and nucleotide ligands studied at relatively low-field strengths.[31]

The discussion above infers that, thus far, ${}^1H$ NMR studies on MT alone have provided no additional evidence that either substantiates or refutes the proposed model for the cluster structures derived on the basis of the ${}^{113}Cd$ NMR studies.[14] Indeed, the ${}^{113}Cd$ homonuclear decoupling experiments still provide the most definitive evidence for the proposed metal cluster structures in MT from *Scylla serrata*.

It is possible that the arrangement of the cysteine residues in these metal clusters may be more clearly defined by using ${}^{113}Cd-{}^{1}H$ chemical shift correlation experiments to correlate cysteine β-proton resonances with ${}^{113}Cd$ resonances. The experimental means we have adopted to pursue this possibility utilizes the indirect detection of ${}^{113}Cd$ via ${}^{1}H-{}^{113}Cd$ multiple quantum coherences.[27,32] Both the execution of these experiments and the analysis of the datasets are critically dependent on the ${}^1H$ NMR analysis presented in this paper.

In summary, in this paper we have presented the first detailed analysis of the ${}^1H$ NMR spectrum of crab MT-1 where we have identified numerous cross-peaks to amino acid type, in particular those arising from its 18 cysteine residues. The range of observed heteronuclear coupling constants (${}^3J_{{}^{113}Cd-{}^{1}H}$) has been deduced, and the consequences of this range of coupling constants on the structure of the metal binding sites has been discussed. Finally, evidence is presented for the presence of a T_1-induced "spin decoupling" of the ${}^{113}Cd-{}^{1}H$ heteronuclear coupling in the 500-MHz ${}^1H$ NMR spectrum of ${}^{113}Cd$ crab metallothionein, which could account for the paucity of bridging cysteines identified and required in the proposed structures of the metal thiolate clusters.

Registry No. Cys, 52-90-4; ${}^{113}Cd$, 14336-66-4.

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Assembly of $[Fe_nS_n(SPh)_4]^{2-}$ (n = 2, 4) and Their Iron-Thiolate Precursors in Aqueous Media

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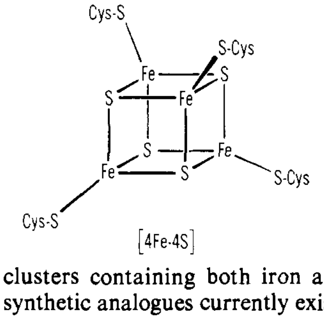
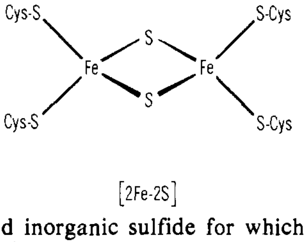
Tetraalkylammonium salts of $[Fe_nS_n(SPh)_4]^{2-}$ (n = 2, 4), which are synthetic analogues of [2Fe-2S] and [4Fe-4S] sites in proteins, can be prepared in >80% yields from 4/1/1 PhSH/$FeCl_3$/S in a medium consisting of 90/5/5 vol % (pH ~8) aqueous buffer/CH_3CN/Triton X-100. For this system the sole assembly pathway appears to be $[Fe(SPh)_4]^{2-} \rightarrow [Fe_2S_2(SPh)_4]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$, which suggests an analogous assembly pathway for [4Fe-4S] sites in ferredoxins. At lower PhSH/Fe mole ratios or at lower pH the $[Fe_4(SPh)_{10}]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$ pathway appears to occur, but to a lesser extent in aqueous Triton than in organic solvents. High yields of the precursors $[Fe(SPh)_4]^{2-}$ and $[Fe_4(SPh)_{10}]^{2-}$ are also achieved in the aqueous-based detergent medium. Evidence from ${}^1H$ and ${}^{19}F$ NMR indicates that the equilibrium $[Fe_4(SPh)_{10}]^{2-} + 6PhS^- \rightleftharpoons 4[Fe(SPh)_4]^{2-}$ is established prior to addition of S. The two most prominent unique features in the 90/5/5 medium are (i) the ability of detergent aggregates in the presence of relatively hydrophobic R_4N^+ to shield $[Fe_2S_2(SPh)_4]^{2-}$ from water, thereby inhibiting the rate of conversion to $[Fe_4S_4(SPh)_4]^{2-}$, and (ii) the facility with which the $[Fe_4(SPh)_{10}]^{2-} \rightleftharpoons [Fe(SPh)_4]^{2-}$ equilibrium can be shifted. Unique feature i leads to the ability to achieve essentially quantitative production of either $[Fe_2S_2(SPh)_4]^{2-}$ or $[Fe_4S_4(SPh)_4]^{2-}$ from the same 4/1/1 PhSH/$FeCl_3$/S reagent ratio. Unique feature ii leads to the production of substantial proportions of $[Fe_2S_2(SPh)_4]^{2-}$ upon reaction of $[Fe_4(SPh)_{10}]^{2-}$ with S, due to a shift of the above equilibrium to the right. Unique feature ii also leads to larger proportions of $[Fe_4S_4(SPh)_4]^{2-}$ at pHs below 6 (below the pK_a of PhSH), apparently due to a shift of the above equilibrium to the left. These two unique features suggest means by which ferredoxins could exert both kinetic and thermodynamic control over assembly of [2Fe-2S] and [4Fe-4S] sites.

Introduction

Iron-sulfur clusters usually function as electron carriers in proteins from diverse bacterial, plant, and mammalian sources. The [2Fe-2S] and [4Fe-4S] centers represent those biological clusters containing both iron and inorganic sulfide for which synthetic analogues currently exist.[1] The title clusters exemplify the latter, having PhS^- in place of Cys-S^-. Although the structural and redox properties of these centers have been thoroughly studied, almost nothing is known about their assembly in vivo, even for the smallest iron-sulfur proteins, generically termed ferredoxins. In fact, until our recent success in aqueous-based media,[2] no one had reported the assembly in and isolation from predominantly aqueous media of synthetic clusters containing the Fe_2S_2 and Fe_4S_4 cores. Previous published results in aqueous solutions containing iron salts, sulfide, and thiols are limited to visible absorption and EPR spectra, which at the time were difficult to interpret in terms of structure.[3,4] However, during subsequent years the stage has been set for reexaminations of these aqueous mixtures by results obtained in the laboratories of Holm and others.[1] For the simple reaction system ≥3.5/1/1 PhS^-/$FeCl_3$/S in methanol or acetonitrile, Hagen et al.[5] have identified two pathways leading to formation of $[Fe_4S_4(SPh)_4]^{2-}$. At 3.5/1 PhS^-/$FeCl_3$, $[Fe_4(SPh)_{10}]^{2-}$, containing an adamantane-like Fe-S framework, is



[4Fe-4S]



[2Fe-2S]

