Mechanism of the Acid-catalysed Substitution of $[Fe_4S_4(SR)_4]^{n-}$ (R = Ph, n = 2 or 3; R = Et or Bu^t, n = 2), and Transient Binding of Small Molecules at $[Fe_4S_4(SEt)_4]^{2-}$ †

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The kinetics of the acid-catalysed substitution of the first thiolate ligand of $[Fe_4S_4(SR)_4]^{n-}$ (R = Ph, n = 2 or 3; R = Et or Bu^t, n = 2) has been studied in MeCN at 25.0 °C using stopped-flow spectrophotometry. Using R′S$^-$ (R′ = Et, Bu^t or Ph, depending on the cluster) as the nucleophile (and base) and $[NHEt_3]^+$ as the acid, the influence of the cluster, the nucleophile and the acid on the rate of the reactions has been defined for the first time. The substitutions are confirmed to be dissociative but, although the reaction is accelerated by acid, protonation of the cluster is never rate-limiting. It has been shown, by a kinetic method, that $[Fe_4S_4(SEt)_4]^{2-}$ {but not $[Fe_4S_4(SPh)_4]^{n-}$} rapidly, but transiently, binds PhS$^-$, CN$^-$, N_2O, Cl$^-$, Br$^-$, N_3^- and CO.

A wide variety of metalloenzymes employ clusters of iron and sulfur atoms to bind substrates; such enzymes include the hydrogenases,[1] nitrogenases,[2] aconitase[3] and certain dehydratases.[4,5] Although the detailed structures of the active sites in some of these systems are, as yet, unknown it is well established that some contain other metals incorporated into the cluster (*e.g.* Ni in hydrogenases and Mo or V in nitrogenases). However, some enzymes such as aconitase employ simple, cubane-based 4Fe–4S clusters as the substrate binding site. Despite this, relatively little is known about the simple substitution patterns for the synthetic analogues of these clusters, $[Fe_4S_4(SR)_4]^{n-}$. In this paper we present a detailed kinetic study on the mechanism of acid-catalysed substitution of the first thiolate ligand in $[Fe_4S_4(SR)_4]^{n-}$ (R = Ph, n = 2 or 3; R = Et or Bu^t, n = 2), and develop this kinetic analysis to show that $[Fe_4S_4(SEt)_4]^{2-}$ is capable of binding a variety of small molecules.

Experimental

All manipulations were performed under an atmosphere of dinitrogen or argon using Schlenk or syringe techniques as appropriate. The clusters used, $[NBu_4]_2[Fe_4S_4(SPh)_4]$,[6] $[NEt_4]_3[Fe_4S_4(SPh)_4]$,[7] $[NPr^n{}_4]_2[Fe_4S_4(SEt)_4]$[8] and $[NMe_4]_2[Fe_4S_4(SBu^t)_4]$,[6] were prepared by the literature methods and recrystallised as described therein. Their purity was established by ^1H NMR spectroscopy; all other methods (*e.g.* elemental analysis and IR spectroscopy) are insufficiently sensitive to be diagnostic of purity. The ^1H NMR spectra were recorded on a JEOL GSX 270 spectrometer using CD_3CN as solvent. Peak heights in the quantitative studies were measured against an internal standard of C_6H_6 (5 mmol dm^{-3}).

The salts $[NEt_4]Cl$, $[NEt_4]Br$ and $[NEt_4][CN]$ (Aldrich) were dried (80 °C, *in vacuo*) prior to use and $[NEt_4][N_3]$ and $[NEt_4][SR]$ (R = Ph, Et or Bu^t) were prepared by the method described for $[NEt_4][SPh]$[9] and their purity established by elemental analysis and ^1H NMR spectroscopy. The salt $[NHEt_3][BPh_4]$ was prepared and analysed as described before.[10]

Acetonitrile used in the kinetic studies was freshly distilled from CaH_2 immediately prior to use. Solutions for the stopped-flow studies were prepared and used within 1 h.

Kinetic Experiments.—All solutions were prepared under an atmosphere of dinitrogen, and transferred by gas-tight glass syringe into the stopped-flow apparatus.

A Hi-Tech Scientific SF-51 stopped-flow spectrophotometer was used, modified to handle air-sensitive solutions. The temperature was maintained at 25.0 °C using a Grant LE8 thermostat tank connected to the spectrophotometer. The apparatus was interfaced to a BBC microcomputer (Acorn Computers, Cambridge) *via* an analogue-to-digital converter operating at 3 kHz. The data were stored on a $5\frac{1}{4}$ in (*ca.* 0.13 m) diskette.

The reactions were studied (usually at λ = 500 nm, but studies at 400 and 450 nm gave identical results) by monitoring the absorbance of the clusters. Since the absorbance *vs.* time curves observed correspond to the sequential substitution of all four thiolate ligands in $[Fe_4S_4(SR)_4]^{n-}$, and are consequently *at least* two exponentials, we restricted our attention to the substitution of the first thiolate ligand corresponding to the initial stages of the absorbance *vs.* time curve. A similar analysis was described by earlier workers on these clusters.[11a] The absorbance *vs.* time curves decrease for substitution of $[Fe_4S_4(SPh)_4]^{n-}$ and increase for $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) (see Figure in SUP 56937). The initial phase of this curve (*ca.* 70% of the total absorbance change) is exponential and corresponds to substitution of the first thiolate. The exponential character of this initial phase was established by (*i*) the linearity of the derived semilogarithmic graphs, and (*ii*) exponential curve fitting (by computer) of the initial 70% of the observed absorbance curve. In addition, analysis of the data by the Guggenheim method[12] gave rate constants in excellent agreement (±5%) with those derived from the exponential analyses.

