Protonation of the Iron–Sulfur Core in $[Fe_4S_4X_4]^{2-}$ (X = Cl or Br): Chemical Precedent for the Elementary Reaction of the Hydrogenases and Nitrogenases

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Kinetic studies on $[Fe_4S_4X_4]^{2-}$ (X = Cl or Br) show that the substitution of the first halide for thiolate is catalysed by protonation of the Fe_4S_4 cubane core, this is the first demonstration that these cores will bind protons.

It is now clear that the substrate binding sites of a variety of metalloenzymes are based on iron–sulfur clusters. Two such classes of enzymes, hydrogenases[1] and nitrogenases,[2] evolve dihydrogen by reduction of protons as a functional part of their biological action. In both cases the presumed precursors to dihydrogen formation are hydridic species, however, no direct evidence for such species has been obtained. Herein we demonstrate unambiguously, for the first time, that protonation of the iron–sulfur core occurs at the $\{Fe_4S_4\}^{2+}$ redox level; thus establishing the chemical precedent for this biochemically fundamental reaction associated with these clusters in a variety of redox levels.

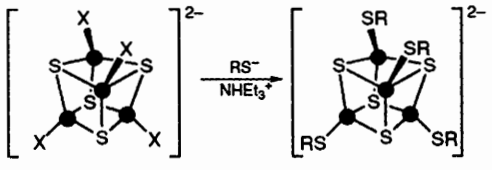


Fig. 1

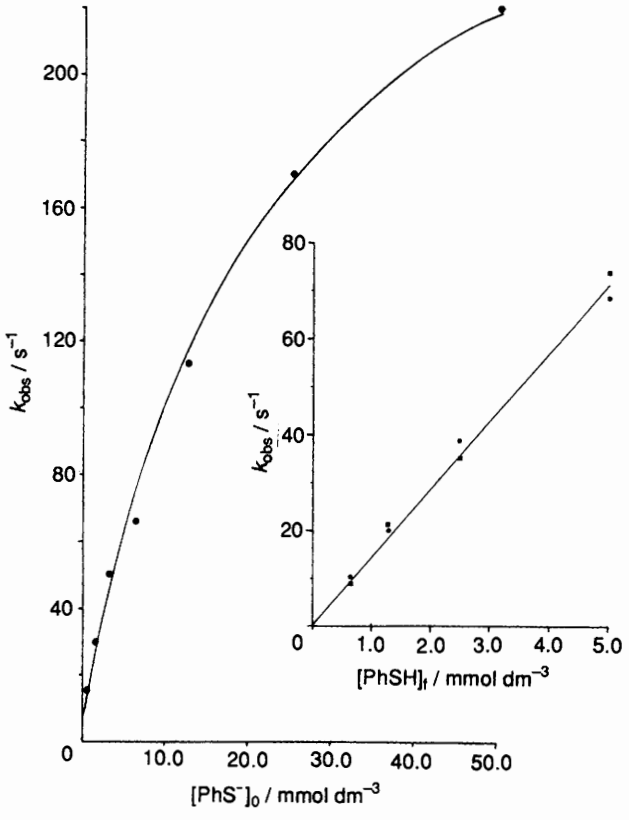


Fig. 2 Graph of k_{obs} (pseudo-first-order rate constant) against the concentration of $[NEt_4]SPh$ for the reaction of $[Fe_4S_4Cl_4]^{2-}$ in MeCN at 25.0 °C. Concentration of cluster = 1×10^{-4} mol dm^{-3}, λ = 600 nm. Curves drawn are those defined by eqn. (1) and the parameters given in the text. The insert shows the graph of k_{obs} against the concentration of PhSH for the reaction with $[Fe_4S_4Cl_4H]^-$ in MeCN at 25.0 °C when $[NHEt_3^+]_f/[NEt_3]_f$ = 10.0 (●) or 15.0 (■).

Previously, we developed the general system shown in Fig. 1 to study the substitution mechanisms of iron–sulfur-based clusters.[4] In this system, the independent variation of the concentations of $[NHEt_3]^+$ and RS^- allows the acid and nucleophile dependencies of the reaction kinetics to be defined. Although the substitution reactions of $[Fe_4S_4(SR)_4]^{2-}$ and $[\{MoFe_3S_4(SR)_3\}_2(\mu\text{-}SR)_3]^{3-}$ (R = Et or Ph) are acid catalysed, the site of protonation is ambiguous and could be either the thiolate ligand or the iron–sulfur core. To simplify the system and remove this ambiguity, we have studied the analogous halo-clusters.