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the only detectable iron–thiolate species. Addition of 1–4 equiv of S produces $[Fe_4S_4(SPh)_4]^{2-}$, apparently in an all-or-nothing stoichiometry. At ≥5/1 $PhS^-/FeCl_3$ the mononuclear tetrahedral $[Fe(SPh)_4]^{2-}$ is the only detectable iron–thiolate species. Upon addition of 1 mol of S/mol of Fe, the [2Fe–2S] synthetic analogue $[Fe_2S_2(SPh)_4]^{2-}$ is the initial identifiable product, which in protic solvents spontaneously and quantitatively converts to $[Fe_4S_4(SPh)_4]^{2-}$.[5-8] This latter process can be aborted by introduction of a tetraalkylammonium salt prior to addition of S, which traps the binuclear complex as the sparingly soluble $(R_4N)_2[Fe_2S_2(SPh)_4]$. The current published procedures for syntheses of the title clusters in methanol from the simple reagents mentioned above both call for a 4/1/1 $PhS^-/FeCl_3/S$ ratio and result in respective yields of 50–55% (n = 2)[6] and ~80% (n = 4).[5,9] The $[Fe(SR')_4]^{2-} \rightarrow [Fe_2S_2(SR')_4]^{2-} \rightarrow [Fe_4S_4(SR')_4]^{2-}$ pathway has also been shown to occur for the system 4/1/1 $EtS^-/FeCl_4^{2-}/S$ in acetonitrile.[10]

These elegant delineations do not directly address the question of how water might affect pathways of cluster assembly in vivo. It is quite likely, at least during some stages of FeS and MoFeS cluster assembly in vivo, that appreciable concentrations of water are present. Certain synthetic clusters containing the [2Fe–2S] and [4Fe–4S] core geometry, when preassembled in organic solvents, have been shown to be stable in either aqueous or aqueous/organic media.[8,11-15] Previous studies in this laboratory had demonstrated that Et_4N^+ salts of $[Fe_nS_n(SPh)_4]^{2-}$ (n = 2, 4) are soluble and hydrolytically stable in an aqueous detergent medium consisting of 90/5/5 vol % aqueous buffer/CH_3CN/Triton X-100.[7,16,17] Subsequently we showed that in the same medium, these clusters can be assembled from the reaction system 4/1/1 $PhS^-/FeCl_3/S$ in yields equal to or exceeding those obtained in organic solvents.[2] The demonstration of high-yield syntheses of $[Fe_4S_4(SPh)_4]^{2-}$ and $[Fe_2S_2(SPh)_4]^{2-}$ in a predominantly aqueous medium puts at our disposal a means of identifying effects on these cluster assembly reactions arising from the presence of water. An aqueous detergent medium such as 90/5/5 may be closer to that in vivo with respect to cluster assembly, especially if such assembly occurs at the surface of a membrane.

Inorganic chemistry in aqueous micellar media has been limited mainly to ligand-substitution[18,19] and electron-transfer reactions, both thermal[20] and photochemical.[21,22] Except for our own work, we are unaware of published studies of transition-metal cluster assembly reactions in aqueous detergent media. Therefore, for the reaction system 4/1/1 $PhSH/FeCl_3/S$ in 90/5/5, we have sought to define (i) unique features compared to organic solvents and (ii) simple techniques that can be used to work up, analyze, and monitor these reactions. Herein we report the results of our investigations into the assembly pathways of the title clusters in these aqueous-based media. The assembly of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ in aqueous media is described in a later paper.[23]

Experimental Section

Preparation of Compounds. $(Et_4N)_2[Fe_2S_2(SPh)_4]$,[6] $(Et_4N)_2[Fe_4S_4(SPh)_4]$,[2] $(Et_4N)_2[Fe_4(SPh)_{10}]$,[5] and $(n\text{-}Bu_4N)_2[Fe_4(SPh)_{10}]$[5] were prepared by published procedures. p-Fluorobenzenethiol and Triton X-100 were both purchased from Aldrich and used without further purification. Bis(p-fluorophenyl)disulfide was prepared by oxidation of p-fluorobenzenethiol with either $K_3Fe(CN)_6$ or I_2 according to the procedure used for preparation of bis(p-(trifluoromethyl)phenyl) disulfide.[15] The product was dried over $CaSO_4$ yielding a yellow oil. This compound has also been prepared by oxidation of the thiol with H_2O_2.[24] DMF was distilled from BaO. Concentrations of stock solutions of $(Et_4N)_2[Fe_2S_2(SPh)_4]$ in DMF were determined from absorption spectra by using published extinction coefficients.[6]

$(Et_4N)_2[Fe_4(S\text{-}p\text{-}C_6H_4F)_{10}]$ was obtained as a crude solid for the purpose of assigning ^{19}F NMR resonances. To 160 mL of methanol were added in order and anaerobically 1.01 g (3.76 mmol) of $FeCl_3{\cdot}6H_2O$, 0.72 g (13 mmol) of NaOMe, 3.36 g (16 mmol) of Et_4NBr, and 1.45 mL (13.2 mmol) of p-fluorobenzenethiol. After the mixture was stirred for 2 h at ambient temperature, the solvent was removed in vacuo. The resulting solid, when dissolved in CD_3OD, yielded an isotropically shifted ^1H NMR resonance at −15.6 ppm at ~22 °C. This resonance is assigned to the m-H of $[Fe_4(S\text{-}p\text{-}C_6H_4F)_{10}]^{2-}$ by analogy to that of $[Fe_4(SPh)_{10}]^{2-}$ (m-H at −16.2 ppm at ~22 °C).[5] In CD_3OD no other resonances were observed in the −10 to −40 ppm region.

Reactions in Aqueous Media. All manipulations were carried out under purified Ar in Schlenk-type glassware. Reagents were transferred via gas-tight syringe or stainless-steel tubing. The following general procedure was used for reactions of $FeCl_3$, PhSH, and S and subsequent workup. To a slurry of 1.01 g (3.75 mmol) of $FeCl_3{\cdot}6H_2O$ in 8 mL of acetonitrile was added 1.6 mL (15 mmol) of PhSH. The resulting dark green solution was added to 8 mL of Triton X-100 (Triton).[16] (For reactions without Triton, an additional 8 mL of buffer was used instead.) This latter solution was transferred to a stirred mixture of 144 mL of 0.2 M sodium N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonate (NaTAPS) at pH 9.0 containing 0.120 g (3.75 mmol) of solid sulfur and 0.1 M R_4NBr (R = Me, Et, n-Pr, n-Bu, or n-pentyl (n-Pe)). The final solvent composition is ~90/5/5 vol % aqueous buffer/CH_3CN/Triton.[17] After being stirred for 8–9 h at room temperature, the mixture was allowed to sit unstirred for 2–10 days at room temperature, while a precipitate gradually formed. The resulting solid was collected by filtration and washed with ~60 mL portions of first ether and then water and finally 2–3 more portions of ether. The solid was then dried in vacuo.

$(Et_4N)_2[Fe_4S_4(SPh)_4]$. Syntheses of this compound in aqueous media have been described previously[2] and consist of following essentially the general procedure but withholding addition of Et_4NBr until after ~7 h of reaction.