The observation that the largest proportion of the total absorbance change associated with the complete (all four thiolates) substitution of the cluster corresponds to the substitution of the first thiolate is consistent with the noted[11a]

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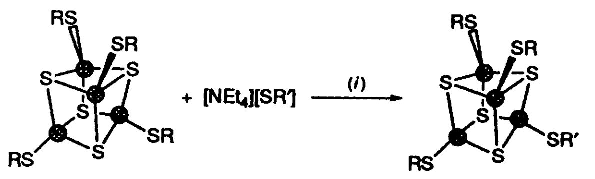
† *Supplementary data available* (No. SUP 56937, 12 pp.): first-order rate constants. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1993, Issue 1, pp. xxiii–xxviii.



Scheme 1 (*i*) $[NHEt_3][BPh_4]$, MeCN

very different visible spectra of any arenethiolate from any alkanethiolate cluster, but the very similar spectra of each class of cluster.

We have not studied in detail the kinetics of the subsequent substitution reactions {of for instance $[Fe_4S_4(SPh)_3(SEt)]^{2-}$} but limited studies indicate similar but slower kinetics to those described for the first substitution.

Results and Discussion

The only major mechanistic studies on the substitution reactions of iron–sulfur clusters, other than that described herein, is on the reactions of $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) with HSC_6H_4R-4 (R = Me, NH_2 or NO_2)[11a] and of $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ with PhSH.[11b] In the former study the authors concluded that (*i*) the substitution at the iron centre is dissociatively activated, and (*ii*) the rate-limiting step was protonation of the cluster by the arenethiol. This second conclusion, that there is a significant barrier to protonation of these clusters, is surprising. After all, the clusters contain four thiolate ligands, each having two lone pairs of electrons, and in addition four bridging sulfur atoms, each formally having a lone pair of electrons. One might consider that protonation of any of these sites would be facile. The problem with the previous studies is that the use of the arenethiol as a reactant leads to ambiguities in the mechanistic interpretation.[11] The thiol is playing, at least, three roles. First, it is the source of the protons, secondly it is the source of the strongest base in the system (RS^-) and thirdly, of course, it is the nucleophile.

To overcome these mechanistic ambiguities we have developed the general system shown in Scheme 1. In this system we separate the acid from the nucleophile (or base) by using the relatively weak acid $[NHEt_3][BPh_4]$ and supplying the thiolate as the salt $[NEt_4][SR]$. By supplying each of these reactants independently, the relative proportions of acid to base, or acid to nucleophile, in the system can be controlled. In addition, the reactions are studied in MeCN, a solvent where the solution properties of this system can, at least in part, be quantified.[13] Thus we know that when $[NHEt_3{}^+] = 1$ mmol dm^{-3}, then $[MeCNH^+] < 1 \times 10^{-10}$ mol dm^{-3}. It is difficult to estimate the amount of PhSH generated in solutions containing $[NHEt_3]^+$ and PhS^- since the pK_a of PhSH in MeCN is unknown. However, we have shown (see below) that, at the concentrations we are using, any thiol generated does not act as an acid in the reaction. Furthermore, the use of $[NHEt_3][BPh_4]$ has the advantage that the associated conjugate base and counter anion are essentially non-co-ordinating.

Using this system we have studied the kinetics for the substitution of the first thiolate ligand of $[Fe_4S_4(SPh)_4]^{n-}$ (*n* = 2 or 3) with EtS^- or Bu^tS^- and of $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) with PhS^-. We will start by discussing the results for the kinetically simpler $[Fe_4S_4(SPh)_4]^{n-}$ and then the $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) species. Subsequently we will show how this system can be developed to investigate the transient binding of small molecules at these clusters.

