The Acid-Base Properties, Hydrolytic Mechanism, and Susceptibility to \( \text{O}_2 \) Oxidation of \( \text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}} \) Clusters

(ferredoxins/high potential iron protein/iron-sulfur cluster compounds/acid labile sulfur/hydrolysis)

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ABSTRACT The iron–sulfur cluster compounds \( \text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}} \) [where \( \text{SR} = -\text{SCH}_3, -\text{S}-(\text{CH}_2)_n \), and \( -\text{S}-(\text{CH}_2)_n-\text{CH}-(\text{CH}_3) \)] have been found to represent the base species of weak acids of \( \text{pK}_a \) comparable to that of carboxylic acids. The acid species \( \text{Fe}_4\text{S}_4(\text{SR})_4\text{H}^+ \) is most subject to reaction with \( \text{O}_2 \) and to acid-catalyzed solvolysis, while the base species \( \text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}} \) most readily undergoes ligand exchange. The kinetics for hydrolysis of the isobutyl mercaptide cluster salt has been investigated in detail and a mechanism involving the stepwise process

\[
\begin{align*}
\text{H}^+ & \quad \text{Fe-S-Fe} \quad \text{Fe-SH-Fe} \\
\end{align*}
\]

has been proposed. The importance of the acid–base equilibria in determining the reactivity of the iron–sulfur clusters and its possible importance as a factor in the determination of the potentials of ferredoxins and high potential iron protein are discussed.

Ferredoxins and other electronically similar non-heme iron–sulfur proteins serve as electron carriers in various biological redox reactions (1, 2). These are characterized by one electron transfers which can be represented by the formalism of Carter (3) (Eq. 1). The recently demonstrated existence of “super-reduced” high potential iron protein (HIPPI-pro-red) (4), and

\[
[\text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}}] \rightleftharpoons [\text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}}] \rightleftharpoons [\text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}}] \\
\]

\[
-0.4V \\
\]

HIPPI-pro-red \( \rightleftharpoons \) HIPPI-pro-red \( \rightleftharpoons \) HIPPI-pro-red \[
+0.35V \\
\]

“super-oxidized” ferredoxin (Fd-ox) (5), gives a unified relationship between the natural redox species and the synthetic analogs. Synthetic analogs of the electron storage site of 4-Fe and 8-Fe ferredoxins and Chromatium high potential protein, which consist of a tetranuclear “cubane” cluster ([Fe-S(\text{SR})_4]^{\text{2-}}), have recently been reported by Holm et al. (6, 7).

Characterization and comparison with natural active sites have shown that these complexes are structural (6–8) and electronic (6, 9–11), but not complete electrochemical (6, 7, 11, 12) analogs of the natural iron–sulfur clusters.

Preliminary to studies involving the synthetic \( \text{Fe}_4\text{S}_4(\text{SR})_4^{\text{2-}} \) complexes in redox reactions, their solution chemistry should be understood in order that rate phenomena may be associated with particular ionic or degradative species. This is particularly important for studies in protic solvents due to the established lability of \( [\text{Fe}_4\text{S}_4(\text{SR})_4]^{\text{2-}} \) compounds to both hydrolysis and ligand exchange. Here we report the results of a study of the acid–base properties and the kinetics of hydrolysis and oxidation of \( [\text{Fe}_4\text{S}_4(\text{SR})_4]^{\text{2-}} \) [\( R = -\text{CH}_2, -\text{CH}_2\text{CH}-(\text{CH}_3)_2, -\text{C}(\text{CH}_3)_4 \)] clusters as a function of \( \text{pH} \) and RSH concentration.