$(n\text{-}Pe_4N)_2[Fe_2S_2(SPh)_4]$. The general procedure was used with R_4N^+ = $n\text{-}Pe_4N^+$. The yield was 87%. Anal. Calcd for $C_{64}H_{108}N_2S_6Fe_4$: C, 63.55; H, 9.00; N, 2.32; Fe, 9.23; S, 15.90. Found: C, 63.28, H, 8.82, N, 2.49, Fe, 9.16; S, 16.08. This salt is soluble in acetone, acetonitrile, THF, and dichloromethane. It is slight soluble in methanol and insoluble in toluene and in water.

$(n\text{-}Bu_4N)_2[Fe(SPh)_4]$. The general procedure (R_4N^+ = $n\text{-}Bu_4N^+$) was modified by omission of S and substitution of 1.89 g (45 mmol) of $LiOH{\cdot}H_2O$ for NaTAPS in 144 mL of water and 4.8 mL (45 mmol) of PhSH, giving a $PhSH/FeCl_3$ ratio of 12. The final pH was 8.7. The product separated as a dark oil, which, when washed with water and ether and dried *in vacuo*, yielded a light green-brown powder. This compound, which is much more air sensitive than either of the above, was obtained in >90% yield. The product was identified by integration of the m-H ^1H NMR resonance at −22.3 ppm[5] in CD_3CN vs. the methylene and methyl resonances of $n\text{-}Bu_4N^+$. Its purity was confirmed by elemental analysis. Anal. Calcd for $C_{56}H_{92}N_2FeS_4$: C, 68.81; H, 9.49; N, 2.87; Fe, 5.71; S, 13.12. Found: C, 68.61; H, 9.66; N, 2.77;, Fe, 5.41; S, 13.02.

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(16) Triton X-100 is a nonionic detergent containing a poly(oxyethylene) chain as the hydrophilic portion and an alkylphenyl group as the hydrophobic portion with, on the average, 9.5 ethylene oxide units per alkylphenyl group: formula, $(CH_3)_3CCH_2C(CH_3)_2C_6H_4O(CH_2CH_2O)_{9.5}H$; M_w, 646; d, 1.08 g/mL.

(17) Hereafter this solvent will be referred to as 90/5/5.

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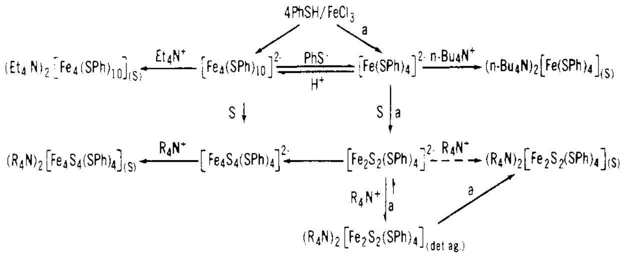

Figure 1. Schematic diagram of cluster assembly pathways and effects thereon for 4/1/1 $PhSH/FeCl_3/S$ in aqueous media. Relative lengths of arrows upon reaction with S are meant to show qualitatively that pathways containing $[Fe(SPh)_4]^{2-} \rightarrow [Fe_2S_2(SPh)_4]^{2-}$ are favored. The dashed arrow refers to a pathway followed mainly in nondetergent media. det. ag. = detergent aggregate. The pathway marked "a" appears to be that followed exclusively in 90/5/5 when R_4N^+ = n-Pe_4N^+.

Reaction and workup in aqueous media of R_4N^+ salts of preassembled $[Fe(SPh)_4]^{2-}$, $[Fe_4(SPh)_{10}]^{2-}$, $[Fe_2S_2(SPh)_4]^{2-}$, and $[Fe_4S_4(SPh)_4]^{2-}$ were carried out according to the general procedure except that stock solutions of the above salts in CH_3CN were used in place of the $FeCl_3$ + PhSH solution. Additional variations are given in the text and figure captions.

Physical Measurements. All samples were prepared and measured under anaerobic conditions. Absorption spectra were obtained on a Perkin-Elmer Model 554 spectrophotometer. NMR spectra were recorded either on a Nicolet NT-300 spectrometer or on a Bruker WM-300 multinuclear spectrometer equipped with a temperature controller and a 10-mm probe for ^{19}F. ^1H NMR spectra were measured at 300 MHz, and ^{19}F NMR spectra at 282.4 MHz. For ^1H NMR spectra CD_2HCN (−1.93 ppm) was used as internal reference in CD_3CN, and HDO (−4.80 ppm) as internal reference in aqueous Triton containing 20% D_2O. For the latter spectra the solvent resonance was suppressed by a 3-s presaturation pulse. Upfield and downfield chemical shifts are designated as positive and negative, respectively. ^{19}F NMR spectra were recorded on samples contained in cylindrical bulbs connected to 5-mm-o.d. stems (Wilmad Glass Co.). These stems permitted injection of samples through tight-fitting rubber septa. The bulbs were placed in 10-mm-o.d. NMR tubes containing either CCl_4 or CH_2Cl_2. When present, the ^1H resonance of the latter solvent was used as both a lock and a shim signal for ^{19}F NMR spectra in aqueous Triton. Otherwise the water resonance was used. $C_6H_4CF_3$ was used as external reference for aqueous Triton solutions and as internal reference for CD_3CN solutions. For NMR spectra obtained in aqueous Triton, intensities of both ^1H and ^{19}F resonances were determined by planimetric integration.

Proportions of $[Fe_2S_2(SPh)_4]^{2-}$ (d^{2-}) and $[Fe_4S_4(SPh)_4]^{2-}$ (t^{2-}) in isolated solids were determined from area ratios of the m-H ^1H NMR resonances in CD_3CN at −8.2 (t^{2-}) and −9.3 (d^{2-}) ppm.[5]

Cloud points[25] of Triton solutions were measured according to a literature method.[27]

Results and Discussion

The scheme in Figure 1 summarizes the cluster assembly pathways found to occur in aqueous media for 4/1/1 $PhSH/FeCl_3/S$ and the effects of various additives. The pathways illustrated in Figure 1 leading to assembly of $[Fe_4S_4(SPh)_4]^{2-}$ are identical with those shown to occur in organic solvents, and the yields of R_4N^+ salts of $[Fe(SPh)_4]^{2-}$, $[Fe_2S_2(SPh)_4]^{2-}$, and $[Fe_4S_4(SPh)_4]^{2-}$ in 90/5/5 compare favorably to those obtained in organic solvents. However, several effects on the reaction system appear to be unique to aqueous media and are discussed below.