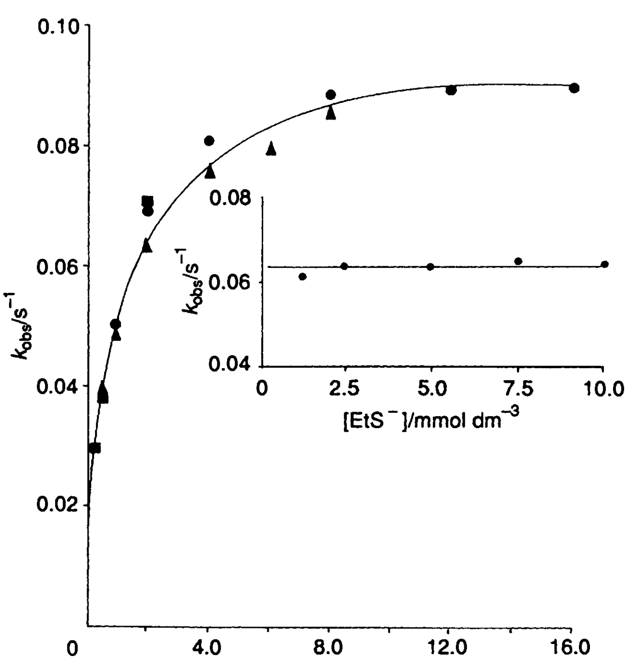


Fig. 1 Kinetic data for the reaction between $[Fe_4S_4(SPh)_4]^{2-}$ (0.1 mmol dm^{-3}) and $[NEt_4][SEt]$ in the presence of $[NHEt_3][BPh_4]$ in MeCN at 25.0 °C. In the main plot the data points correspond to: $[EtS^-] = 2.5$, $[NHEt_3{}^+] = 2.5$–40 (●); $[EtS^-] = 5.0$, $[NHEt_3{}^+] = 2.5$–40 (▲); $[EtS^-] = 10.0$, $[NHEt_3{}^+] = 5.0$–40 mmol dm^{-3} (■). The curve drawn is that defined by equation (2). For the insert $[NHEt_3{}^+]/[EtS^-] = 2.0$

Substitution at $[Fe_4S_4(SPh)_4]^{2-}$.—In the absence of acid the reaction between $[Fe_4S_4(SPh)_4]^{2-}$ and EtS^- or Bu^tS^- exhibits a simple first-order dependence on the cluster concentration, but is independent of the concentration and nature of the thiolate ion, giving rise to the simple rate law (1). The addition

$$-d[Fe_4]/dt = (1.0 \pm 0.2) \times 10^{-2}[Fe_4] \qquad (1)$$

of $[NHEt_3]^+$ increases the rate of substitution as shown in Fig. 1. It is clear from the Figure that the rate of reaction is defined solely by the ratio $[NHEt_3{}^+]/[RS^-]$, and not by the absolute concentration of either reagent. The dependence of the reaction rate on this ratio is complicated. At low values of the ratio the rate exhibits a first-order dependence on this quotient, but at higher values the rate becomes independent of both the value and identity of the ratio. Analysis of the data by the normal double-reciprocal plot[14] allows the derivation of the rate law (2). This rate law describes not only the substitution kinetics in

$$\frac{-d[Fe_4]}{dt} = \left\{(1.0 \pm 0.2) \times 10^{-2} + \frac{a[NHEt_3{}^+]/[RS^-]}{(1 + b[NHEt_3{}^+][RS^-]^{-1})}\right\}[Fe_4] \qquad (2)$$

the presence of acid, but also the acid-independent route defined in equation (1). When R = Et, $a = (9.6 \pm 0.1) \times 10^{-2}\ s^{-1}$, $b = 1.20 \pm 0.1$, and when R = Bu^t, $a = (9.5 \pm 0.1) \times 10^{-2}\ s^{-1}$, $b = 1.15 \pm 0.1$.

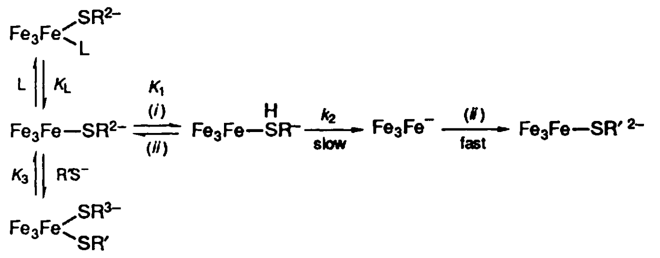
We were concerned that, under our experimental conditions, varying and significant amounts of RSH present were contributing to the reaction rate. However studies on the reactions between $[Fe_4S_4(SPh)_4]^{2-}$ and EtSH or Bu^tSH (1–10 mmol dm^{-3}) showed that in this concentration range the rate of the reaction was that described by equation (1). Thus any thiol EtSH or Bu^tSH generated in our system does not contribute to the reaction, at least not as an acid. Similarly in the studies on $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) described below the acid-catalysed rate is not attributable to the presence of any PhSH.

Before proceeding any further it is important to clarify the

Table 1 Summary of the elementary rate constants and equilibrium constants for the kinetics of reactions between $[Fe_4S_4(SR)_4]^{n-}$ and $R'S^-$ in the presence of $[NHEt_3]^+$ in MeCN at 25.0 °C*

Cluster	$R'S^-$	k_0/s^{-1}	K_1	k_2/s^{-1}	$K_3/dm^3\ mol^{-1}$
$[Fe_4S_4(SPh)_4]^{2-}$	EtS^-	$(1.0 \pm 0.2) \times 10^{-2}$	1.20 ± 0.1	$(8.0 \pm 0.1) \times 10^{-2}$	—
	Bu^tS^-	$(1.0 \pm 0.2) \times 10^{-2}$	1.15 ± 0.1	$(8.2 \pm 0.1) \times 10^{-2}$	—
$[Fe_4S_4(SPh)_4]^{3-}$	EtS^-	$(1.0 \pm 0.2) \times 10^{-2}$	1.15 ± 0.1	0.23 ± 0.02	—
	Bu^tS^-	$(1.0 \pm 0.2) \times 10^{-2}$	1.18 ± 0.1	0.23 ± 0.02	—
$[Fe_4S_4(SEt)_4]^{2-}$	PhS^-	—	0.29 ± 0.02	2.48 ± 0.1	67.4 ± 2.0
$[Fe_4S_4(SBu^t)_4]^{2-}$	PhS^-	—	0.29 ± 0.02	0.22 ± 0.02	67.8 ± 2.0

* All kinetics studied at λ = 500 nm.