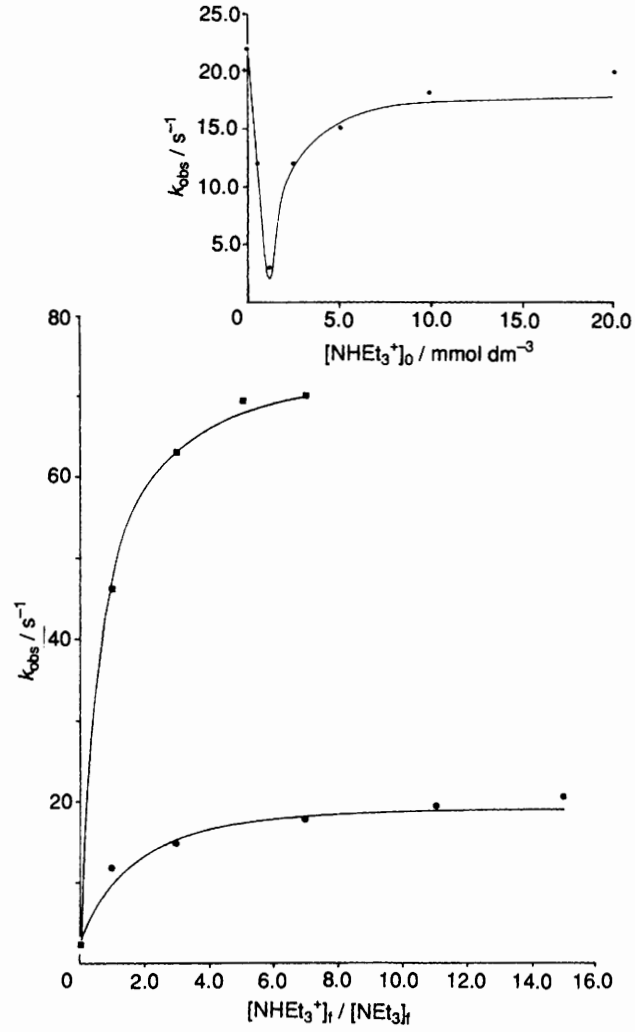
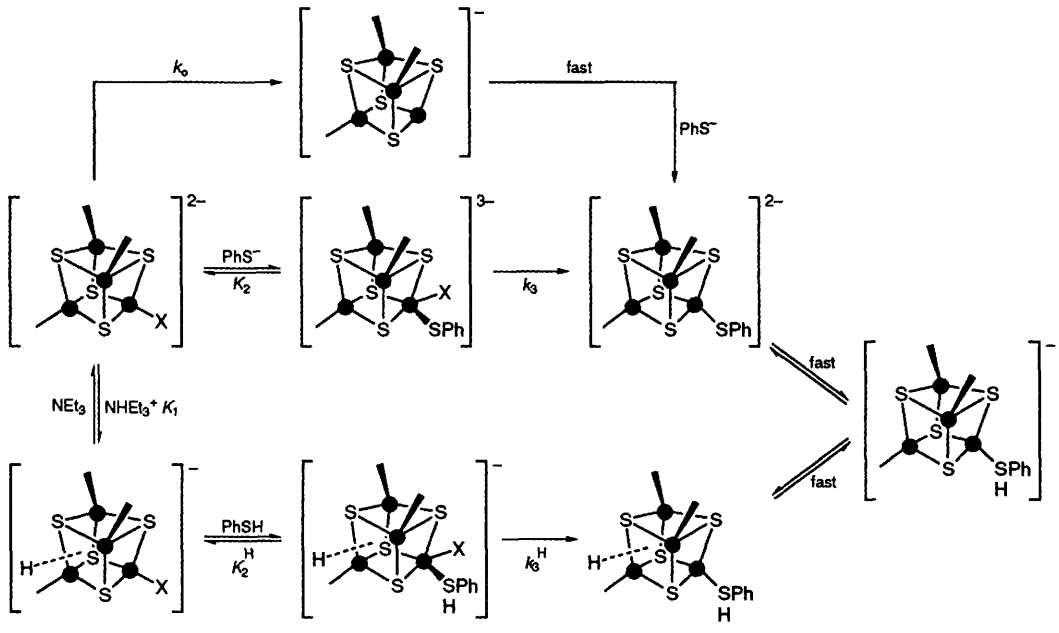


