The rates of binding protons and substrates to $[Fe_4S_4Cl_4]^{2-}$ †

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The kinetics of the substitution reaction between $[Fe_4S_4Cl_4]^{2-}$ and Bu^tNC to form $[Fe_4S_4Cl_2(CNBu^t)_6]$, in the presence of $[H_2N(CH_2)_3CH_2]^+$, $[NHEt_3]^+$ or $[lutH]^+$ (lut = 2,6-dimethylpyridine) have been studied. With the weakest acid, $[H_2N(CH_2)_3CH_2]^+$ (pK_a = 21.5), Bu^tNC binds before the transfer of the proton. Analysis of the kinetics yields the rate constant for the binding of Bu^tNC to $[Fe_4S_4Cl_4]^{2-}$ ($k = 2.1 \pm 0.5 \times 10^3$ dm^3 mol^{-1} s^{-1}). With the stronger acids, $[NHEt_3]^+$ (pK_a = 18.5) and $[lutH]^+$ (pK_a = 15.4), two protons bind to the cluster. Earlier work indicated that for a thermodynamically favourable proton-transfer reaction, the rate of the first proton transfer to $[Fe_4S_4Cl_4]^{2-}$ is at least 10^4 times slower than the diffusion-controlled limit (*i.e.* $k = ca.$ 1×10^6 dm^3 mol^{-1} s^{-1}). Studies presented herein on the reactions between $[Fe_4S_4Cl_4]^{2-}$ and Bu^tNC or Br^- indicate that the rate of the second proton transfer is about 10^7 times slower than the diffusion-controlled limit (*i.e.* $k = ca.$ 1×10^3 dm^3 mol^{-1} s^{-1}). One consequence of these slow rates of proton transfer to $[Fe_4S_4Cl_4]^{2-}$ is that the rate of binding Bu^tNC or Br^- to the cluster is slower than the initial proton transfer, but faster than the second one. The results of these studies are compared with those reported earlier for the reaction of $[Fe_4S_4Cl_4]^{2-}$ with PhSH in the presence of $[lutH]^+$, where the poor nucleophilicity of the thiol results in PhSH binding to the cluster only after diprotonation. The possible relevance of these studies to the mechanisms of substrate binding and transformation at both synthetic and natural Fe–S clusters is discussed.

Introduction

It is now well established that Fe–S-based clusters are the substrate-binding sites in enzymes such as nitrogenases,[1] hydrogenases[2] and certain hydratases.[3,4] However, the factors which control how rapidly molecules and ions (hereafter referred to as substrates) bind to Fe–S clusters are still largely undefined. As we, and others, have pointed out before,[5,6] the binding of substrates to intact Fe–S-based clusters is difficult to study directly because these reactions are often associated with small spectroscopic changes. Herein, we focus on the relative rates of substrate and proton binding to $[Fe_4S_4Cl_4]^{2-}$, which allows the order that these species bind to the cluster to be identified, and the consequences for substrate binding and transformation at Fe–S clusters.

Establishing the order that substrates and protons bind to Fe–S clusters is crucial in understanding the reactivity of hydrogenases and nitrogenases. For example, in the absence of dinitrogen, the nitrogenases reduce protons to dihydrogen.[7] Introduction of dinitrogen results in the formation of ammonia and a concomitant decrease in dihydrogen production. However, proton reduction is never entirely supressed, even at high pressures of dinitrogen,[8] and all nitrogenases evolve dihydrogen during nitrogen fixation. Analogous behaviour has been observed in the transformation of C_2H_2 into C_2H_4 by $[Fe_4S_4(SPh)_4]^{3-}$ in the presence of weak acids.[9–11] Dihydrogen production always occurs, even at high concentrations of C_2H_2. In order to understand the factors controlling the reduction of protons on the one hand and reduction of substrates on the other, at both synthetic and natural Fe–S-based clusters, it is crucial to understand the fundamental chemistry of Fe–S clusters under conditions where both protons and substrates can bind.

Experimental

All manipulations were routinely performed under an atmosphere of dinitrogen using Schlenk and syringe techniques as appropriate. The compounds $[NEt_4]SPh$,[12] $[lutH]BPh_4$,[11] $[NHEt_3]BPh_4$,[13] $[H_2N(CH_2)_3CH_2]BPh_4$,[14] $[Fe_4S_4Cl_2(CNBu^t)_6]$[15] and $[NEt_4]_2[Fe_4S_4Cl_4]$[12] were prepared by the literature methods. Bu^tNC (Aldrich) was stored at 5 °C under dinitrogen and used as received. $[Et_4N]Cl$, $[Et_4N]Br$ and $[Et_4N]CN$ (Aldrich) were used without purification, but dried under vacuum at 80 °C for 24 h prior to use. Solvents were dried over the appropriate drying agents and distilled immediately prior to use; MeCN (CaH_2), thf (sodium–benzophenone), diethyl ether (sodium).

$[NDEt_3]BPh_4$ was prepared by a method analogous[13] to that used for $[NHEt_3]BPh_4$, except MeOD and D_2O were used in place of MeOH and H_2O, respectively. The presence of N–D was evidenced in the IR spectrum of $[NDEt_3]BPh_4$ by a band at $2330 cm^{-1}$. The ^1H NMR spectrum of $[NHEt_3]BPh_4$ in CD_3CN shows a broad resonance δ 2.5 ppm. The ^1H NMR spectrum of $[NDEt_3]BPh_4$ shows no resonance in this position (although the rest of the spectrum is identical). Comparison of the spectra of $[NHEt_3]BPh_4$ and $[NDEt_3]BPh_4$ indicates at least 95% N–D labelling in the latter.