Scheme 2 (*i*) $[NHEt_3]^+$; (*ii*) $R'S^-$

meaning of the ratio $[NHEt_3{}^+]/[RS^-]$ since it will recur in all the subsequent studies reported in this and the succeeding paper. The first, and most important, feature is that in the presence of $[NHEt_3]^+$ the rate of substitution is significantly faster than in its absence, or indeed in the reactions with RSH. This indicates a role for $[NHEt_3]^+$ in protonating the cluster. In the absence of quantitative information on the pK_a of PhSH in MeCN it seems most likely that the inverse dependence on the concentration of added RS^- represents the fact that both RS^- and RSH can act as bases in this system. Certainly we know that RSH does not act as a proton source under the conditions of our experiments. What this ratio corresponds to, then, is the total acid to the total base present in the system.

The kinetics described by equation (2) demonstrates, unambiguously, that the reaction is not rate-limited by protonation, since at high values of $[NHEt_3{}^+]/[RS^-]$ the rate of the reaction becomes independent of the acid concentration. The simplest mechanism consistent with the observed kinetics is shown in Scheme 2. In the absence of acid the substitution at the cluster involves rate-limiting dissociation of the co-ordinated thiolate to give a cluster in which one iron centre has a vacant site. Rapid attack by the large excess of RS^- present completes the substitution. In the presence of acid another pathway for substitution is available, involving rapid protonation of the cluster (probably at a co-ordinated thiolate sulfur atom), followed by rate-limiting dissociation of the thiol to generate the intermediate containing a single iron centre with a vacant site. Subsequent rapid attack by RS^- then completes the substitution, as before.

If we assume that protonation of the cluster involves a rapidly established equilibrium, with $[NHEt_3]^+$ being the acid, effectively RS^- being the base, and dissociation from the cluster the slowest steps, then the rate law (3) can be derived.

$$\frac{-d[Fe_4]}{dt} = \left\{k_0 + \frac{k_2K_1[NHEt_3{}^+]/[RS^-]}{(1 + K_1[NHEt_3{}^+][RS^-]^{-1})}\right\}[Fe_4] \quad (3)$$

This analysis indicates that at large values of $[NHEt_3{}^+]/[RS^-]$ stoichiometric and hence detectable concentrations of the protonated cluster accumulate. Although we see no spectrophotometric evidence for the accumulation of this protonated species this is not unexpected since the UV/VIS absorption spectrum of these clusters is dominated by the Fe_4S_4 chromophore. Comparison of equations (2) and (3) allows the determination of the elementary rate constants and equilibrium constant summarised in Table 1. Clearly the rate of the protonation step is too rapid to be determined by the stopped-flow technique, but we can estimate $k_1 > 1 \times 10^7$ dm^3 mol^{-1} s^{-1}. There is no evidence, in this or the subsequently described studies, for the addition of more than one proton to a cluster prior to dissociation of a thiolate ligand.

In general, these systems were not studied at a constant ionic strength. However, studies on the reactions of $[Fe_4S_4(SEt)_4]^{2-}$ [see section on the binding of small molecules at these clusters (see below) and Table 2 in SUP 56937] with $[NHEt_3]/[PhS] = 2.0$ showed that for 0–40 mmol dm^{-3} = $[NBu^n{}_4][BF_4]$ the rate of substitution of the initial thiolate ligand was independent of the concentration of this inert electrolyte ($k_{obs} = 0.92 \pm 0.03$ s^{-1}).

Substitution at $[Fe_4S_4(SPh)_4]^{3-}$.—Essentially identical kinetics is observed in the reaction of $[Fe_4S_4(SPh)_4]^{3-}$ with EtS^- or Bu^tS^- in the presence of $[NHEt_3]^+$ as was observed with $[Fe_4S_4(SPh)_4]^{2-}$. Consequently the rate law is that of equation (3) with the corresponding rate constants and equilibrium constant shown in Table 1.

The protonation constant K_1 associated with $[Fe_4S_4(SPh)_4]^{3-}$ is essentially the same that for $[Fe_4S_4(SPh)_4]^{2-}$, presumably because the negative charges on these clusters are delocalised over the entire cube. However, the rate constant (k_2) for dissociation of the thiol from $[Fe_4S_4(SPh)_4]^{3-}$ is nearly three times larger than that for $[Fe_4S_4(SPh)_4]^{2-}$.