General Features of the Cluster Assembly Reactions in Aqueous Media. At the reagent concentrations used in the general procedure given in the Experimental Section, the 4/1/1 $PhSH/FeCl_3/S$ in 90/5/5 reaction system at room temperature is probably best characterized as an emulsion. Portions of the reaction mixture diluted 5-fold with 90/5/5 are, however, homogeneous and transparent such that absorption spectra are readily obtained during the course of the reaction. The 96% yield of $[Fe_4S_4(SPh)_4]^{2-}$ calculated spectrophotometrically[2] in the above reaction mixture prior to addition of R_4N^+ corresponds to 5.6 mM, about 10 times the calculated micelle concentration in 5 vol % (84 mM) Triton, assuming 140–150 Triton molecules/micelle.[26] The solubilization in the 5-fold diluted solutions cited above gives a $[Fe_4S_4(SPh)_4]^{2-}$/micelle ratio of ~2. This ratio is not unreasonable considering the estimated micellar radius of 43 Å or even the radius of the hydrophobic core of the micelle at 28 Å.[26]

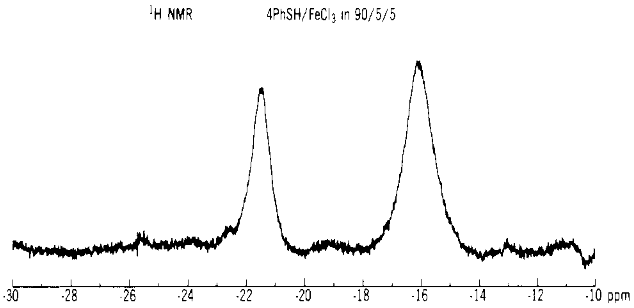

Figure 2. ^1H NMR spectrum in the m-H region at 278 K of the reaction system 4/1 $PhSH/FeCl_3$ in 90/5/5 prepared by using the general procedure omitting S and R_4N^+ and containing 20% D_2O. A total of 2859 transients were collected. Each accumulation was preceded by a 3-s presaturation pulse at −4.80 ppm. No other resonances were observed further downfield to −60 ppm.

$(Et_4N)_2[Fe_4S_4(SPh)_4]$ can be isolated analytically pure without recrystallization in 82% yield from the above reaction mixture by addition of Et_4NBr after ~7 h of reaction and filtration.[2] When 0.1 M R_4NBr is included in the aqueous buffer at the outset of the reaction, a bluish black opalescent emulsion forms upon mixing with the $FeCl_3$ + PhSH solution in CH_3CN/Triton. When solid sulfur is present, a purple color emanates from the particles, which disappear within 2 h. In contrast to those without R_4N^+, dilutions of these latter reaction mixtures remain cloudy. With the exception of R_4N^+ = Me_4N^+, these emulsions gradually break down over the course of several days at room temperature to give dark solids plus faintly colored solutions. When R = n-Pe, the solid consists of analytically pure $(n\text{-}Pe_4N)_2[Fe_2S_2(SPh)_4]$. Product distributions in the presence of other R_4N^+ salts are discussed below.

The turbidity of these solutions is most likely due to an increase in micellar weight related to the "cloud point" phenomenon of Triton.[25] We have confirmed that 100 mM PhSH in 5 vol % Triton in aqueous Tris-Cl at pH 8.3 lowers the cloud point from 63.0 °C to 20.5 °C. Storage of the reaction mixtures at 4 °C significantly reduces their turbidities and prevents the precipitation referred to above.

We have also shown previously that $(Et_4N)_2[Fe_4S_4(SPh)_4]$ can be isolated in 71% yield from aqueous media in the absence of Triton by using the procedure in which addition of Et_4NBr is withheld for several hours.[2] The reaction mixture without Triton contains solid material throughout the course of the reaction. Similarly, reactions in the absence of Triton but containing at the outset 0.1 M R_4NBr are inhomogeneous, resulting in essentially complete precipitation within 2 h. Product distributions in these latter cases are discussed below.

Premixing of $FeCl_3$ and PhSH and CH_3CN prior to addition to water leads to reduction of Fe^{III} to Fe^{II} and production of PhSSPh. This procedure minimizes hydrolytic polymerization of $Fe^{3+}(aq)$. A later paper[23] shows that premixing can be avoided by substitution of Fe^{II} salts for $FeCl_3$.

Fe–SPh Precursors Present in the Reaction Mixture. Having successfully demonstrated the assembly of $[Fe_nS_n(SPh)_4]^{2-}$ (n = 2, 4) in aqueous media, we turned our attention to the distribution of Fe–SPh species present in the aqueous reaction mixture prior to addition of S. The two complexes previously identified in organic solvents are $[Fe(SPh)_4]^{2-}$ and $[Fe_4(SPh)_{10}]^{2-}$.[5] Figure 2 shows the ^1H NMR spectrum in the m-H region of the reaction system 4/1 $PhSH/FeCl_3$ in 90/5/5. The reaction was assembled according to the general procedure, omitting S and R_4NBr, and

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(25) The cloud point is the sudden onset of turbidity upon increasing the temperature past a certain critical temperature. The hydration of the oxyethylene groups is the primary reason that Triton X-100 is water soluble, and increased temperature is believed to cause partial dehydration of the chains. At the cloud point temperature the solution separates into two isotropic phases, one enriched and the other depleted in surfactant.[26]

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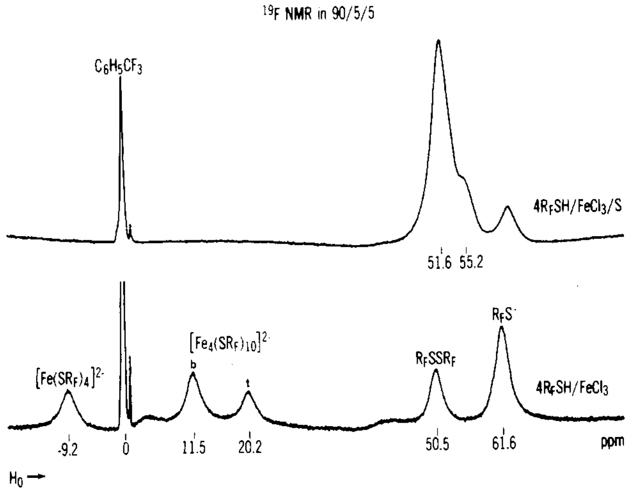

Figure 3. ^{19}F NMR spectra at 297 K of (bottom) a portion of a reaction mixture in 90/5/5 containing 4/1 $R_FSH/FeCl_3$ ($R_F = p\text{-}C_6H_4F$) prepared according to the general procedure but omitting S and R_4N^+ and (top) a portion of the same reaction mixture a few minutes after addition of 1 mol of S/mol of Fe. The aqueous phase contained 1 mol of LiOH/mol of R_FSH, pH ~8. 512 transients were collected for each spectrum, which took ~6 min.

contained 20% D_2O. Two major peaks are present at −21.4 and −15.9 ppm relative to the HDO resonance, which was assigned to −4.80 ppm. On the basis of their similar chemical shifts to those in CD_3CN, we assign the −21.4 ppm resonance to $[Fe(SPh)_4]^{2-}$ (−22.5 ppm in CD_3CN at 22 °C) and the −15.9 ppm resonance to $[Fe_4(SPh)_{10}]^{2-}$ (−16.2 ppm in CD_3CN at 22 °C; average of bridging and terminal resonances). The relative broadness of the −15.9 ppm resonance is most likely due to overlap of the bridging and terminal resonances. The ratio of integrated intensity of the −21.4 ppm resonance to that of the −15.9 ppm resonance in Figure 2 is 0.61. Taking into account the 2/5 ratio of m-H's leads to a mole ratio $[Fe(SPh)_4]^{2-}/[Fe_4(SPh)_{10}]^{2-}$ ~ 1.5 for the $4PhSH/FeCl_3$ system in 90/5/5 near pH 8.