Kinetic studies

All kinetic studies were performed using an Applied Photophysics SX.18MV stopped-flow spectrophotometer, modified to handle air-sensitive solutions. The temperature was maintained at 25.0 ± 0.1 °C using a Grant LT D6G thermostatted recirculating pump.

All solutions were prepared under an atmosphere of dinitrogen and transferred by gas-tight, all-glass syringes into the stopped-flow spectrophotometer. Solutions of mixtures of reagents were prepared from freshly prepared stock solutions and used within 1 h. Kinetics were studied under pseudo-first-
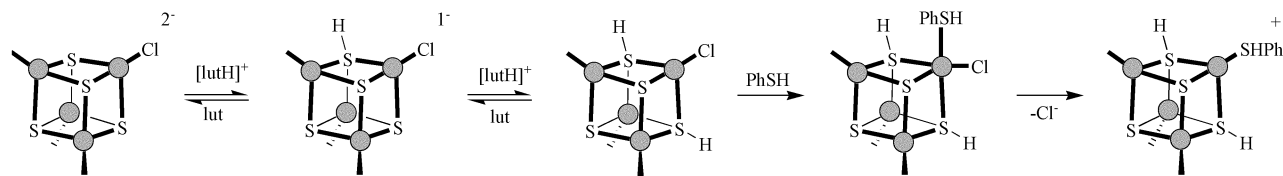

Fig. 1 The sequence of elementary steps in the substitution of the first chloro ligand of $[Fe_4S_4Cl_4]^{2-}$ by PhSH in the presence of $[lutH]^+$ in MeCN.

order conditions with all reagents in a large excess (>10-fold) over the concentration of the cluster.

For all the reactions described in this paper, under all conditions, the absorbance–time curves were excellent fits to a single exponential. For the reactions with an excess of Bu^tNC, the initial absorbance corresponds to $[Fe_4S_4Cl_4]^{2-}$ and the final absorbance to $[Fe_4S_4Cl_2(CNBu^t)_6]$. The reaction between $[Fe_4S_4Cl_4]^{2-}$ and Br^- is an equilibrium reaction and, consequently, the final absorbance depends on the relative concentrations of Cl^- and Br^- in the mixture. The initial absorbance corresponds to $[Fe_4S_4Cl_4]^{2-}$ and the final absorbance corresponds to a mixture of $[Fe_4S_4Cl_4]^{2-}$ and what we (reasonably) assume is $[Fe_4S_4BrCl_3]^{2-}$.

In all cases, the absorbance–time curves were analysed using the Applied Photophysics computer programme and the values of the observed rate constants (k_{obs}) were obtained from the analysis. The dependence of the rate of reaction on the concentrations of the various reagents was determined by the usual graphical analyses, as shown throughout the text.

Results and discussion

Our earlier studies[16] showed that in the presence of an excess of $[lutH]^+$ (lut = 2,6-dimethylpyridine; $pK_a = 15.4$), the kinetics of the substitution of the chloro ligands in $[Fe_4S_4Cl_4]^{2-}$ by PhSH involve diprotonation of the cluster prior to binding of PhSH. The substitution is completed by dissociation of chloride (Fig. 1). Thus, both proton transfers to $[Fe_4S_4Cl_4]^{2-}$ must be faster than the rate of binding PhSH.

Although the rate[14] of the first thermodynamically favourable proton transfer to $[Fe_4S_4Cl_4]^{2-}$ has been estimated ($k = ca.\ 1 \times 10^6$ dm^3 mol^{-1} s^{-1}), the rate of the second proton transfer has not been measured. In this paper, we report studies on the kinetics of the reactions between $[Fe_4S_4Cl_4]^{2-}$ and Br^- or Bu^tNC in the presence of $[H_2N(CH_2)_3CH_2]^+$, $[NHEt_3]^+$ or $[lutH]^+$, which allow, for the first time, determination of: (i) the rate of proton transfer to $[Fe_4S_3(SH)Cl_4]^-$ and (ii) comparison of the rates of binding Bu^tNC to $[Fe_4S_4Cl_4]^{2-}$ and $[Fe_4S_3(SH)Cl_4]^-$.

Reactions of $[Fe_4S_4Cl_4]^{2-}$ with Bu^tNC in the presence of acids

The rate of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Bu^tNC (eqn. 1), in the absence of Cl^-, exhibits[17] first-order dependences on the concentrations of cluster and Bu^tNC with $k = 11.5 \pm 0.5$ dm^3 mol^{-1} s^{-1}.

$$[Fe_4S_4Cl_4]^{2-} + 6Bu^tNC \longrightarrow [Fe_4S_4Cl_2(CNBu^t)_6] + 2Cl^- \quad (1)$$

In the presence of $[H_2N(CH_2)_3CH_2]^+$ ($pK_a = 21.5$), the rate of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Bu^tNC increases (Fig. 2). The reaction exhibits a first order dependence on the concentration of the cluster and Bu^tNC, but the dependence on the concentration of acid is complicated. At low concentrations of $[H_2N(CH_2)_3CH_2]^+$, the reaction is first order in acid and, at high

concentrations of $[H_2N(CH_2)_3CH_2]^+$, the reaction is independent of acid. The data can be fitted to the rate law shown in eqn. 2.

Eqn. 2 is analogous to that observed in earlier studies[14] on the reaction between $[Fe_4S_4Cl_4]^{2-}$ and PhS^- in the presence of $[H_2N(CH_2)_3CH_2]^+$, where it was shown that because $[H_2N(CH_2)_3CH_2]^+$ is such a weak acid, protonation is slow and hence PhS^- binds initially to the cluster. Only after PhS^- has bound does the cluster protonate, and substitution ensues.