Substitution at $[Fe_4S_4(SR)_4]^{2-}$ (R = Et *or* Bu^t).—Studies on the kinetics of substitution of the first alkanethiolate ligand from $[Fe_4S_4(SR)_4]^{2-}$ by PhS^- shows essentially the same behaviour as described above for $[Fe_4S_4(SPh)_4]^{n-}$ with two important differences. First, the uncatalysed pathway is not observed and secondly the rate of the reaction depends not only on the ratio $[NHEt_3{}^+]/[PhS^-]$ but also on the concentration of PhS^-. This second point is evident from Fig. 2. In the insert it is shown that, for any given value of $[NHEt_3{}^+]/[PhS^-]$, increasing $[PhS^-]$ decreases the rate of the reaction. Note again here that we cannot distinguish between this being a dependence on PhS^- or PhSH. Inspection of these lines shows that the ratio gradient/intercept is a constant (67.4 ± 2.0). Correcting the observed first-order rate consant k_{obs} for this effect, $k'_{obs} = k_{obs}(1 + 67.4[PhS^-])$ gives the usual dependence on $[NHEt_3{}^+]/[PhS^-]$, shown in Fig. 2 (main). The rate law derived from this curve is shown in equation (4). For

$$\frac{-d[Fe_4]}{dt} = \left\{\frac{a[NHEt_3{}^+]/[PhS^-]}{(1 + b[NHEt_3{}^+][PhS^-]^{-1})(1 + c[PhS^-])}\right\}[Fe_4] \quad (4)$$

$[Fe_4S_4(SEt)_4]^{2-}$, $a = 0.72 \pm 0.03$ s^{-1}, $b = 0.29 \pm 0.02$, $c = 67.4 \pm 2.0$ dm^3 mol^{-1}; for $[Fe_4S_4(SBu^t)_4]^{2-}$, $a = 6.5 \pm 0.2 \times 10^{-2}$ s^{-1}, $b = 0.29 \pm 0.02$, $c = 67.8 \pm 2.0$ dm^3 mol^{-1}. This rate law reflects essentially the same mechanism as already

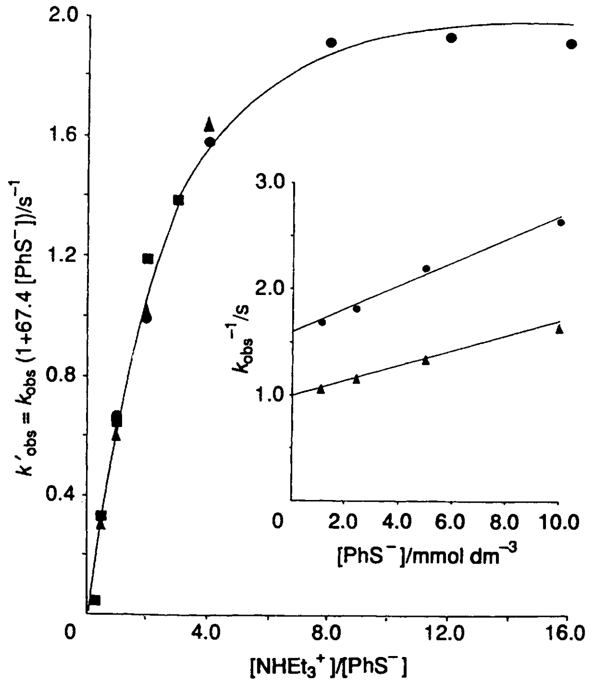


Fig. 2 Kinetic data for the reaction between $[Fe_4S_4(SEt)_4]^{2-}$ (0.1 mmol dm^{-3}) and $[NEt_4][SPh]$ in the presence of $[NHEt_3][BPh_4]$ in MeCN at 25.0 °C. In the main plot the data points correspond to: [PhS$^-$] = 2.5, [NHEt$_3$] = 2.5–40 (●); [PhS$^-$] = 5.0, [NHEt$_3^+$] = 2.5–40 (▲); [PhS$^-$] = 10.0, [NHEt$_3^+$] = 2.5–30 mmol dm^{-3} (■). The curve drawn is that defined by equation (4). For the insert the data points correspond to [NHEt$_3^+$]/[PhS$^-$] = 1.0 (●) and 2.0. The lines drawn are those defined by equation (4).

described for $[Fe_4S_4(SPh)_4]^{n-}$, and shown in Scheme 2. The inhibition afforded to the reaction by the excess of PhS$^-$ is attributable to binding of this thiolate ion to the intact cluster *in competition with the protonation step.* However, this binding does not give rise to an energetically viable associative pathway for substitution, at least not one which is able to compete with the facile protonation, dissociation route with which we have become familiar in these studies.

Again, assuming all equilibria in Scheme 2 are rapidly established, and that the rate-limiting step is dissociation of the

$$\frac{-d[Fe_4]}{dt} = \left\{\frac{k_2K_1[NHEt_3^+]/[PhS^-]}{(1 + K_1[NHEt_3^+]/[PhS^-]^{-1})(1 + K_3[PhS^-])}\right\}[Fe_4] \qquad (5)$$

alkanethiol ligand, the rate law (5) can be derived, and comparison with equation (4) permits the determination of the elementary rate constants and equilibrium constants for $[Fe_4S_4(SR)_4]^{2-}$ (R = Et or Bu^t) shown in Table 1.

