_2H_5)_4]$ with $(CH_3O)_3PO$ nicely illustrate the effect of charge on methyl transfer capability. The $[Fe_4S_4(SC_2H_5)_4]^{3-}$ cluster reacted

at least 200 times faster than the more oxidized complex $[Fe_4S_4(SC_2H_5)_4]^{2-}$. The fact that a more negatively charged cluster reacts with an electrophile with a higher rate constant was not unexpected, but the magnitude of the difference is surprising. Under identical conditions, the $[Zn(SC_6H_5)_4]^{2-}$ dianion reacted only 15 times faster with $(CH_3O)_3PO$ than $[Zn(SC_6H_5)_3(MeIm)]^-$ (MeIm is 1-methylimidazole) (Table 1). The difference in reactivity of at least 200 between $[Zn(SC_6H_5)_3(MeIm)]^-$ and $[Zn(SC_6H_5)_2(MeIm)_2]$, however, was more pronounced (Table 1). In the reactions of these zinc complexes, the active nucleophile is thiolate dissociated from the metal complexes, rather than a zinc-bound thiolate.[28] The different rate constants reflected varied degrees of ligand dissociation. Perhaps this explanation holds true for the iron complexes examined here (vide infra).

The dianionic clusters $[Fe_4S_4(SR)_4]^{2-}$ (R = C_6H_5 and C_2H_5) exhibit rate constants of methyl transfer approximately 100-fold lower than those of $[M(SC_6H_5)_4]^{2-}$ (M = Zn, Co, Cd, Hg; Table 1). The latter four complexes are also dianions, each having four thiolates. We attribute the pronounced kinetic differences to the delocalized electronic state of the $\{Fe_4S_4\}^{2+}$ core.[50] The negative charge of $[Fe_4S_4(SR)_4]^{2-}$ is distributed over the entire unit, resulting in lower charge density at any one {Fe(SR)} corner relative to that in mononuclear $[M(SR)_4]^{2-}$ anions. Thus, the $[Fe_4S_4(SR)_4]^{2-}$ clusters have overall decreased nucleophilicity and a lesser tendency for ligand dissociation, either property of which would decrease the rate constant for dealkylating $(CH_3O)_3PO$ relative to that of the mononuclear complexes. The $[Fe(SC_2H_5)_4]^{2-}$ dianion thus exhibited a rate constant not only higher than that of the dianions $[Fe_4S_4(SR)_4]^{2-}$ (R = C_6H_5 and C_2H_5) but also higher than that of the trianion $[Fe_4S_4(SC_2H_5)_4]^{3-}$ (Table 1).

The pK_a of HSC_2H_5 is approximately 4 units higher than that of HSC_6H_5, which may provide an explanation for the 200-fold difference in rate constants for $[Fe(SC_2H_5)_4]^{2-}$ and $[M(SC_6H_5)_4]^{2-}$ (M = Zn, Co, Cd, Hg).[51] The greater basicity of the $^-SC_2H_5$ ligand will enhance its reactivity relative to $^-SC_6H_5$ and is likely to result in higher rate constants for all $[M(SC_2H_5)_4]^{2-}$ complexes. The reactivities of both $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5) were examined in order to measure the effect of thiolate ligand type on reactivity. Unfortunately, the rate constants for these two compounds were low, making a quantitative comparison difficult.

We now turn to the question of whether the active thiolate nucleophile in these studies is bound to iron or dissociated. The low rate constants for methyl transfer from $(CH_3O)_3PO$ to $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5) suggest that, if the observed reactivity were due entirely to dissociated thiolate, the degree of dissociation must be low relative to that of $[(CH_3)_4N]_2[Zn(SC_6H_5)_4]$ (≥75% dissociation of one ligand)[28] and $[(CH_3)_4N]_2[Hg(SC_6H_5)_4]$ (approximately 100% dissociation of one thiolate).[29] As little as 0.1% dissociation of a ligand from $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$ could account for the methyl transfer that occurs.[28] Previous experiments revealed that the cuboidal structures of $[(C_2H_5)_4N]_3[Fe_4S_4(SC_6H_5)_4]$ and $[(C_2H_5)_4N]_3[Fe_4S_4(SCH_2C_6H_5)_4]$ are preserved in CH_3CN solution.[52] An examination of thiolate ligand exchange in $[(C_6H_5)_4As]_2\{Fe_4S_4[SC(CH_3)_3]_4\}$ indicated the rate-determining step to be protonation of a coordinated thiolate by the added thiol.[53] We cannot rule out, however, that transiently dissociated thiolate serves as the nucleophile toward $(CH_3O)_3PO$.