Eqn. 2 is consistent with the mechanism shown in Fig. 3. Initial binding of Bu^tNC to the cluster is followed by protonation of the cluster by $[H_2N(CH_2)_3CH_2]^+$. Dissociation of Cl^- completes the substitution. Assuming that $[Fe_4S_4Cl_4(CNBu^t)]^{2-}$ is a steady-state intermediate, the rate law shown in eqn. 3 can be derived from this mechanism. Comparison of eqn. 2 and 3 gives $k_1^{BuNC}k_3^{BuNC}/(k_{-1}^{BuNC} + k_3^{BuNC}) = 11.5 \pm 0.5$ dm^3 mol^{-1} s^{-1}, $k_1^{BuNC} = 2.1 \pm 0.5 \times 10^3$ dm^3 mol^{-1} s^{-1} and $k_2^{BuNC}/(k_{-1}^{BuNC} + k_3^{BuNC}) = 0.85 \pm 0.05$ dm^3 mol^{-1}. Contrasting these results with the corresponding values[14] from the reaction with PhS^- shows that PhS^- binds 90.5 times more rapidly than Bu^tNC to $[Fe_4S_4Cl_4]^{2-}$.

$$\frac{-d[Fe_4S_4Cl_4^{2-}]}{dt} = \qquad\qquad (3)$$

$$\frac{k_1^{BuNC}\{k_3^{BuNC} + k_2^{BuNC}[H_2N(CH_2)_3CH_2^+]\}[Bu^tNC][Fe_4S_4Cl_4^{2-}]}{k_{-1}^{BuNC} + k_3^{BuNC} + k_2^{BuNC}[H_2N(CH_2)_3CH_2^+]}$$

The kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Bu^tNC in the presence of $[NHEt_3]^+$ or $[lutH]^+$ are similar to those in the presence of $[H_2N(CH_2)_3CH_2]^+$: a first order dependence on both cluster and Bu^tNC and a non-linear dependence on the concentration of acid. The data is illustrated in Fig. 2 and the rate laws presented in eqn. 4. Analysis of the data gives $k_4^{BuNC} = (8.0 \pm 0.7) \times 10^3$ dm^3 mol^{-1} s^{-1} and $k_5^{BuNC}/k_{-4}^{BuNC} = 0.31 \pm 0.05$ dm^3 mol^{-1} for the studies with $[NHEt_3]^+$, and $k_4^{BuNC} = (8.0 \pm 0.7) \times 10^3$ dm^3 mol^{-1} s^{-1} and $k_5^{BuNC}/k_{-4}^{BuNC} = 0.60 \pm 0.05$ dm^3 mol^{-1} for the studies with $[lutH]^+$.

$$\frac{-d[Fe_4S_4Cl_4^{2-}]}{dt} = \frac{k_4^{BuNC}k_5^{BuNC}[NHR_3^+][Bu^tNC][Fe_4S_4Cl_4^{2-}]}{k_{-4}^{BuNC} + k_5^{BuNC}[NHR_3^+]} \quad (4)$$

Although the kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Bu^tNC are similar for all three acids, it is notable that the reaction is faster in the presence of $[NHEt_3]^+$ and $[lutH]^+$ than with $[H_2N(CH_2)_3CH_2]^+$. Furthermore, at high concentrations of $[NHEt_3]^+$ and $[lutH]^+$, the rates (k_4^{BuNC}) are identical, and different to the limiting value observed with $[H_2N(CH_2)_3CH_2]^+$. In order to interpret these observations, it is necessary to consider earlier studies on the substitution reactions of $[Fe_4S_4Cl_4]^{2-}$ in the presence of acid.

All previous studies on the substitution reactions of $[Fe_4S_4Cl_4]^{2-}$ in the presence of $[NHEt_3]^+$ have involved replace-
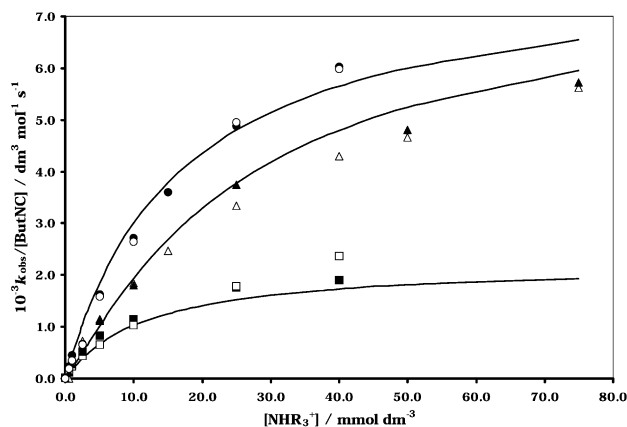
$$\frac{-d[Fe_4S_4Cl_4^{2-}]}{dt} = \frac{\{(11.5 \pm 0.5) + (1.9 \pm 0.4) \times 10^3[H_2N(CH_2)_3CH_2^+]\}[Bu^tNC][Fe_4S_4Cl_4^{2-}]}{1 + (0.85 \pm 0.05)[H_2N(CH_2)_3CH_2^+]} \quad (2)$$

Fig. 2 Kinetics for the reaction of $[Fe_4S_4Cl_4]^{2-}$ (0.15 mmol dm^{-3}) with an excess of Bu^{t}NC in the presence of acids, in MeCN at 25.0 °C. Dependence on the concentration of acid. Data corresponds to $[H_2\overline{N(CH_2)_3CH_2}]^+$: [Bu^{t}NC] = 13.5 (■), 27.0 mmol dm^{-3} (□); $[NHEt_3]^+$: [Bu^{t}NC] = 13.5 (▲), 27.0 mmol dm^{-3} (△); [lutH]$^+$: [Bu^{t}NC] = 13.5 (●), 27.0 mmol dm^{-3} (○). Curves drawn are defined by eqn. 2 $\{[H_2\overline{N(CH_2)_3CH_2}]^+\}$ or eqn. 4 $\{[NHEt_3]^+$ and [lutH]$^+\}$, using the values given in the text.