At this stage it is not entirely clear why PhS$^-$ (or PhSH) binds to the alkanethiolate clusters, whereas RS$^-$ does not bind significantly to $[Fe_4S_4(SPh)_4]^{n-}$. What is clear though is that this difference cannot be attributed to any greater nucleophilicity of PhS$^-$ over RS$^-$, or any role of PhSH acting as a proton source, but rather it is a characteristic of the alkanethiolate clusters. This will become clear when the interaction of a variety of small molecules with these clusters is discussed in the next section. However, before we move on to that aspect it is interesting to compare our results with those observed earlier. The two sets of results are not directly comparable since the earlier study[11a] used various arenethiols as the reactants, and consequently saw much simpler kinetics than ours. The system we have developed permits a distinction between the role of the acid and the various roles of the thiolate ion, with correspondingly more complicated rate laws. Nonetheless an important conclusion from our studies is that the rate of substitution of a cluster is limited by the rate of dissociation of the thiol ligand (k_2). Irrespective of the acid or the nucleophile, the rate of the reaction cannot exceed this value. For $[Fe_4S_4(SBu^t)_4]^{2-}$ this limiting rate constant is k_2 = 0.22 s^{-1}, and for $[Fe_4S_4(SEt)_4]^{2-}$, k_2 = 2.48 s^{-1}. Consistent with our mechanism, the earlier studies[11a] with these clusters and a variety of arenethiols all gave rates well below these limits, with one exception: the reactions with $HSC_6H_4NO_2$-4 exceed the limit by a factor of *ca.* 5. We attempted to clarify this discrepancy by studying the reaction of $[Fe_4S_4(SBu^t)_4]^{2-}$ with $HSC_6H_4NO_2$-4 but the intense colour of the thiol masked the absorbance changes associated with the reaction of the cluster. We would suggest, tentatively, that the earlier workers were looking at an oxidation of the thiol due to the presence of adventitious air, or possibly with $HSC_6H_4NO_2$-4 an associatively activated substitution of the cluster occurs.

Table 2 Binding constants, K_L for the interactions of small molecules with $[Fe_4S_4(SEt)_4]^{2-}$ in MeCN at 25.0 °C

L	K_L[a]/dm^3 mol^{-1}
Cl$^-$	201.9
Br$^-$	96.2
N$_3^-$	48.1
CN$^-$	380.8
PhS$^-$	67.6
N$_2$O	*ca.* 380[b]
CO	*ca.* 300[b]

[a] The value of K_L is independent of the cluster concentration in the range 0.1–0.01 mmol dm^{-3} [Fe$_4$]. It is also independent of the ratio [NHEt$_3^+$]/[PhS$^-$] = 1.0–4.0. Routinely, studies were performed at [NHEt$_3^+$]/[PhS$^-$] = 2.0. [b] Limited solubility ([gas] < 5 mmol dm^{-3}) restricts the accuracy.

Binding of Small Molecules at Clusters.—Having developed the basic kinetics and mechanism for the substitution reactions of $[Fe_4S_4(SR)_4]^{n-}$ it is possible to use this system to investigate the binding of a variety of small molecules at the clusters. This approach results directly from our observation that PhS$^-$ binds to the alkanethiolate clusters. The strategy adopted is as follows. If the substitution reaction of any cluster is studied at a constant concentration of $[NHEt_3]^+$ and a constant concentration of R′S$^-$, then the rate of the reaction will be a constant. However, the addition of a species L will result in a change in the rate of this reaction if L interacts with the cluster. Using this approach we have studied the reactions of $[Fe_4S_4(SPh)_4]^{n-}$ or $[Fe_4S_4(SEt)_4]^{2-}$ with L = Cl$^-$, Br$^-$, N$_3^-$, CN$^-$, N$_2$O, CO, C$_2$H$_2$, PhC≡CH, C$_2$H$_4$, N$_2$ and H$_2$. It is important to remember in all these studies that L *is never incorporated into the final product.* What we are looking at is the substitution of, for instance, an alkanethiolate ligand for an arenethiolate ion, and the way in which the interaction of L with the cluster perturbs these kinetics.

For $[Fe_4S_4(SEt)_4]^{2-}$ we see from the data in Fig. 3 and Table 2 that with L = Cl$^-$, Br$^-$. N$_3^-$, N$_2$O, CN$^-$ or CO the rate of the acid-catalysed substitution reaction is inhibited. This inhibition is not attributable to an ionic strength effect for the following reasons. (*i*) The extent of the inhibition is a function of both the concentration and *nature* of L. (*ii*) Both neutral species and anions inhibit the reaction. (*iii*) The effect is specific for $[Fe_4S_4(SEt)_4]^{2-}$ and no perturbation to the kinetics of $[Fe_4S_4(SPh)_4]^{2-}$ is observed at the same concentrations of L. (*iv*) Over the concentration range 2.5–40 mmol dm^{-3} $[NBu_4][BF_4]$ there is no effect on the kinetics.