In order to take advantage of improved resolution and lack of solvent interference, we substituted p-fluorobenzenethiol (R_FSH) for PhSH and used ^{19}F NMR spectroscopy. Thus, a reaction mixture consisting of 4/1 $R_FSH/FeCl_3$ in 90/5/5 prepared according to the general procedure (substituting 1.55 mL of R_FSH for 1.6 mL of PhSH) but omitting S and R_4NBr gave the ^{19}F NMR spectrum shown at the bottom of Figure 3. Five major resonances are observed. On the basis of previous ^{19}F NMR studies in N-methylformamide, we assign the −9.2 and 61.6 ppm resonances to $[Fe(SR_F)_4]^{2-}$ and R_FS^-, respectively.[28] We have found that bis(p-fluorophenyl)disulfide (R_FSSR_F) gives a single ^{19}F resonance at 51.9 ppm in CD_3CN at 297 K. On this basis, we assign the resonance at 50.5 ppm to R_FSSR_F, generated from reaction of $FeCl_3$ and R_FS^-. The remaining resonances at 11.5 and 20.2 ppm we assign to bridging and terminal thiolates, respectively, of $[Fe_4(SR_F)_{10}]^{2-}$. These assignments are based on observations of ^{19}F resonances at 11.9 and 23.0 ppm in CD_3OD of $(Et_4N)_2[Fe_4(SR_F)_{10}]$ prepared independently. No ^{19}F resonances were observed in the 0 to −20 ppm range for this latter material. The ratio of the integrated intensity of the −9.2 ppm resonance to the sum of the integrated intensities of the 11.5 and 20.2 ppm resonances of Figure 3 is 0.50, close to the corresponding ratio of 0.61 for the m-H ^1H resonances of Figure 2. These data support the existence in 90/5/5 of equilibrium 1, which at room

$$[Fe_4(SPh)_{10}]^{2-} + 6PhS^- \rightleftharpoons 4[Fe(SPh)_4]^{2-} \qquad (1)$$

temperature is apparently established slowly on the NMR time scale. This equilibrium was previously suggested but not observed to occur in organic solvents.[5] Additional ^{19}F NMR studies show that at pH 6 the resonance assigned to $[Fe(SR_F)_4]^{2-}$ had disappeared, consistent with a shift to the left of the equilbrium analogous to (1) by protonation of R_FS^-. By analogy the same effect would occur with PhSH (pK_a 6.4–6.8[29]) as illustrated in Figure 1. Addition of 1 mol S/mol Fe to the 4/1 $R_FSH/FeCl_3$ reaction mixture in 90/5/5 causes complete loss of the $[Fe(SR_F)_4]^{2-}$ and $[Fe_4(SR_F)_{10}]^{2-}$ resonances within the few minutes time between addition of S and collection of the top spectrum of Figure 3. This rapid loss is likely caused by a combination of $Fe^{II} \rightarrow Fe^{III}$ oxidation, which would tend to shift the resonances out of the range shown, and conversion of $[Fe(SR_F)_4]^{2-}$ to $[Fe_2S_2(SR_F)_4]^{2-}$ and $[Fe_4(SR_F)_{10}]^{2-}$ to $[Fe_4S_4(SR_F)_4]^{2-}$. The resonance at 51.6 ppm is likely due to a combination of R_FSSR_F and $[Fe_2S_2(SR_F)_4]^{2-}$, with the shoulder at 55.2 ppm due to $[Fe_4S_4(SR_F)_4]^{2-}$. These assignments are consistent with chemical shifts measured in N-methylformamide for the latter two species.[28]

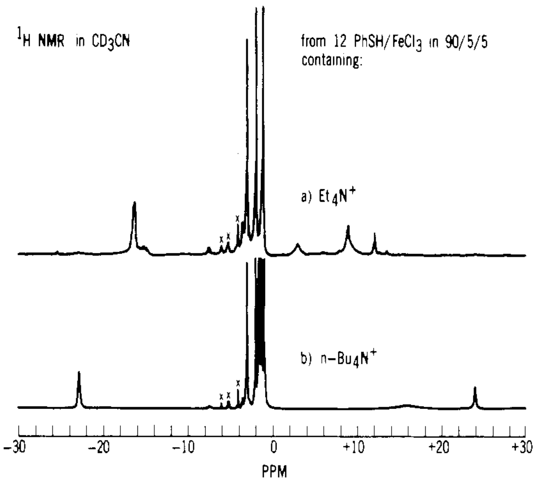

Figure 4. ^1H NMR spectra in CD_3CN at ~22 °C of unrecrystallized solids isolated from the reaction system 12/1 $PhSH/FeCl_3$ in 90/5/5 containing 0.1 M Et_4NBr (a) or 0.1 M n-Bu_4NBr (b). Reactions were conducted according to the procedure described in the Experimental Section by using 1 mol of LiOH/mol of PhSH in place of buffer. From low to high field the major resonances outside the 0 to −10 ppm range are due to m-, o-, and p-H's, respectively, of $[Fe_4(SPh)_{10}]^{2-}$ (a) or $[Fe(SPh)_4]^{2-}$ (b).[5] × denotes solvent impurity. Multiplet near −7.5 ppm is due to PhSSPh.

We have been unsuccessful in obtaining well-resolved ^1H NMR spectra in 90/5/5 in the presence of R_4N^+. As discussed above, R_4N^+ induces the formation of larger detergent aggregates, which may increase correlation times sufficiently to prevent observation of NMR resonances.

Figure 4 shows that the nature of R_4N^+ does have a striking effect on the Fe–SPh species that can be isolated from 90/5/5. At a mole ratio of 12/1 PhS^-/Fe^{3+} in acetonitrile or methanol, $[Fe(SPh)_4]^{2-}$ is essentially the only iron–thiolate species.[5] Analogously in 90/5/5, $(n\text{-}Bu_4N)_2[Fe(SPh)_4]$ can be isolated analytically pure in nearly quantitative yield in the presence of excess n-Bu_4NBr as described in the Experimental Section and as shown in Figure 4b. However, under identical conditions in 90/5/5 essentially only $(Et_4N)_2[Fe_4(SPh)_{10}]$ (identified by ^1H NMR in Figure 4a) can be isolated in ~76% yield when Et_4NBr is substituted for n-Bu_4NBr. These results demonstrate that equilibrium 1 is quite facile in 90/5/5 and can be explained as due to precipitation of the least soluble R_4N^+ salt.