ment of the chloro ligands by thiols, thus necessitating the addition of thiolates to the reaction medium. In MeCN, mixtures of thiolates and an excess of $[NHEt_3]^+$ result in the protolytic equilibrium shown in eqn. 5 and the formation of NEt$_3$. Consequently, studies in the presence of thiolate are typically performed with $[NHEt_3^+]/[NEt_3] = 0$–20. In the studies presented herein, the nucleophiles (Bu^{t}NC and Br$^-$) are insufficiently basic to be protonated and thus the ratio range is much larger: $[NHEt_3^+]/[NEt_3] = [NHEt_3^+]/[Fe_4S_4Cl_4^{2-}] = 0$–750.

$$[NHEt_3]^+ + RS^- \rightleftharpoons NEt_3 + RSH \qquad (5)$$

Using the pK_as of $[Fe_4S_3(SH)Cl_4]^-$ (pK_a = 18.8)[18] and $[Fe_4S_2(SH)_2Cl_4]$ (pK_a = 16.6),[19] we can calculate that at all concentrations of [lutH]$^+$, the cluster is doubly protonated, and even with the weaker acid $[NHEt_3]^+$, diprotonation is extensive (although not exclusive) at all concentrations of acid. For example, when $[NHEt_3^+]$ = 5.0 mmol dm^{-3}, 32% of the cluster is diprotonated and when $[NHEt_3^+]$ = 75 mmol dm^{-3}, 84% of the cluster is diprotonated.

The kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Bu^{t}NC in the presence of [lutH]$^+$ or $[NHEt_3]^+$ (eqn. 4) indicate the addition of a single proton. However, this cannot correspond to the transfer of the first proton. Earlier work[14] indicated that with [lutH]$^+$ or $[NHEt_3]^+$, the rate of the first proton transfer to $[Fe_4S_4Cl_4]^{2-}$ falls in the range, $2 \times 10^5 \leq k \leq 4.8 \times 10^6$ dm^3 mol^{-1} s^{-1}. Thus, under all conditions employed in the studies with Bu^{t}NC, the first proton is transferred within the dead time of the stopped-flow apparatus. The dependence on the concentration of acid observed in these experiments must therefore correspond to the transfer of the second proton.

The mechanism shown in Fig. 4 is consistent with the arguments presented above and the rate law (eqn. 4). After transfer of the first proton, Bu^{t}NC binds and then the second proton is transferred, finally, dissociation of the chloro ligand completes the substitution. Clearly, addition of the second proton must be slower than binding of Bu^{t}NC, and hence we can estimate a limit for the transfer of the second proton, $k_5^{BuNC} \leq 8 \times 10^3$ dm^3 mol^{-1} s^{-1}. The transfer of the second proton is thus at least 100 times slower than the transfer of the first. This correlates with the difference in pK_as of $[Fe_4S_3(SH)Cl_4]^-$ and $[Fe_4S_2(SH)_2Cl_4]$ (ΔpK_a = 2.2).[19] The transfer of the second proton is at least 10^6 times slower than the diffusion-controlled limit, despite the process being thermodynamically favourable. In the studies with Br$^-$ described below, the slowness of this proton-transfer reaction is confirmed.

Previously, we have suggested[14] that bond-length reorganisation within the cluster is the major intrinsic barrier in proton transfer to $[Fe_4S_4Cl_4]^{2-}$, and consistent with this proposal we have shown that the rate of proton transfer from $[H_2\overline{N(CH_2)_3CH_2}]^+$ to $[Fe_4S_4Cl_4(SC_6H_4R\text{-}4)]^{3-}$ is facilitated by electron-withdrawing R-substituents.[20] It is to be expected that the rate of proton transfer from $[NHR_3]^+$ to $[Fe_4S_3(SH)Cl_4]^-$ is also slow because of bond-length reorganisation within the cluster. Intuitively, it seems likely that bond-length changes are energetically more demanding for the second protonation than the first, resulting in a slower rate of proton transfer.

From the kinetics of the reactions between Bu^{t}NC and $[Fe_4S_4Cl_4]^{2-}$ in the presence of $[H_2\overline{N(CH_2)_3CH_2}]^+$ and [lutH]$^+$, we can calculate that the rate of binding of Bu^{t}NC to $[Fe_4S_3(SH)Cl_4]^{1-}$ ($k_4^{BuNC} = 8.0 \times 10^3$ dm^3 mol^{-1} s^{-1}) is ca. 4 times faster than the rate of binding to $[Fe_4S_4Cl_4]^{2-}$ ($k_1^{BuNC} = 2.1 \times 10^3$ dm^3 mol^{-1} s^{-1}). Whilst this is not a large effect, it is in line with earlier studies. It has been argued[20] that as a substrate approaches the anionic cluster there is an unfavourable build-up of negative charge in the transition state, which is dissipated by electron-withdrawing substituents. Since protonation of the cluster decreases the negative charge on the cluster, it seems reasonable that protonation will also facilitate substrate binding.