Four important features of these data deserve further comment. (*i*) For all the inhibitions the data can be fitted by the

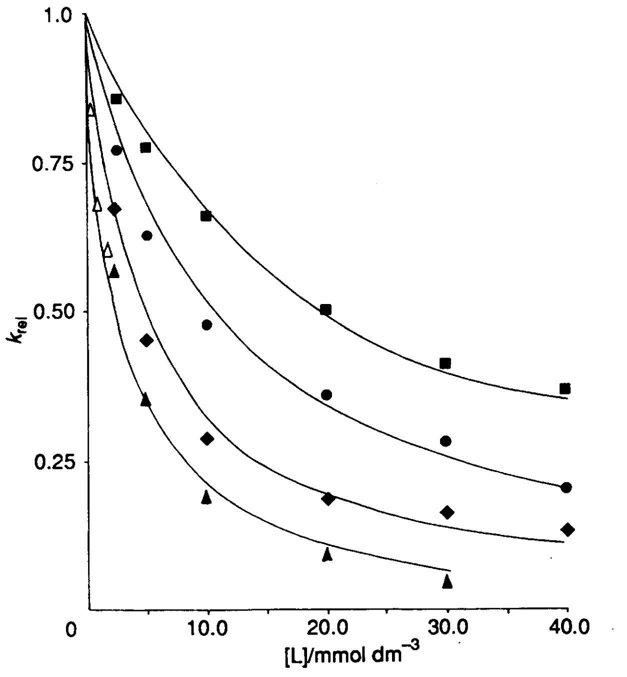
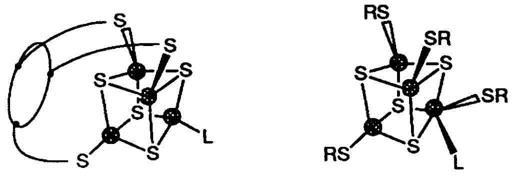


Fig. 3 Kinetic data for the reaction between $[Fe_4S_4(SEt)_4]^{2-}$ and PhS^- with $[NHEt_3]^+$, in the presence of various small molecules L [N_3^-, ■; Br^-, ●; Cl^-, ◆; CN^-, ▲; N_2O (△)] in MeCN at 25.0 °C. The curves drawn are those defined by equation (6), where $k_{rel} = k_{obs}/k'$, using the values in Table 2



Scheme 3

function (6), where k' is the rate constant observed in the

$$k_{obs} = k'/(1 + K_L[L]) \qquad (6)$$

absence of L. This equation assumes only 1 mole equivalent of L is binding to the cluster. Thus binding of L at one site suppresses substitution at all four iron atoms in the cluster. This strongly indicates that the origin of the inhibition is electronic and not steric. (*ii*) Restrictions due to solubility of the salts and gases means that we can only study the inhibitions up to [L] = 40 mmol dm^{-3}. Consequently it is not possible to tell if the binding of L to the cluster switches off substitution at all iron centres completely, or whether the curves limit to a finite rate, associated with slower substitution at the L-bound cluster. (*iii*) The ligand L is binding to an intact cluster, *without displacing a thiolate ligand*, since the derived value of K_L is independent of the concentration of cluster. (*iv*) No L-catalysed pathways are observed.

There is no perturbation to the rate of reaction in the presence of N_2, H_2, C_2H_2, C_2H_4 or PhC≡CH, and hence we conclude that these molecules do not interact with the clusters.

These kinetically based studies complement the synthetic studies on the binding of small molecules at 'differentiated iron subsite clusters'[15] as shown in Scheme 3. Together these two types of study demonstrate the ways in which these simple clusters can interact with substrates. With the 'site-differentiated' clusters using tripodal ligands, isolable complexes have been obtained by displacing a ligand at the unique iron position, whereas in our studies L binds only transiently to an intact complex. For the systems where the substrates interact with the cluster by ligand substitution the binding is relatively slow and energy consuming. In contrast the studies described herein show that the four-co-ordinate iron centres within the cluster are capable of binding rapidly a variety of small molecules, albeit only transiently. The rapidity of this binding is emphasised when it is appreciated that it competes with a protonation reaction. If a metalloenzyme can bind and activate a substrate without the dissociation of an amino acid side-chain ligand then, energetically, this would clearly be advantageous.