Fig. 3 Graph of k_{obs} against the ratio, $[NHEt_3^+]_f/[NEt_3]_f$, in the reaction of $[Fe_4S_4Cl_4]^{2-}$ with $[NEt_4]SPh$ in MeCN at 25.0 °C. Concentration of cluster = 1×10^{-4} mol dm^{-3}, λ = 600 nm. Data points correspond to $[PhSH]_f$ = 1.25 mmol dm^{-3} (●), $[PhSH]_f$ = 5.0 mmol dm^{-3} (■). Curves drawn are those defined by eqn. (2), and the parameters given in the text. The insert shows the dependence of k_{obs} on $[NHEt_3^+]_0$ for the reaction of $[Fe_4S_4Cl_4]^{2-}$ with $[NEt_4]SPh$, $[PhS^-]_0$ = 1.25 mmol dm^{-3}. In this representation both the acid-catalysed and the inhibition of the non-acid-catalysed pathways are illustrated.



Scheme 1

When monitored on a stopped-flow spectrophotometer, the reaction between $[Fe_4S_4Cl_4]^{2-}$ and an excess of PhS^- is associated with a single exponential corresponding to the substitution of the first chloride. The subsequent three substitutions are much slower and occur over the course of several minutes. The dependence on the concentration of PhS^- is shown in Fig. 2 and is consistent with the top two pathways shown in Scheme 1, involving parallel dissociative or associative pathways. The rate law for these substitution pathways is given in eqn. (1), with $k_0 = 2.0 \pm 0.3\ s^{-1}$, $k_3 = (2.5 \pm 0.1) \times 10^2\ s^{-1}$ and $K_2 = 68.4 \pm 1.2\ dm^3\ mol^{-1}$.

$$-d[Fe_4S_4]/dt = \{k_0 + k_3K_2[PhS^-]_0/(1 + K_2[PhS^-]_0)\}[Fe_4S_4] \quad (1)$$

The influence of acid, $[NHEt_3]^+$ on the rate of the substitution is complicated, and is shown in Fig. 3. As the acid concentration is increased, the rate of the reaction decreases to a minimum value (at $[NHEt_3^+]/[PhS^-] = 1.0$), after which the rate increases. This behaviour is consistent with the lower pathway shown in Scheme 1. At a constant initial concentration of PhS^- (Fig. 3, insert), increasing the concentration of $[NHEt_3]^+$ progressively decreases the concentration of PhS^- until at $[NHEt_3^+]/[PhS^-] = 1.0$ all the PhS^- is converted to PhSH. Over this acid concentration range the rate of the reaction correspondingly decreases as the contribution from the associative (K_2k_3) pathway is progressively diminished. If this was the only effect of $[NHEt_3^+]$, the rate of the reaction would become independent of the acid concentration and remain at the value of k_0. However, the subsequent increase in the reaction rate as the concentration of $[NHEt_3]^+$ is increased further is a consequence of protonation of the cluster.

The rate law for the acid-dependent pathways shown in Scheme 1 is given by eqn. (2), bearing in mind that at low concentrations of PhS^-, $K_2[PhS^-] << 1$†. The first two terms of the numerator describe the acid-independent pathways [eqn. (1)], and the last term defines the reactivity of the protonated cluster. Analysis of the data in Fig. 3 gives $k_3^HK_2^H = (1.5 \pm 0.2) \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$ and $K_1 = 2.2 \pm 0.1$.‡

Protonation could occur either at the chloro-group or the HOMO of the $\{Fe_4S_4\}^{2+}$ core. The former protonation site seems unlikely, and this is confirmed by studies on $[Fe_4S_4Br_4]^{2-}$. This bromo-complex behaves the same as the chloro-analogue with, $k_0 = 60 \pm 5\ s^{-1}$, $k_3 = (2.3 \pm 0.2) \times 10^2\ s^{-1}$, $K_2 = (2.9 \pm 0.2) \times 10^2\ dm^3\ mol^{-1}$, $k_3^HK_2^H = (2.8 \pm 0.2) \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$ and $K_1 = 2.5 \pm 0.1$. The similarity of the values of K_1 for both the chloro- and bromo-species is most consistent with protonation of the $\{Fe_4S_4\}^{2+}$ core. We can calculate the equilibrium constant for the simple reaction of $[NHEt_3]^+$ with X^- in MeCN [eqn. (3)]: $X = Cl^-$ $K = 2.6 \times 10^{-10}$; $X = Br^-$, $K = 1.02 \times 10^{-13}$. Thus, if the halo-group was

$$NHEt_3^- + X^- \rightleftharpoons NEt_3 + HX \quad (3)$$

the site of protonation, the $\{Fe_4S_4\}^{2+}$ core would be making the chloro-group *ca.* 10^{10} times more basic than free Cl^-, and (even more improbably) the core would have to make the bromo-ligand more basic than the chloro-residue.