MATERIAL AND METHODS

The \( [\text{Fe}_4\text{S}_4(\text{SR})_4]^{\text{2-}} \) complexes were prepared by a modification of the method of Holm et al. (7). The diaminon were prepared as the tetrabutylammonium salts. All studies were carried out in 60/40 (v/v %) N-methylpyrroldinione (NMP)–H_2O solutions at 30°C with ionic strength \( \mu = 0.1 \) at all \( \text{pH} \) values above 1.0. Pseudo-first-order conditions were maintained throughout with substrate \([\text{Fe}_4\text{S}_4(\text{SR})_4]^{\text{2-}} [\text{R} = -\text{CH}_2, -\text{CH}_2\text{CH}-(\text{CH}_3)_2, -\text{C}(\text{CH}_3)_4] \) concentrations at 40 \( \mu \)M and total buffer concentrations, aside from HCl, maintained at 58 mM. The buffers employed were HCl (\( \text{pH} \) 0.05–0.799), tri-chloroacetate (\( \text{pH} \) 1.15–2.15), chloroacetate (\( \text{pH} \) 2.9–4.15), formate (\( \text{pH} \) 4.55–5.05), and acetate (\( \text{pH} \) 5.45–6.50). All kinetic runs were carried out under a nitrogen atmosphere with degassed samples in Thunberg cuvettes. Reactions were followed by observing the decrease in absorbance of the \( [\text{Fe}_4\text{S}_4(\text{SR})_4]^{\text{2-}} \) cluster at 425 nm. Data were obtained in the form of initial rates (slope = \( \Delta A/\Delta t \)) which, at all \( \text{pH} \) values, were obtained prior to the first 15% of the reactions. An absorbance versus \( \text{pH} \) acid titration curve was obtained by extrapolation to absorbance at \( t = 0 \) using initial rate slopes. Rates of \( \text{O}_2 \) oxidation were obtained in the same manner, employing solvent saturated with \( \text{O}_2 \) at 30°C. Under these conditions the rates of oxidation were much greater than those for hydrolysis.

A correction factor of \( \text{pH}_{\text{app}} - 0.875 \) to adjust the \( \text{pH} \) meter reading to a test \( \text{pH} \) was obtained from standard HCl solution in 60/40 NMP–H_2O by plotting \( \text{pH} \) calculated from formal HCl concentrations versus measured \( \text{pH} \) in the mixed solvent. This electrode correction was judged to be valid at lower acidities, since the plot of absorbance at \( t_0 \) versus \( \text{pH} \) for reaction solutions fit a theoretical titration curve which was independent of buffer type (see Fig. 2).

Abbreviations: NMP, \( \text{N}-\text{methylpyrroldinione} \); HIPPI, high potential iron protein.

* A portion of the material is to be submitted by R.M. for the Ph.D. in Chemistry, University of California at Santa Barbara.
RESULTS

The time course for the disappearance of Fe₄S₄(SR)_4 species from solution was followed under conditions of constant pH and total buffer concentration. The buffer ([B⁺] = [B] + [BH⁻]) was maintained in great excess over substrate. Due to the occurrence of turbidity at ≈t/µ the pseudo-first order rate constants were computed from the initial rate of change of absorbance with time (Eq. 2) where

\[
A_1 = \text{initial absorbance and } k \text{ is in sec}^{-1}. \text{Ten-fold changes in buffer concentrations (58-5.8 mM) at pH 2.9 (chloroacetate) and 5.05 (formate) were found to provide only 1.5- to 2-fold changes in } k_{\text{obs}} \text{ for the disappearance of Fe₄S₄([SCH₂CH(CH₃)]₄)₄}^{−3}. \text{In mixed solvents of the variety employed in this study, such small changes in rate constants are to be anticipated from specific ion effects (13). It is concluded, therefore, that the values of } k_{\text{obs}} \text{ are related only to changes in concentration of lyate species. In Fig. 1, the log of the values of } k_{\text{obs}} \text{ is plotted versus pH for Fe₄S₄([SCH₂CH(CH₃)]₄)₄}^{−3}. \text{The line of Fig. 1 was generated from the empirical expression of Eq. 3 where } A = 8.5 \times 10^{-2}; B = 7.0 \times 10^{-4}; C = 1.0; D = 8.1 \times 10^{-4}; \text{and } E = 8.8 \times 10^{-15} \text{and } a_κ \text{ is hydrogen}.

\[
k_{\text{obs}} = \frac{Aa_κ^2 + B a_κ^3}{Ca_κ^3 + Da_κ + E}
\]

ion activity. Changing the R of Fe₄S₄(SR)_4⁴⁻ from —CH₃ to —C(CH₃)₄ at pH values of 0.5, 4.1, and 6.0 provided 1.5- to 2-fold changes in k_{\text{obs}}. The rate constants as a function of R and pH are given in Table 1. Holm et al. have found that ligand exchange equilibria are also largely independent of the steric bulk of —SR (14). Since the pH values employed pertain to positions on the log k_{\text{obs}} versus pH-profile of slopes −1, 0, and 1, one may conclude that all solvolytic processes occurring in the pH range investigated are insensitive to steric effects. Much larger changes in rate would be observed if solvolysis of the clusters were due to nucleophilic displacement. For example, in the S₈2 reaction of Br⁻ + Br⁻ → Br⁻ + Br⁻, an 18,000-fold difference in rate between methyl and tert-butyl substituents is observed (15).