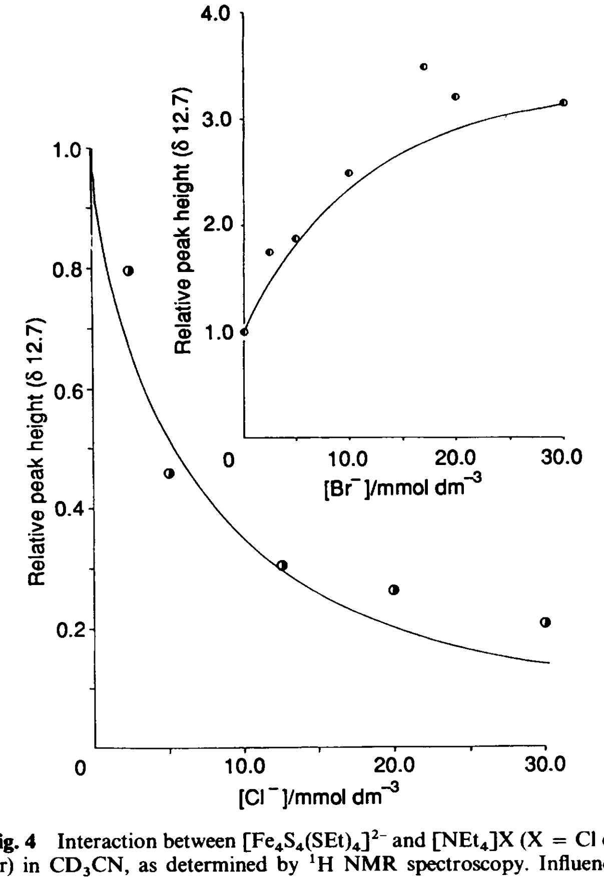


Fig. 4 Interaction between $[Fe_4S_4(SEt)_4]^{2-}$ and $[NEt_4]X$ (X = Cl or Br) in CD_3CN, as determined by ^1H NMR spectroscopy. Influence on the *height* of the signal due to SCH_2Me (δ 12.6) of various concentrations of Cl^- (main plot) or Br^- (insert); $[Fe_4S_4(SEt)_4{}^{2-}]$ = 5.0 mmol dm^{-3}. The curves drawn are those defined by using the binding constants K_L, shown in Table 2, together with the assumptions that for X = Cl the peak broadens so much that it collapses into the baseline, and for X = Br the peak reaches a maximum height four times that of $[Fe_4S_4(SEt)_4]^{2-}$, at high concentrations of X^-

The ligands on the cluster impart a high degree of control on the binding capability of the iron centres. Thus with $[Fe_4S_4(SPh)_4]^{n-}$ (*n* = 2 or 3) none of the small molecules is observed to perturb the kinetics and hence demonstrate its binding to the cluster. The reasons for this are not entirely clear, but are sufficiently general that the same characteristics are observed in the heterometal-substituted clusters $[\{MFe_3S_4(SR)_3\}_2(\mu\text{-}SR)_3]^{3-}$ (M = Mo or W, R = Ph or Et).[16]

We have looked to other techniques to confirm the binding of L at the clusters, as identified by the kinetic analysis. Probably the most diagnostic spectroscopic feature of these iron–sulfur clusters is their contact-shifted ^1H NMR spectra.[17] We have observed that for $[Fe_4S_4(SEt)_4]^{2-}$ the signal associated with the SCH_2Me protons (δ 12.6) in the ^1H NMR spectrum is perturbed by the presence of $[NEt_4]Cl$ or $[NEt_4]Br$ as shown in Fig. 4. Notice here that we are looking at signals associated with protons which are as close as possible to the expected binding sites of L, and hence most likely to be affected.

In the presence of various concentrations of Cl^- the *height* of the signal due to SCH_2Me becomes markedly smaller (because of a broadening of the peak), whilst with corresponding concentrations of $\mathrm{Br^-}$ its *height* increases (because of a sharpening of the peak). Whilst it is difficult to be sure that these effects are attributable solely to the formation of $[\mathrm{Fe_4S_4(SEt)_4X}]^{3-}$ (X = Cl or Br), it is at least gratifying that the magnitude of these variations in the linewidth reflect the binding constants derived from the kinetic analysis (see Fig. 4). However, it is clear that the response of the $\mathrm{SC}H_2\mathrm{Me}$ signal to the presence of L can vary, either broadening or sharpening the signal. This problem is further amplified in studies on $[\{\mathrm{MFe_3S_4(SEt)_3}\}_2(\mu\text{-}\mathrm{SEt})_3]^{3-}$ (M = Mo or W).[16] Kinetic studies, similar to those described in this paper, indicate extensive binding of $\mathrm{Cl^-}$ to the cluster, but ^{1}H NMR spectroscopic studies show no detectable perturbation to the $\mathrm{SC}H_2\mathrm{Me}$ signals (δ 55.2).

Conclusion

We have developed a general system for establishing the kinetics and mechanisms of substitution at iron–sulfur-based clusters. By using this system it has been possible to demonstrate the following.

(*i*) Protonation is not slow for these $[\mathrm{Fe_4S_4(SR)_4}]^{2-}$ clusters. The proton affinities of the clusters in MeCN can be determined semiquantitatively (using the ratio $[\mathrm{NHEt_3}^+]/[\mathrm{RS}^-]$) and is essentially independent of the cluster type.

(*ii*) The substitution at these essentially tetrahedral iron sites is dissociatively activated, dissociation of a co-ordinated thiol being the rate-limiting step in the acid-catalysed substitution pathway.

(*iii*) The lability of the protonated clusters falls in the sequence $[\mathrm{Fe_4S_4(SEt)_4}]^{2-} > [\mathrm{Fe_4S_4(SBu^t)_4}]^{2-} \approx [\mathrm{Fe_4S_4(SPh)_4}]^{3-} > [\mathrm{Fe_4S_4(SPh)_4}]^{2-}$. For the unprotonated clusters the order is $[\mathrm{Fe_4S_4(SPh)_4}]^{2-} = [\mathrm{Fe_4S_4(SPh)_4}]^{3-} \gg [\mathrm{Fe_4S_4(SR)_4}]^{2-}$ (R = alkyl).

(*iv*) A variety of small molecules, $\mathrm{Cl^-}$, $\mathrm{Br^-}$, $\mathrm{N_3}^-$, $\mathrm{CN^-}$, $\mathrm{N_2O}$ or CO, bind rapidly, but transiently to $[\mathrm{Fe_4S_4(SEt)_4}]^{2-}$, but not $[\mathrm{Fe_4S_4(SPh)_4}]^{n-}$.

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