Analogous studies on $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SEt)_3]^{3-}$ [$k_3 = 82.9 \pm 0.5\ s^{-1}$, $K_2 = (2.1 \pm 0.1) \times 10^2\ dm^3\ mol^{-1}$, $k_3^HK_2^H = (4.0 \pm 0.3) \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$, $K_1 = 1.2 \pm 0.1$] and $[Fe_4Se_4Cl_4]^{2-}$ [$K_2k_3 = (1.7 \pm 0.2) \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$, $k_3^HK_2^H = (1.8 \pm 0.2) \times 10^4\ dm^3\ mol^{-1}\ mol^{-1}\ s^{-1}$, $K_1 = 0.75 \pm 0.05$] show similar behaviour, indicating that protonation of these cluster cores is a general phenomenon.

We have shown that these clusters are capable of binding protons at the $\{Fe_4S_4\}^{2+}$ core by monitoring how the kinetics of a substitution reaction are perturbed by the presence of an acid, in a solvent where the acid–base characteristics can be quantified. The drawback of this kinetic approach is that we cannot define the exact position of the protonation (*i.e.* Fe or S or a face of the cluster). Attempts to obtain ^1H or ^{33}Se NMR spectroscopic confirmation of the protonated cluster have been unsuccessful, probably in part because of the broad, contact-shifted spectra associated with these clusters. However the kinetic behaviour of the seleno-cluster is revealing in defining the position of protonation of these clusters. As might be expected, the value of K_1 for $[Fe_4Se_4Cl_4]^{2-}$ is smaller than that of the analogous sulfido-complex, but only by a factor of 3.1. This relatively small effect does not reflect the expected large difference in basicity of sulfido- and seleno-species,[6] and is more consistent with the

$$-d[Fe_4S_4]/dt = \frac{\{k_0 + k_3K_2[PhS^-]_f + k_3^HK_2^HK_1[NHEt_3^+]_f/[NEt_3]_f[PhSH]_f\}[Fe_4S_4]}{1 + K_1[NHEt_3^+]_f/[NEt_3]_f} \quad (2)$$

proton binding to a HOMO containing a significant contribution from the iron centres.

Previous discussion[1,2] on the protonation of these type of biological clusters has tended to emphasise metal hydride species. However, provided protonation occurs on the $\{MFe_3S_4\}^{n+}$ core, whether it be an iron atom, the hetero-metal, M, a sulfur atom or just associated with a face of the cluster, subsequent electron transfer and further protonation could give rise to dihydrogen.

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Footnotes

† Previous studies[3,4] on iron–sulfur-based clusters have defined the dependence of the reaction rate on the ratio, $[NHEt_3^+]/[PhS^-]$ or $[NHEt_3^+]/[PhSH]$, but it is only from the studies on the halo-clusters described here that the quantitative acid-base behaviour of eqn. (4) can be defined.

$$NHEt_3^+ + PhS^- \rightleftharpoons NEt_3 + PhSH \quad (4)$$

The symbols used in the text are as follows: $[NHEt_3^+]_0$ and $[PhS^-]_0$ are the total concentrations of the respective reagents. In mixtures of the two reagents the equilibrium concentrations of the compounds are are follows. $[NHEt_3^+]_f = [NHEt_3^+]_0 - [PhS^-]_0$ and $[PhSH]_f = [NEt_3]_f$.

Hence, when $[NHEt_3^+]_0 > [PhS^-]_0$, the ratio, $[NHEt_3^+]_f/[PhSH]_f = [NHEt_3^+]_f/[NEt_3]_f$.

‡ It is important to emphasise that these reactions were studied at a constant ionic strength, $[NBu_4^N]BF_4 = 0.1$ mol dm^{-3}. In addition, the observations that: (i) the minimum value of k_{obs} occurs when $[NHEt_3^+]_0/[PhS^-]_0 = 1.0$ and (ii) the rate of the reaction depends on the ratio, $[NHEt_3^+]_f/[NEt_3]_f$ are inconsistent with the observed kinetics being due to medium or ion-pairing effects.

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