The formal oxidation states of the metal ions may be important in determining both the thiolate dissociation constants and overall cluster nucleophilicity. In the case of $[M(SR)_4]^{2-}$ (M = Zn, Co, Cd, Hg, or Fe) anions, the metals are all in the 2+ state. The $[Fe_4S_4(SR)_4]^{2-}$ (R = C_6H_5 and C_2H_5) and $[Fe_4S_4(SC_6H_5)_4]^{3-}$ clusters all have Fe(III) character, which should cause them to bind thiolate ligands with higher affinity and to have lower equilibrium constants for dissociation. If the thiolate nucleophile for alkyl transfer is dissociated, the greater Fe(III) character of $[(C_4H_9)_4N]_2[Fe_4S_4(SR)_4]$ (R = C_6H_5 and C_2H_5) may enhance ligand binding and explain the lower observed kinetic results.

Potential Biological Implications. The present results suggest that tetranuclear iron−sulfur centers in proteins could repair DNA alkylation damage. The kinetic data, however, indicate that all clusters would not perform this task equally well. Presumably, the $[Zn(S\text{-cys})_4]^{2-}$ center of Ada is optimal for DNA repair and was selected through evolution. Here, we find that the complex $[Fe_4S_4(SC_6H_5)_4]^{2-}$ reacts with $(CH_3O)_3PO$ with a rate constant about 200 times smaller than that of the analogous mononuclear zinc species $[Zn(SC_6H_5)_4]^{2-}$. Although iron−sulfur centers appear to be capable of alkylphosphotriester repair, use of these moieties may not be compatible with a biological time scale. Repair involves formation of a transient protein−DNA complex, and alkyl transfer must occur rapidly.[54] The more reduced complex $[Fe_4S_4(SC_2H_5)_4]^{3-}$, however, has a more suitable rate constant for methyl transfer (Table 1) and could be an appropriate center for repairing DNA alkylation damage. Its potential instability with respect to oxidation, however, may select against such a role for this cluster.

Previously, we concluded that the low nucleophilicity of $[Zn(SC_6H_5)_2(MeIm)_2]$ and, by analogy, $[Zn(S\text{-cys})_2(N\text{-his})_2]$ sites contributed to their suitability for folding protein secondary structure.[28,30] Although $[Fe_4S_4(S\text{-cys})_4]^{2-}$ centers are charged, the present experiments similarly demonstrate a low reactivity for these moieties. Neutral $[Zn(SC_6H_5)_2(MeIm)_2]$ is less reactive toward $(CH_3O)_3PO$ than $[Fe_4S_4(SR)_4]^{2-}$ (R = C_6H_5 and C_2H_5), but all react rather slowly. From the present results we suggest that $[Fe_4S_4(S\text{-cys})_4]^{2-}$ clusters may be suitable for folding protein structures, but less so than the $[Zn(S\text{-cys})_2(N\text{-his})_2]$ center because of limited reactivity toward electrophiles in the cell.

Another interesting case to consider is methyl transfer in the carbon monoxide dehydrogenase/acetyl coenzyme A synthase system.[55] A methyl cation from a methylated corrinoid−iron−sulfur protein, carbon monoxide, and coenzyme A, which contains a thiol ("HSR") group, condense to form acetyl coenzyme A ($CH_3-CO-SR$). This reaction is catalyzed by the so-called "A-cluster", an $\{Fe_4S_4\}$ core coupled to a nickel center. Among the many issues that remain to be understood in acetyl coenzyme A synthesis is the exact site of methyl cation binding at the A-cluster. Although the present experiments cannot provide information on where $\{CH_3\}^+$ binds the A-cluster, our data do indicate where the alkyl equivalent must *not* bind, namely, the cysteine thiolate ligands of the $\{Fe_4S_4\}$ cluster. As we have shown, such a reaction creates thioether products. The stability of these products will preclude further reaction of the