Interestingly, the values k_5^{BuNC}/k_{-4}^{BuNC} depend on the identity of the acid (with $[NHEt_3]^+$, k_5^{BuNC}/k_{-4}^{BuNC} = 0.31 dm^3 mol^{-1} and with [lutH]$^+$, k_5^{BuNC}/k_{-4}^{BuNC} = 0.60 dm^3 mol^{-1}). Since k_{-4}^{BuNC} must be constant, the rate of the second proton transfer to the cluster depends on acid. The small difference does not reflect the large difference in pK_as of $[NHEt_3]^+$ and [lutH]$^+$ (ΔpK_a = 3.1) and is probably due to different steric interactions with each acid during the transfer of the proton.

Reactions of $[Fe_4S_4Cl_4]^{2-}$ with Br$^-$ in the presence of $[NHEt_3]^+$ or [lutH]$^+$

Studies on the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Br$^-$ in the presence of $[NHEt_3]^+$ or [lutH]$^+$ give further insight into the protonation of the cluster. In the absence of acid, the reaction of $[Fe_4S_4Cl_4]^{2-}$ with Br$^-$ to form $[Fe_4S_4Cl_3Br]^{2-}$ is an
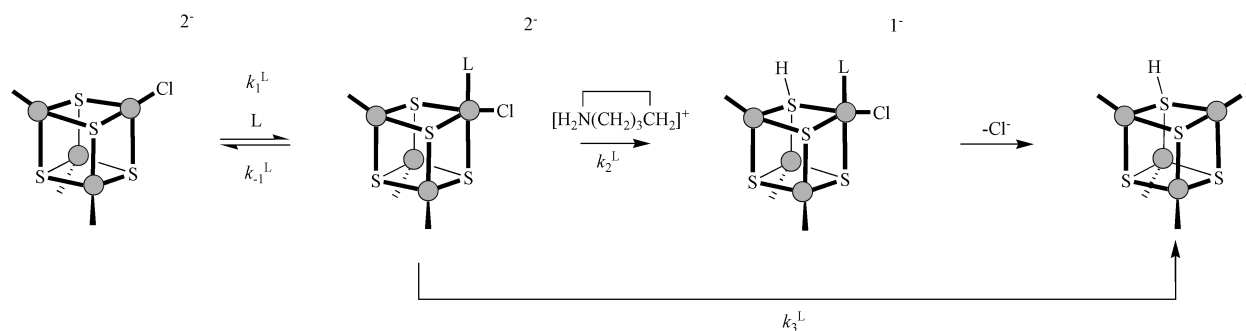
Fig. 3 The mechanism of the substitution of the first chloro ligand of $[Fe_4S_4Cl_4]^{2-}$ by Bu^{t}NC in the presence of $[H_2\overline{N(CH_2)_3CH_2}]^+$ in MeCN.
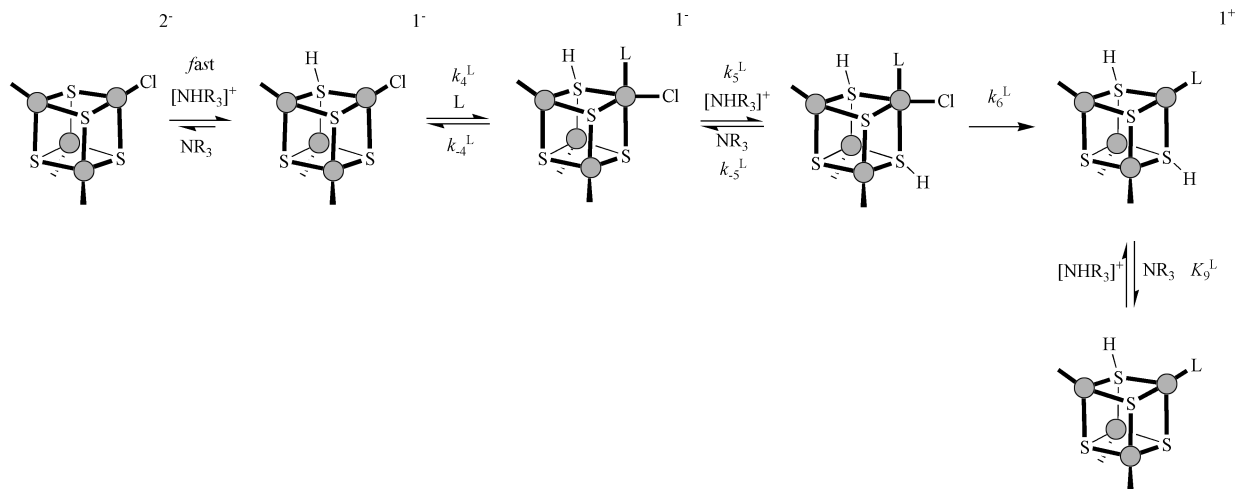
Fig. 4 The mechanism of the substitution of the first chloro ligand of $[Fe_4S_4Cl_4]^{2-}$ by L = Bu^{t}NC or Br$^-$ in the presence of $[NHEt_3]^+$ or $[lutH]^+$ in MeCN.

equilibrium reaction. Thus, when studied at a single wavelength ($\lambda = 320$ nm) using stopped-flow spectrophotometry, the magnitude of the absorbance change increases as the concentration of Br$^-$ increases. The equilibrium nature of the reaction is reflected in the rate law (eqn. 6).[21]

$$\frac{-d[Fe_4S_4Cl_4^{2-}]}{dt} = 5.1 \pm 0.6 \times 10^2[Br^-] + 5.2 \pm 0.4 \times 10^3[Cl^-] \quad (6)$$

Eqn. 6 is consistent with a mechanism involving two coupled equilibria (Fig. 5), in which binding of Br$^-$ to the cluster to form
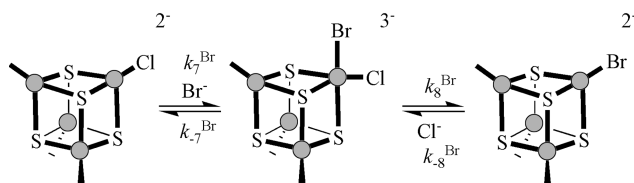

Fig. 5 The mechanism of substitution of the first chloro ligand in $[Fe_4S_4Cl_4]^{2-}$ by Br$^-$ in MeCN.