To test the effect of PhSH/Fe ratio on the distribution of iron–thiolate species, solids were isolated from 90/5/5 containing 0.1 M n-Bu_4NBr and either 4/1 $PhSH/FeCl_3$ or preassembled $(n\text{-}Bu_4N)_2[Fe_4(SPh)_{10}]$. The ^1H NMR spectra in the m-H region of these unrecrystallized solids dissolved in CD_3CN contain the resonance due to $[Fe(SPh)_4]^{2-}$ at −22.8 ppm as the major feature. In addition, however, prominent m-H resonances centered at −16.2

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[28] Mascharak, P. K.; Smith, M. C.; Armstrong, W. H.; Burgess, B. K.; Holm, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7056–7060.

[29] (a) Jencks, W. P.; Salveson, K. *J. Am. Chem. Soc.* **1971**, *93*, 4433–4436. (b) Moss, R. A.; Dix, F. M. *J. Org. Chem.* **1981**, *46*, 3029–3035. (c) Danehy, J. P.; Parameswaran, K. N. *J. Chem. Eng. Data* **1968**, *13*, 386–389.

ppm due to $[Fe_4(SPh)_{10}]^{2-}$ are observed as are five other less intense resonances in the −10 to −30 ppm region. Though firm assignments are not currently possible, some or all of these resonances[30] are presumably due to metastable Fe–SPh intermediates in equilibrium 1. Thus taken, together with that in Figure 4b, these results are consistent with the idea that decreasing the PhSH/Fe ratio shifts equilibrium 1 to the left. Consistent with the result in Figure 4a, a mixture of preassembled $(Et_4N)_2[Fe_4(SPh)_{10}]$ and 0.1 M Et_4NBr in 90/5/5 yields a solid containing essentially only the starting material. Omission of Triton from aqueous mixtures of PhSH and $FeCl_3$ does not greatly affect the distribution of Fe–SPh species in the isolated solids.

Reactions of Preassembled $[Fe(SPh)_4]^{2-}$ and $[Fe_4(SPh)_{10}]^{2-}$ with S in 90/5/5. In acetonitrile, salts of $[Fe(SPh)_4]^{2-}$ and $[Fe_4(SPh)_{10}]^{2-}$ react with S to give, respectively, $[Fe_2S_2(SPh)_4]^{2-}$ (d^{2-}) and $[Fe_4S_4(SPh)_4]^{2-}$ (t^{2-}) in nearly quantitative yields.[5] Similarly, reaction of 1 mol of S with 1 mol of $(n\text{-}Bu_4N)_2[Fe(SPh)_4]$ in 90/5/5 (0.1 M $n\text{-}Bu_4NBr$) yields a mole ratio $d^{2-}/t^{2-} \simeq 50$ based on the ^1H NMR spectrum of the unrecrystallized solid dissolved in CD_3CN. However, in 90/5/5 both Et_4N^+ and $n\text{-}Bu_4N^+$ salts of $[Fe_4(SPh)_{10}]^{2-}$ upon reaction with S also yield substantial proportions of $[Fe_2S_2(SPh)_4]^{2-}$. An ^1H NMR spectrum of the unrecrystallized solid dissolved in CD_3CN shows that reaction of 1 mol of $(n\text{-}Bu_4N)_2[Fe_4(SPh)_{10}]$ with 4 mol of S in 90/5/5 (0.1 M R_4N^+) results in a mole ratio $d^{2-}/t^{2-} \simeq 3$. The analogous reaction with $(Et_4N)_2[Fe_4(SPh)_{10}]$ yields $d^{2-}/t^{2-} \simeq 0.5$. These results suggest that, at least in the presence of $n\text{-}Bu_4N^+$, the major portion of the reaction of $[Fe_4(SPh)_{10}]^{2-}$ with S in 90/5/5 occurs via $[Fe_4(SPh)_{10}]^{2-} \rightleftharpoons [Fe(SPh)_4]^{2-} + S \rightarrow [Fe_2S_2(SPh)_4]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$. This preference could derive from faster reaction of S with $[Fe(SPh)_4]^{2-}$ (reaction 2) than with $[Fe_4(SPh)_{10}]^{2-}$ (reaction 3), thereby shifting equilibrium 1 to the right. The higher

$$2[Fe(SPh)_4]^{2-} + 2S \rightarrow [Fe_2S_2(SPh)_4]^{2-} + PhSSPh + 2PhS^- \quad (2)$$

$$[Fe_4(SPh)_{10}]^{2-} + 4S \rightarrow [Fe_4S_4(SPh)_4]^{2-} + 3PhSSPh \quad (3)$$

d^{2-}/t^{2-} mole ratio obtained with $(n\text{-}Bu_4N)_2[Fe_4(SPh)_{10}]$ than with $(Et_4N)_2[Fe_4(SPh)_{10}]$ is explained below.

Effects on d^{2-}/t^{2-} Mole Ratio in the 4/1/1 PhSH/$FeCl_3$/S Reaction System. (a) R_4N^+. Figure 1 indicates that in addition to reactions 1–3, reaction 4 should influence the d^{2-}/t^{2-} mole ratio.

$$2[Fe_2S_2(SPh)_4]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-} + PhSSPh + 2PhS^- \quad (4)$$

In aqueous media reaction 4 appears to be quantitative and irreversible.[7,8] However, with excess R_4N^+ the extent of reaction 4 in 90/5/5 is influenced by the nature of R. Increasing the chain length of the R group leads to a monotonic increase in the proportion of $[Fe_2S_2(SPh)_4]^{2-}$ in the solid reaction product until with R = *n*-Pe no $[Fe_4S_4(SPh)_4]^{2-}$ is detectable. This trend is illustrated in Figure 5. As described in the Experimental Section, the latter solid consists of analytically pure $(n\text{-}Pe_4N)_2[Fe_2S_2(SPh)_4]$ and is obtained in nearly quantitative yield. No $[Fe(SPh)_4]^{2-}$ or $[Fe_4(SPh)_{10}]^{2-}$ was detected in any of the spectra of Figure 5. These results indicate that the reaction system of 4/1/1 PhSH/$FeCl_3$/S in 90/5/5 yields essentially only R_4N^+ salts of $[Fe_2S_2(SPh)_4]^{2-}$ and $[Fe_4S_4(SPh)_4]^{2-}$.