For the Fe₄S₄([SCH₂CH(CH₃)]₄)₄⁻² substrate, the values of k_{\text{obs}} were found to change 2- to 5-fold at pH 0.5 and 4.1- and 3.5-fold at pH 6.0 when the disappearance of substrate was followed in the presence of a concentration of (CH₃)₄CHCH₃SH exceeding the initial concentration of substrate by 20-fold. From this result one may conclude that no portion of the log k_{\text{obs}} versus pH profile represents a competitive pre-equilibrium exchange of lyate species for mercaptan (Eq. 4). At pH

\[
\text{Fe}^{2+} + \text{L} \rightleftharpoons \text{Fe}^{3+} + \text{RS(H)}
\]

values greater than 6.5, significant ligand exchange for lyate species occurs. This can be shown by the fact that: (1) the absorbance of reaction solutions in this pH range increases rather than decreases with time as in the case of solvolysis; and (2) in the presence of RSH (100-fold excess over substrate) the degree of absorbance increase with time is greatly diminished. Since the diamines Fe₄S₄(SR)_4⁴⁻ represent conjugate bases of what could be rather weak acids (Eq. 5), a spectrophotometric titration of Fe₄S₄([SCH₂CH(CH₃)]₄)₄⁻² was carried

\[
K_{a₁} + H⁺ \rightleftharpoons Fe₄S₄(SR)_4^{−1} H⁺ \rightleftharpoons Fe₄S₄(SR)_4 H⁺ \rightleftharpoons Fe₄S₄(SR)_4 H⁺
\]

out. For this purpose the absorbance at t = 0 was obtained in the following manner. An aliquot of a solution of the isobutyl cluster salt in 6/40 NMP-H₂O was tipped into a buffered solution (t) at the desired pH and the change of absorbance was recorded for a period of a few minutes. Extrapolation of the plot of absorbance to t = 0 provided the initial absorbance of the ionic species of the iron-sulfur cluster. In Fig. 2 these values of initial absorbance are plotted versus pH. The curve of Fig. 2 has been computer generated to represent the dissociation of an acid of pKₐ 3.92.

**TABLE 1.** Observed rate constants of Fe₄S₄(SR)_4⁴⁻ hydrolysis as a function of alkyl steric bulk

<table>
<thead>
<tr>
<th>R</th>
<th>pH</th>
<th>k_{\text{obs}} (10⁻¹ sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—CH₃</td>
<td>0.50</td>
<td>17.8</td>
</tr>
<tr>
<td>—C(CH₃)₃</td>
<td>0.50</td>
<td>19.8</td>
</tr>
<tr>
<td>—CH₄</td>
<td>4.20</td>
<td>2.43</td>
</tr>
<tr>
<td>—C(CH₃)₃</td>
<td>5.75</td>
<td>0.541</td>
</tr>
<tr>
<td>—C(CH₃)₃</td>
<td>5.75</td>
<td>0.296</td>
</tr>
</tbody>
</table>

**FIG. 1.** Log k_{\text{obs}} versus pH rate profile for the solvolysis of the isobutylthiol tetranuclear complex. The inset represents the k_{\text{obs}} versus pH profile for the oxidation of the isobutylthiol tetranuclear complex in solutions saturated with O₂ at 30°. All reactions carried out at 30° in 60/40 (v/v %) N-methylpyrrolidinone in H₂O, µ = 0.1.

**FIG. 2.** Spectrophotometric titration of the isobutyl tetranuclear cluster [30°, solvent 60/40 (v/v %) N-methylpyrrolidinone in H₂O, µ = 0.1].
Initial studies of the pH-dependence of the O₂ oxidation of the isobutyl cluster salt are provided in the insert to Fig. 1, which represents a plot of the initial rate of absorbance decrease versus pH for a 60/40 (v/v %) N-methylpyrrolidinone–H₂O solution saturated (30°C) with O₂ and containing 40 μM Fe₄S₄(SR)₄H⁻. The sigmoid curve drawn through the points was generated from Eq. 6, where kₒ, apparent rate constant for Fe₄S₄(SCH₂CH(CH₃)₂)₄, is 0.032 sec⁻¹;

\[
k₁ = \frac{kₒK_{app}}{K_{app} + aH} + \frac{kₒ_aH}{K_{app} + aH}
\]  

(6)
kₒ, H (apparent rate constant for Fe₄S₄(SCH₂CH(CH₃)₂)₄H⁻) = 0.13 sec⁻¹ and pKₐₜₜ = 4.5. Though pKₐₜₜ differs from the true pKₐ by 0.6 pH units it must be recalled that kinetically apparent dissociation constants often do not coincide with thermodynamic constants and that this may often be rationalized in terms of the details of mechanism (16).