$[Fe_4S_4Cl_4Br]^{3-}$ is followed by dissociation of chloride.[22] Analogous mechanisms have been proposed for the substitution reactions of a variety of other Fe–S-based clusters.[1,6,14,16–20] The rate law for the mechanism in Fig. 5 is shown in eqn. 7, which simplifies to the same form as eqn. 6 when $k_{-7}^{Br} \gg k_7^{Br}[Br^-]$. Comparison of eqn. 6 and 7 gives $k_{-8}^{Br} = 5.2 \pm 0.4 \times 10^3$ dm^3 mol^{-1} s^{-1} and $k_7^{Br}k_8^{Br}/k_{-7}^{Br} = 5.1 \pm 0.6 \times 10^2$ dm^3 mol^{-1} s^{-1}.

$$\frac{-d[Fe_4S_4Cl_4^{2-}]}{dt} = \frac{k_7^{Br}k_8^{Br}[Br^-]}{k_{-7}^{Br} + k_7^{Br}[Br^-]} + k_{-8}^{Br}[Cl^-] \quad (7)$$

Studies on the kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Br$^-$ in the presence of $[NHEt_3]^+$ or $[lutH]^+$ show that the rate is independent of the concentration of Br$^-$ and exhibits a non-linear dependence on the concentration of acid (Fig. 6). The addition of Cl$^-$ had only a minor effect on the rate of the reaction. Thus, when $[lutH^+]$ or $[NHEt_3^+] = 10$ mmol dm^{-3}, k_{obs} = 30 ± 5 s^{-1}. Whilst the data indicate a trend in which the rate increases with increasing concentration of Cl$^-$, we attribute this to salt effects.

The kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ and Br$^-$ in the presence of $[NHEt_3]^+$ or $[lutH]^+$ are indistinguishable, with both systems described by the same rate law (eqn. 8). The mechanism of the reaction is shown in Fig. 4. As we have discussed above, for the studies with Bu^{t}NC, initial protonation of $[Fe_4S_4Cl_4]^{2-}$ by $[NHEt_3]^+$ or $[lutH]^+$ is rapid and, under the
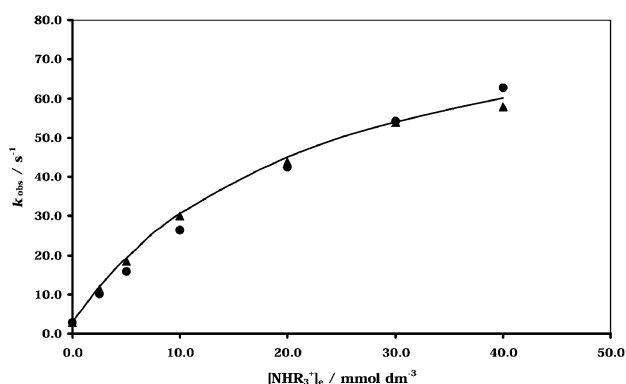

Fig. 6 Kinetics for the reaction of $[Fe_4S_4Cl_4]^{2-}$ (0.15 mmol dm^{-3}) with an excess of Br$^-$ in the presence of acids, in MeCN at 25.0 °C. Dependence on the concentration of acid. Data corresponds to: $[NHEt_3]^+$, $[Br^-] = 5.0$ mmol dm^{-3} (▲); $[lutH]^+$, $[Br^-] = 5.0$ mmol dm^{-3} (●). Curves drawn are defined by eqn. 8.

$$\frac{-d[Fe_4S_4Cl_4^{2-}]}{dt} = \frac{3.0 \pm 0.4 \times 10^3[NHR_3^+][Fe_4S_4Cl_4^{2-}]}{1 + 35 \pm 7[NHR_3^+]} \quad (8)$$

conditions employed in this study, complete within the dead time of the stopped-flow apparatus. In addition, since the rate is independent of the concentration of Br$^-$, binding of Br$^-$ has presumably occurred within the dead time of the stopped-flow apparatus. Thus, the kinetics correspond to the reactivity of $[Fe_4S_3(SH)Cl_4Br]^{2-}$.

Eqn. 8 is consistent with protonation of $[Fe_4S_3(SH)Cl_4Br]^{2-}$ being rate limiting at low concentrations of $[NHEt_3]^+$ or $[lutH]^+$. The rate of protonation with both $[NHEt_3]^+$ (pK_a = 18.5)[23] and $[lutH]^+$ (pK_a = 15.4)[23] is identical ($k_5^{Br} = 3.0 \pm 0.4 \times 10^3$ dm^3 mol^{-1} s^{-1}) as expected for thermodynamically favourable proton transfer reactions to $[Fe_4S_3(SH)Cl_4Br]^{2-}$. The value of k_5^{Br} is similar to that estimated in the reactions of $[Fe_4S_4Cl_4]^{2-}$ with Bu^{t}NC. At high concentrations of acid, dissociation of Cl$^-$ from $[Fe_4S_2(SH)_2Cl_4Br]^-$ becomes rate limiting ($k_6^{Br} = 86 \pm 4$).