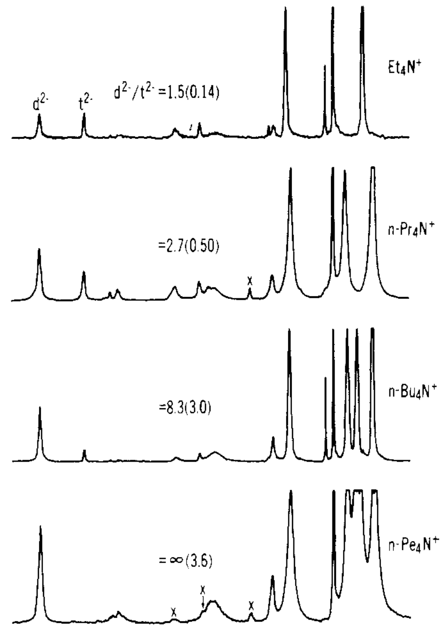

Figure 5. ^1H NMR spectra in CD_3CN at ~22 °C of unrecrystallized solids isolated from the reaction system of 4/1/1 PhSH/$FeCl_3$/S in 90/5/5 containing the R_4N^+ ion listed nearest each spectrum. Reactions were conducted by using the general procedure described in the Experimental Section. *m*-H resonances of $[Fe_2S_2(SPh)_4]^{2-}$ (d^{2-}) at −9.3 ppm and $[Fe_4S_4(SPh)_4]^{2-}$ (t^{2-}) at −8.2 ppm are labeled on the top spectrum. d^{2-}/t^{2-} mole ratios determined from the intergrated areas of the *m*-H resonances are listed near each spectrum. Numbers in parentheses are ratios obtained for reactions conducted in the absence of Triton. No resonances other than those shown were observed in any of the spectra from −60 to +60 ppm. The multiplet near −7.5 ppm is due to PhSSPh. Peaks upfield of −3.4 ppm are due to cation, CD_2HCN (−1.93), or water (−2.1). × denotes solvent impurity.

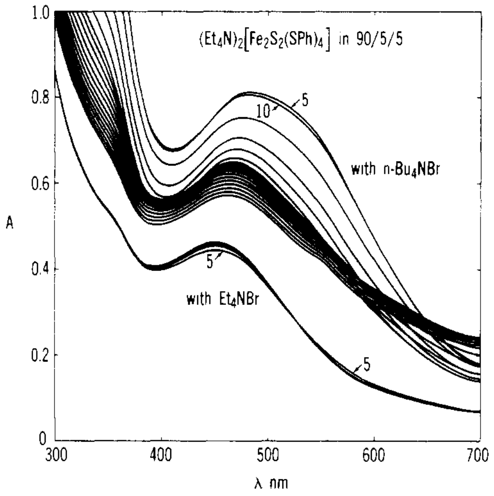

Figure 6. Absorption spectra resulting from dilutions of a 20.8 mM stock solution of $(Et_4N)_2[Fe_2S_2(SPh)_4]$ in DMF with Triton followed by aqueous 0.20 M Tris-sulfate at pH 8.5 containing (upper spectra) 8.9 mM $n\text{-}Bu_4NBr$ or (lower spectra) 8.9 mM Et_4NBr. Final solvent composition is ~90/5/5 vol % aqueous buffer/DMF/Triton. Numbers near spectra refer to minutes after mixing. Unlabeled spectra were recorded thereafter every 30 min for the upper ($n\text{-}Bu_4NBr$) set and every 20 min for the lower (Et_4NBr) set. Cell path length = 0.5 mm.

An explanation for the trend of Figure 5 is that the cation influences the rate of reaction 4. Evidence for this influence is contained in Figure 6. Shown are absorption spectra of a stock solution of a predetermined concentration of $(Et_4N)_2[Fe_2S_2(SPh)_4]$ in DMF diluted to a final solvent composition of ~90/5/5 vol % buffer/DMF/Triton. The $[R_4N^+]/[Fe]$ ratio used for these spectra is the same as used in the general procedure, but the concentrations are diluted ~11-fold. With excess Et_4NBr, the spectrum obtained 5 min after mixing in 90/5/5 is nearly identical with that of $[Fe_4S_4(SPh)_4]^{2-}$ in the same medium, having λ_{max} at 452 nm and A_{452}/A_{550} = 2.04.[7] Using the final absorbance and ϵ_{454} = 17 000 M^{-1} cm^{-1} [7] gives a 100% yield of $[Fe_4S_4(SPh)_4]^{2-}$, assuming transformation according to reaction 4. In contrast a solution prepared identically but containing $n\text{-}Bu_4NBr$ in place of Et_4NBr gives a spectrum 5 min after mixing (λ_{max}, 484 nm,

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(30) One of these, a *m*-H resonance at −14.7 ppm (~22 °C), has a temperature dependence opposite to those of $[Fe_4(SPh)_{10}]^{2-}$, consistent with its assignment to $[Fe_2(SPh)_6]^{2-}$, a previously unidentified species.[5,31] Thus, the transformation in equilibrium 1 may occur via $[Fe_4(SPh)_{10}]^{2-} + 2PhS^- \rightleftharpoons 2[Fe_2(SPh)_6]^{2-}$ and $[Fe_2(SPh)_6]^{2-} + 2PhS^- \rightleftharpoons 2[Fe(SPh)_4]^{2-}$.

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Table I. Distributions and Yields of $[Fe_4S_4(SPh)_4]^{2-}$ (t^{2-}) and $[Fe_2S_2(SPh)_4]^{2-}$ (d^{2-}) at Various pHs for the Reaction System 4/1/1 $PhSH/FeCl_3/S$ in 90/5/5[a]

final pH[b]	R_4N^+ [c]	product[d]	combined yield,[e] %
~10.5	none	t^{2-}	(73)
~10.5	n-Pe_4N^+	d^{2-}/t^{2-} ~20	*f*
8.2	none	t^{2-}	82 (96)
7.9	Et_4N^+	d^{2-}/t^{2-} = 1.5	*f*
7.9	n-Pe_4N^+	d^{2-}	87
4.8	none	t^{2-}	48
4.8	Et_4N^+	t^{2-}	52
4.5	n-Pe_4N^+	d^{2-}/t^{2-} = 7.1	57

[a] Reactions were conducted and products isolated by using the general procedure described in the Experimental Section. [b] Initially the "buffers" were as follows: pH ~10.5, 0.1 M LiOH; pH 8.2, 0.5 M NaTAPS pH 8.5; pH 7.9, 0.2 M NaTAPS pH 9.0; pH 4.5 or 4.8, 0.2 M NaMES pH 6.0. [c] Where "none" is entered, the product was isolated as the Et_4N^+ salt, the cation being added at 7–9 h reaction time. [d] Where both d^{2-} and t^{2-} were obtained, the mole ratio determined by NMR is given. [e] Yields in parentheses were determined spectrophotometrically. Others are by weight, on the basis of the starting amount of Fe and the d^{2-}/t^{2-} ratio. [f] Not determined.

sh ~540 nm) closely resembling that of $[Fe_2S_2(SPh)_4]^{2-}$ in DMF (λ_{max}, 490 nm, sh ~540 nm).[32] Over the course of ~9 h, the spectra gradually approach that of $[Fe_4S_4(SPh)_4]^{2-}$. When reaction 4 is allowed to proceed to completion as shown in Figure 6, but without excess R_4N^+, addition of n-Pe_4NBr to a concentration of 0.1 M does not reverse the reaction.