**DISCUSSION**

The most interesting aspect of the present investigation is the finding that the tetranuclear iron–sulfur cluster complexes exist in solution as acid–base pairs (Fig. 2). The pKₐ of the conjugate acid [Eq. 7, R = CH₂CH(CH₃)₂] is comparable to pKₐ of formic acid. This finding has significant implications. Thus, (Box-H)⁻ and (Box)⁻² would be expected to differ not only in their rates of reaction with a particular reagent but in their oxidation potential and actual mode of reaction. In this study we establish a difference in mechanism of reaction of (Box-H)⁻ and (Box)⁻² with lyate species (solvolysis versus ligand exchange, respectively) and establish the acid species to be most susceptible to oxidation by O₂ [kₒ, for (Box-H)⁻/kₒ, for (Box)⁻² = 4.06; see Results]. Differences in the E₁/₂ values of (Box-H)⁻ and (Box)⁻² will be described in a subsequent publication. It now becomes obvious that in the evaluation of the oxidation potentials of ferredoxins and HIPIT, consideration must be accorded to the control, by the protein molety, of the pKₐ of the tetranuclear iron–sulfur cluster, which in turn controls the (Box-H)⁻/(Box)⁻² at a given pH. As protein functional groups go, (Box-H)⁻ is a relatively strong acid and it is conceivable that it could play a role as a general acid in enzyme catalysis. This, therefore, may be the function of the iron–sulfur structure in aconitase, which is a nonoxidative enzyme (17).

The mechanistic rationalisation of the pH-dependence of solvolysis of the tetranuclear iron–sulfur clusters must take into account the presence of the acidic and basic species of the substrate. The pH-log k_{obs} profile for the disappearance of the thi-isobutyl complexed tetranuclear iron–sulfur cluster is provided in Fig. 1, the unusual profile being generated by the empirical Eq. 3. Inspection of Eq. 3 reveals that there must be at least two proton association equilibria and at least one acid-catalyzed rate-determining step. One of the two acid base equilibria must pertain to the established pre-equilibrium protonation of the dianionic cluster and the other to an intermediate. The additional restraint is that any mechanistic scheme that may be devised must not directly reflect the true pKₐ of the cluster in the derived log k_{obs} versus pH profile. That is to say, the inflections in the pH-log k_{rate} profile (Fig. 1) in going from slopes -1, 0, to -2 must not be required to occur at the determined pKₐ of the cluster. The scheme of Eq. 8 rather uniquely fits these criteria,

\[
\begin{align*}
&\text{Box}^{-2} \xrightarrow{+H^+} \text{Box}-H \rightleftharpoons A \xrightarrow{+H^+} \text{AH} \rightleftharpoons B \xrightarrow{k_{obs}} \text{Products} \\
&K_{a1} \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \q
atom. If this were so then HO⁻ would be a most effective nucleophile (as would the buffer bases) and the rate law (Eq. 9) would be of a different form. The lack of a significant steric effect on the rates of solvolysis in the pH ranges corresponding to slopes -1, 0, and -2 (Fig. 1) when R = -CH₃ and -C(CH₃)₃ would be in accord with our suggestion of spontaneous opening of the tetranuclear cluster through Fe-S bond scission, either Fe₄S₄(SR)₄⁻² or AH → B). The ratio of the rate constants for degradation of B can be determined to be k₃/k₂ = 0.082.

Since the basicity of the isolated thiol functions which form either the ligands or the alternate >S corners of the Fe₄S₄(SR)₄⁻² species could not account for the determined pKa of (Box-H)⁻, it must be assumed that the proton associates with one of the six faces of [Box]⁻. We suggest that this occurs through a hydrogen-bridged structure involving the filled d-orbitals above a face of the cluster. Evidence has been presented by Ford et al. (18) for d-orbital protonation of Ru¹¹ in the acid-catalyzed equation of Ru(NH₃)₆⁺². The transition states for the hydrolytic steps Box-H⁻ → A and AH → B may be reached by simultaneous movement of the proton to S with concerted breaking of the bonds of the involved S to neighboring iron atoms. Since there exists six essentially identical proton bonding faces, the proton could be found on any face. Because each of the six modes of proton association should be energetically equivalent, it is conceivable that the proton hops or tunnels from face to face around the periphery of the iron-sulfur cluster.

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