It is anticipated that dissociation of Cl$^-$ from $[Fe_4S_2(SH)_2Cl_4Br]^-$ results in a decrease in the basicity of the cluster and consequent proton release, as shown in Fig. 4. Knowing that for $[lutH]^+$, pK_a = 15.4, and estimating that for $[Fe_4S_2(SH)_2Cl_3Br]$, pK_a = 16.6 (the same as that of $[Fe_4S_2(SH)_2Cl_4]$), we can calculate k_9^{lutH} = 0.063. Thus, in the presence of $[lutH]^+$, the product is exclusively diprotonated (> 99%) $[Fe_4S_2(SH)_2Cl_3Br]$ at all concentrations of acid. In the studies with $[NHEt_3]^+$, k_9^{NHEt3} = 79.4, and the product is an equilibrium mixture containing

approximately equal concentrations of [Fe₄S₂(SH)₂Cl₃Br] and [Fe₄S₃(SH)Cl₃Br]⁻ at the concentrations of acid used.

Isotope effects

In the reactions between $[Fe_4S_4Cl_4]^{2-}$ and Bu^{t}NC or Br$^-$, binding of the second proton is, under certain conditions, rate limiting. We have studied the kinetics of these reactions using [NDEt]₃⁺, and the results are shown in Fig. 7. Clearly, the

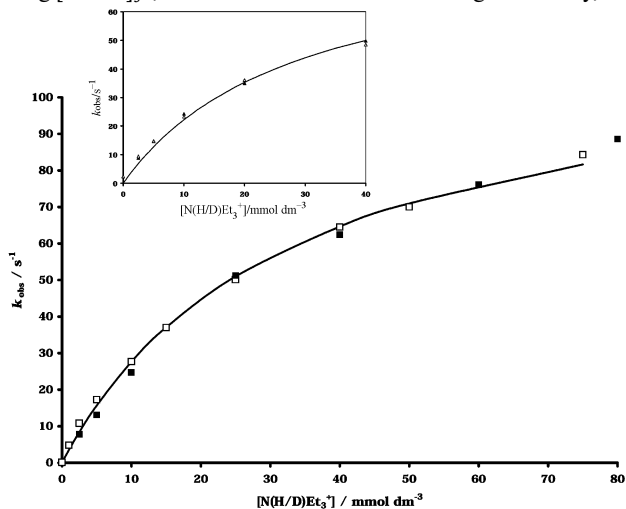**Fig. 7** The kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ (0.15 mmol dm⁻³) and Bu^{t}NC (13.5 mmol dm⁻³) in the presence of [NHEt₃]⁺ (■) or [NDEt₃]⁺ (□). Curve drawn is defined by eqn. 4. Inset: the kinetics of the reaction between $[Fe_4S_4Cl_4]^{2-}$ (0.15 mmol dm⁻³) and Br⁻ (5.0 mmol dm⁻³) in the presence of [NHEt₃]⁺ (▲) or [NDEt₃]⁺ (Δ). Both reactions were studied in MeCN at 25.0 °C. Curve drawn is defined by eqn. 8.

kinetics in the presence of [NHEt₃]⁺ and [NDEt₃]⁺ for both reactions are indistinguishable: there is no discernable primary isotope effect in these reactions.

The small isotope effect in these reactions is perhaps not too surprising. Earlier work[20] has shown that the rate of proton transfer is modulated by the electron-withdrawing and -accepting properties of the terminal ligands on the cluster. This indicates that bond-length movement is an appreciable barrier to proton transfer and the effect on the rate of changing from deuterium to hydrogen in the acid is small in comparison.

The order that protons and substrates bind to Fe–S clusters

The studies reported in this paper, together with our earlier investigations, reveal some important aspects concerning the rates of proton transfer and substrate binding to Fe–S clusters, and the ordering of these fundamental reactions.

As we have noted above, all our earlier studies involved thiols as the nucleophile. The kinetics of the reactions with [Fe₄S₄-Cl₄]²⁻ and PhSH in the presence of an excess of [lutH]⁺ are consistent with a mechanism involving initial diprotonation of the cluster followed by binding of PhSH and then dissociation of the Cl⁻ (Fig. 1). This order is what one might anticipate from common experience of the reactivity of complexes. Thus, when the rates of proton transfer are diffusion controlled, diprotonation would be expected to precede the slower binding of the nucleophile. However, the rates of proton transfer to Fe–S clusters are appreciably slower than the diffusion-controlled limit, even for thermodynamically favourable reactions. The rate of the first proton transfer[14] to $[Fe_4S_4Cl_4]^{2-}$ is $k = ca.$ 1×10^6 dm³ mol⁻¹ s⁻¹, whilst the addition of the second proton is even slower, $k = ca.$ 1×10^3 dm³ mol⁻¹ s⁻¹. Consequently, with nucleophiles such as Bu^{t}NC or Br⁻ the rate of substrate binding can exceed the second protonation step. The rates that protons and substrates bind to Fe–S clusters could have important repercussions on the way enzymes containing these sites operate.

In the enzyme nitrogenase, the active site is an Fe–S-based cluster of composition MoFe₇S₉, referred to as FeMo-cofactor.[7,24] In addition to binding dinitrogen and other substrates of the enzyme, the cluster also binds protons and, in the absence of other substrates, all the available electrons go into producing dihydrogen. When dinitrogen binds to the cofactor, electrons are diverted away from dihydrogen production and into ammonia formation. However, even at high pressures of dinitrogen, proton reduction cannot be entirely suppressed. Although the binding of protons and dinitrogen to FeMo-cofactor have been investigated by calculation,[25–28] the results from the studies described herein give some important insights into the reactivity of the cluster.