On the basis of these results, the correlation of alkyl chain length with proportion of $[Fe_2S_2(SPh)_4]^{2-}$ illustrated in Figure 5 can be explained as an influence of R_4N^+ on the partitioning of $[Fe_2S_2(SPh)_4]^{2-}$ between water-rich and water-poor regions of the solvent. The increasing hydrophobicity of R_4N^+ with increasing alkyl chain length would by ion pairing increasing the solubility of $[Fe_2S_2(SPh)_4]^{2-}$ in hydrophobic regions of the Triton micelles or aggregates, which are also likely to contain higher concentrations of PhSH. Lack of exposure to water and high PhSH concentrations are both known to inhibit the rate of $[Fe_2S_2(SPh)_4]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$ conversion.[7,8] This interpretation is consistent with the increased solubility of $(n\text{-}Pe_4N)_2[Fe_2S_2(SPh)_4]$ compared to $(Et_4N)_2[Fe_2S_2(SPh)_4]$ in less polar solvents such as THF and CH_2Cl_2.

The d^{2-}/t^{2-} mole ratios obtained by using the general procedure but omitting Triton are listed in parentheses in Figure 5 for each R_4N^+. Although the same trend is observed, the R_4N^+ ions are not nearly so effective at increasing the proportion of $[Fe_2S_2(SPh)_4]^{2-}$ in the absence of Triton. These results support the above conclusions concerning inhibition of reaction 4. In the absence of Triton the trend of increasing proportion of $[Fe_2S_2(SPh)_4]^{2-}$ with increasing alkyl chain length of R_4N^+ is probably due to faster and more complete precipitation of $(R_4N)_2[Fe_2S_2(SPh)_4]$.[33]

These results strongly suggest that for the 4/1/1 $PhSH/FeCl_3/S$ in 90/5/5 reaction system near pH 8, $[Fe(SPh)_4]^{2-} \rightarrow [Fe_2S_2(SPh)_4]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$ represents the sole assembly pathway and that, in the presence of n-Pe_4N^+, pathway a of Figure 1 is followed exclusively.

(b) pH. Table I lists d^{2-}/t^{2-} mole ratios and combined yields obtained for the reaction system 4/1/1 $PhSH/FeCl_3/S$ in 90/5/5 at various pHs. As can be seen, the optimal combination of yield and selectivity occurs in the region near pH 8 for both $[Fe_2S_2(SPh)_4]^{2-}$ and $[Fe_4S_4(SPh)_4]^{2-}$. The somewhat decreased yields at low pH are not unexpected in light of previous studies demonstrating decreased stability of the Fe_4S_4 core below pH 6.5.[12,13] Apparently precipitation and/or solubilization in hydrophobic regions of Triton aggregates sufficiently stabilize these clusters at low pH so that yields in the range of 50% are obtainable. The fact that uncomplexed PhSH would be mostly protonated is obviously no great deterrent to cluster assembly at pH 6.0 and below.[34] Table I also shows that a smaller proportion of $[Fe_2S_2(SPh)_4]^{2-}$ is obtained at these lower pHs. This latter result can be explained as due to protonation of PhS^-, which would tend to shift equilibrium 1 to the left, thereby favoring the $[Fe_4(SPh)_{10}]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$ pathway.[35]

Summary. Figure 1 summarizes most of the results discussed above. The two most prominent unique features of the $PhS^-/FeCl_3/S$ reaction system in 90/5/5 compared to organic solvents are (i) the ability of detergent aggregates to shield $[Fe_2S_2(SPh)_4]^{2-}$ from water in the presence of relatively hydrophobic R_4N^+, thereby inhibiting the rate of conversion to $[Fe_4S_4(SPh)_4]^{2-}$, and (ii) the facility with which equilibrium 1 can be shifted. Unique feature i leads to the ability to achieve essentially quantitative production of either $[Fe_2S_2(SPh)_4]^{2-}$ or $[Fe_4S_4(SPh)_4]^{2-}$ from the same 4/1/1 $PhSH/FeCl_3/S$ reagent ratio. Unique feature ii leads to production of substantial proportions of $[Fe_2S_2(SPh)_4]^{2-}$ upon reaction of $[Fe_4(SPh)_{10}]^{2-}$ with S and to larger proportions of $[Fe_4S_4(SPh)_4]^{2-}$ at pHs below the pK_a of PhSH. These two unique features suggest means by which ferredoxins coupled with various conditions within living cells could exert both kinetic and thermodynamic control over [2Fe–2S] vs. [4Fe–4S] site selection. However, our results strongly suggest that, upon reaction of Fe–SPh species with S, the $[Fe(SPh)_4]^{2-} \rightarrow [Fe_2S_2(SPh)_4]^{2-} \rightarrow [Fe_4S_4(SPh)_4]^{2-}$ pathway is thermodynamically favored in aqueous media under a wide range of conditions and occurs exclusively near pH 8. The existence of $[Fe(S\text{–}Cys)_4]^{2-}$, $[Fe_2S_2(S\text{–}Cys)_4]^{2-}$ and $[Fe_4S_4(S\text{–}Cys)_4]^{2-}$ sites in rubredoxins and ferredoxins may be a biological exploitation of this thermodynamic tendency. Studies are in progress to determine the extent to which our results apply when water-soluble aliphatic thiols replace PhSH in aqueous reaction systems containing iron salts and sulfur or sulfide.

Acknowledgment. This research was supported by NSF Grant PCM-8216447. The Nicolet NT-300 NMR spectrometer was purchased with funds from NSF equipment Grant CHE-8209709. We thank Dr. Dee Hua Huang and R. David Scott for obtaining the NMR spectra of aqueous solutions and for helpful discussions. We thank Mark T. Werth and Wayne K. Jones for experimental assistance.

Registry No. $(Et_4N)_2[Fe_4(S\text{-}p\text{-}C_6H_4F)_{10}]$, 97826-13-6; $(Et_4N)_2[Fe_4S_4(SPh)_4]$, 55663-41-7; $(n\text{-}Pe_4N)_2[Fe_2S_2(SPh)_4]$, 88915-80-4; $(n\text{-}Bu_4N)_2[Fe(SPh)_4]$, 97826-14-7; $[Fe_4S_4(SPh)_4]^{2-}$, 52325-39-0; $[Fe_2S_2(SPh)_4]^{2-}$, 55939-69-0; $FeCl_3$, 7705-08-0; PhSH, 108-98-5; S, 7704-34-9.

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(33) The selectivity of n-Pe_4N^+ for $[Fe_2S_2(SPh)_4]^{2-}$ is apparently confined to aqueous media. A reaction conducted according to the general procedure with R_4N^+ = n-Pe_4N^+, but substituting NaOMe in MeOH for aqueous buffer, gave a 77% yield of $(n\text{-}Pe_4N)_2[Fe_4S_4(SPh)_4]$ whose ^1H NMR spectrum in CD_3CN showed only a trace of $[Fe_2S_2(SPh)_4]^{2-}$.

(34) No $[Fe(SPh)_4]^{2-}$ or $[Fe_4(SPh)_{10}]^{2-}$ was detected in the reaction products at these lower pHs.

(35) In 90/5/5 $(Et_4N)_2[Fe_2S_2(SPh)_4]$ can be metathesized to $(n\text{-}Pe_4N)_2[Fe_2S_2(SPh)_4]$ quantitatively at pH 5, which argues against an increase in the rate of reaction 4 as an explanation for the higher proportion of $[Fe_4S_4(SPh)_4]^{2-}$ at lower pH.