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methyl cation unless specific activation by the protein is enabled in a manner analogous to that proposed for ether dealkylation in the Ada protein.[56]

We were intrigued by the proposal that acetyl coenzyme A synthase methylation involves the oxidized, rather than the reduced, A-cluster state.[57] Our data indicate that a one-electron-reduced $\{Fe_4S_4\}$ cluster is more susceptible to alkylation by a factor of at least 200 (Table 1). We therefore suggest that methylation of the A-cluster may occur at a site other than a thiolate sulfur atom. The enzyme might have evolved to accept the methyl cation without cluster reduction in order to avoid the facile thiolate alkylation shown here. Consistent with such reasoning is an earlier report in which the reduced enzyme was incubated with methylated corrinoid−iron−sulfur protein and *S*-methylcysteine formation was observed.[58]

Site-Specifically Modified Clusters. By using the tridentate ligand 1,3,5-tris((4,6-dimethyl-3-mercaptophenyl)thio)-2,4,6-tris-(*p*-tolylthio)benzene ($L{\cdot}(SH)_3$), a series of $\{Fe_4S_4\}^{2+}$ complexes were prepared in which three iron atoms are bound by $L{\cdot}(S^-)_3$ and the fourth iron is ligated by various mono-, di-, and tridentate ligands.[46−48] These studies have provided the only means so far of directing ligand substitution chemistry to one corner of the iron−sulfur cube and isolating the site-differentiated cluster. The present methyl transfer reactions similarly remove one thiolate ligand and afford the $\{Fe_4S_4(SR)_3\}^-$ cluster. Thus, methylation with $(CH_3O)_3PO$ may provide a facile route to the site-specifically modified clusters $\{Fe_4S_4(SR)_3[(CH_3O)_2PO_2]\}^{2-}$ ($R = C_6H_5$ and C_2H_5). Previous studies have shown, however, that electrophilic and ligand substitution reactions of $[Fe_4S_4(XAr)_4]^{2-}$ ($X = O, S$, $Ar = C_6H_5$, C_6H_4-*p*-CH_3) clusters afforded statistical distributions among $[Fe_4S_4(XAr)_{4-n}(L)_n]^{2-}$ products.[38,59,60] Such species were observable by ^1H NMR spectroscopy. Either different *p*-CH_3 resonances for each species present or contact-shifted resonances for both XAr and L ligands were observed. In our studies, only the $^-SC_6H_5$ and $^-SC_2H_5$ ligands displayed ^1H NMR resonances attributed to $\{Fe_4S_4\}^{2+}$ core binding. These peaks were broad, however, and did not permit us to gain insight about the statistical nature of the products. Thus, $\{Fe_4S_4(SR)_3[(CH_3O)_2PO_2]\}^{2-}$ ($R = C_6H_5$ and C_2H_5) could exist as the series of $\{Fe_4S_4(SR)_{4-n}[(CH_3O)_2PO_2]_n\}^{2-}$ complexes. Attempts to isolate site-differentiated complexes in the solid state afforded only $[(C_4H_9)_4N]_2[Fe_4S_4(SC_6H_5)_4]$. As before,[28] these results may only attest to the stability of tetrathiolate species in the solid state and not exclude the existence of the desired reaction products in solution.

Conclusions

We have provided a detailed study on the alkylation reactions of iron−sulfur complexes. The kinetic results of thiolate methylation permitted a comparison of the general nucleophilic character of each complex. Although tetranuclear iron−sulfur species may be capable of repairing DNA alkylation damage, mononuclear tetrathiolate metal centers appear more adept at such reactions. Models for $\{Fe_4S_4\}^{2+}$ protein sites were fairly unreactive and, as such, may be better suited for the structural roles which have recently been identified. Our studies show a continued parallel between the development of zinc and iron thiolate protein chemistry. Thiolate moieties of both metals appear proficient at accepting alkyl groups and stabilizing protein secondary structure.

Acknowledgment. This research was supported by Grant CA34992 from the National Cancer Institute. J.J.W. was a predoctoral trainee under NCI training grant CA09112. We thank Profs. H. B. Gray and R. H. Grubbs for access to facilities required to carry out experiments addressing comments of the referees while J.J.W. was a postdoctoral fellow at Caltech.

IC9808899

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