If the cofactor binds two successive protons prior to the binding of the substrate, then dihydrogen production can occur, by coupling of the two hydrogen atoms, before the substrate binds. An easy mechanism to suppress dihydrogen production is to bind the substrate before the release of dihydrogen. Clearly, if the rates of proton-transfer reactions to the cluster were diffusion controlled, the substrate could not bind before either protonation step. However, proton-transfer reactions in Fe–S clusters can be slow.

The initial binding of a proton to the cofactor could be advantageous. As we have shown herein, protonation of [Fe₄S₄-Cl₄]²⁻ facilitates the binding of Bu^{t}NC (albeit only by a factor of 4), since it diminishes the build-up of negative charge as the substrate approaches the cluster. However, with only one proton bound to the cofactor, production of dihydrogen is not possible. Having bound one proton and the substrate to the cofactor, further protonation can now occur. The second protonation of the cluster core is very slow ($k = ca.$ 1×10^3 dm³ mol⁻¹ s⁻¹). Thus, there is a good chance that the bound substrate protonates faster than the cluster core. In particular, if the bound substrate contains a lone pair of electrons, a thermodynamically favourable protonation of the substrate is expected to be diffusion controlled.

In all enzymes where transformation of the substrate involves coupled proton- and electron-transfer reactions, reduction of protons to dihydrogen is invariably a complicating side reaction. The studies reported in this paper indicate that FeMo-cofactor could suppress dihydrogen production, at least in part, because proton transfer to the cluster (dihydrogen-producing site) is slow, thus allowing the substrate to bind before binding of the second proton and the subsequent production of dihydrogen. Furthermore, because protonation of the cluster is slow, a lone pair of electrons on the bound substrate can be the kinetically favoured site of protonation.

References

1 D. J. Evans, R. A. Henderson, B. E. Smith, in *Bioinorganic Catalysis*, ed. J. Reedijk and E. Bouwman, Marcel Dekker, New York, 1999, ch. 7; and refs. therein.

2 M. Frey, *Struct. Bonding*, 1998, **90**, 97 and refs. therein.

3 D. H. Flint and R. M. Allen, *Chem. Rev.*, 1996, **96**, 2315 and refs. therein.

4 R. H. Holm, P. Kennepohl and E. I. Solomon, *Chem. Rev.*, 1996, **96**, 2239 and refs. therein.

5 K. L. C. Gronberg, C. A. Gormal, M. C. Durrant, B. E. Smith and R. A. Henderson, *J. Am. Chem. Soc.*, 1998, **120**, 10613.

6 R. A. Henderson and K. E. Oglieve, *J. Chem. Soc., Dalton Trans.*, 1993, 1467.

7 B. K. Burgess and D. J. Lowe, *Chem. Rev.*, 1996, **96**, 2983 and refs. therein.

8 F. F. Simpson and R. H. Burris, *Science*, 1984, **224**, 1095.

9 R. S. McMillan, J. Renaud, J. G. Reynolds and R. H. Holm, *J. Inorg. Biochem.*, 1979, **11**, 213.

10 L. J. Laughlin and D. Coucouvanis, *J. Am. Chem. Soc.*, 1995, **117**, 3118.

11 K. L. C. Gronberg, R. A. Henderson and K. E. Oglieve, *J. Chem. Soc., Dalton Trans.*, 1998, 3093.

12 R. E. Palermo, P. P. Power and R. H. Holm, *Inorg. Chem.*, 1982, **21**, 173.

13 J. R. Dilworth, R. A. Henderson, P. Dahlstrom, T. Nicholson and J. A. Zubieta, *J. Chem. Soc., Dalton Trans.*, 1987, 529.
14 R. A. Henderson and K. E. Oglieve, *J. Chem. Soc., Dalton Trans.*, 1999, 3927.
15 J. A. Weigel, K. K. P. Srivastava, E. P. Day, E. Munck and R. H. Holm, *J. Am. Chem. Soc.*, 1990, **112**, 8015.
16 R. A. Henderson and K. E. Oglieve, *J. Chem. Soc., Dalton Trans.*, 1998, 1731.
17 R. A. Henderson and A. J. Dunford, submitted for publication.
18 K. L. C. Gronberg and R. A. Henderson, *J. Chem. Soc., Dalton Trans.*, 1996, 3667.
19 V. R. Almeida, C. A. Gormal, K. L. C. Gronberg, R. A. Henderson, K. E. Oglieve and B. E. Smith, *Inorg. Chim. Acta*, 1999, **291**, 212.
20 A. J. Dunford and R. A. Henderson, *Chem. Commun.*, 2002, 360.

21 J. H. Espenson, *Chemical Kinetics and Reaction Mechanisms*, McGraw-Hill, New York, 1981, p. 42.
22 R. G. Wilkins, *Kinetics and Mechanism of Reactions of Transition Metal Complexes*, VCH, Weinheim, 2nd edn., 1991, p. 34.
23 K. Izutsu, *Acid-Base Dissociation Constants in Dipolar Aprotic Solvents*, Blackwell Scientific, Oxford, 1990.
24 B. E. Smith, M. C. Durrant, S. A. Fairhurst, C. A. Gormal, K. L. C. Gronberg, R. A. Henderson, S. K. Ibrahim, T. Le Gall and C. Pickett, *Coord. Chem. Rev.*, 1999, **185–186**, 669 and refs. therein.
25 I. Dance, *Chem. Commun.*, 1997, 165.
26 K. K. Stavner and M. C. Zerner, *Int. J. Quantum Chem.*, 1998, **70**, 1159.
27 M. C. Durrant, *Inorg. Chem. Commun.*, 2001, **4**, 60.
28 T. H. Rod and J. K. Nørskov, *J. Am. Chem. Soc.*, 2000, **122**, 12751.