The Acid-Base Properties, Hydrolytic Mechanism, and Susceptibility to O₂ Oxidation of Fe₄S₄(SR)₄⁻² Clusters

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

THOMAS C. BRUCE, RICHARD MASKIEWICZ*, AND ROBERT JOB

Department of Chemistry, University of California, Santa Barbara, Calif. 93106

Contributed by Thomas C. Bruce, October 31, 1974

ABSTRACT The iron-sulfur cluster compounds Fe₄S₄(SR)₄⁻² [where —SR = —SCH₃, —S—C(CH₃)₃, and —S—CH₂—CH(CH₃)₂] have been found to represent the base species of weak acids of pK_a comparable to that of carboxylic acids. The acid species Fe₄S₄(SR)₄H⁺ is most subject to reaction with O₂ and to acid-catalyzed solvolysis, while the base species Fe₄S₄(SR)₄⁻² 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

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 (HIPIP_{s-red}) (4), and

"super-oxidized" ferredoxin (Fd_{s-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 ([Fe₄S₄(SR)₄]⁻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 Fe₄S₄(SR)₄⁻² 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 [Fe₄S₄(SR)₄]⁻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 [Fe₄S₄(SR)₄]⁻² [R = —CH₃, —CH₂CH(CH₃)₂, —C(CH₃)₃] clusters as a function of pH and RSH concentration.

MATERIAL AND METHODS

The [Fe₄S₄(SR)₄]⁻² complexes employed were prepared by a modification of the method of Holm *et al.* (7). The dianions were prepared as the tetrabutylammonium salts. All studies were carried out in 60/40 (v/v %) *N*-methylpyrrolidinone (NMP)—H₂O solutions at 30° with ionic strength μ = 0.1 at all pH values above 1.0. Pseudo-first-order conditions were maintained throughout with substrate [Fe₄S₄(SR)₄]⁻² [R = —CH₃, —CH₂CH(CH₃)₂, —C(CH₃)₃] concentrations at 40 μM and total buffer concentrations, aside from HCl, maintained at 58 mM. The buffers employed were HCl (pH 0.05-0.60), trichloroacetate (pH 1.15-2.15), chloroacetate (pH 2.90-4.15), formate (pH 4.55-5.05), and acetate (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 [Fe₄S₄(SR)₄]⁻² cluster at 425 nm. Data were obtained in the form of initial rates (slope = ΔA/Δt) which, at all pH values, were obtained prior to the first 15% of the reactions. An absorbance versus pH acid titration curve was obtained by extrapolation to absorbance at t = 0 using initial rate slopes. Rates of O₂ oxidation were obtained in the same manner, employing solvent saturated with O₂ at 30°. Under these conditions the rates of oxidation were much greater than those for hydrolysis.

A correction factor of pH_{app} - 0.875 to adjust the pH-meter reading to read pH was obtained from standard HCl solution in 60/40 NMP-H₂O by plotting pH calculated from formal HCl concentrations versus measured pH in the mixed solvent. This electrode correction was judged to be valid at lower acidities, since the plot of absorbance at t₀ versus pH for reaction solutions fit a theoretical titration curve which was independent of buffer type (see Fig. 2).

Abbreviations: NMP, *N*-methylpyrrolidinone; HIPIP, 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.

FIG. 1. Log k_{obs} versus pH rate profile for the solvolysis of the isobutylthiol tetranuclear complex. The inset represents the k_{obs} versus pH profile for the oxidation of the isobutylthiol tetranuclear complex in solutions saturated with O_2 at 30° . All reactions carried out at 30° in 60/40 (v/v %) *N*-methylpyrrolidinone in H_2O , $\mu = 0.1$.

RESULTS

The time course for the disappearance of $\text{Fe}_4\text{S}_4(\text{SR})_4$ species from solution was followed under conditions of constant pH and total buffer concentration. The buffer ($[\text{B}_\text{T}] = [\text{B}] + [\text{BH}]$) was maintained in great excess over substrate. Due to the occurrence of turbidity at $\approx t_{1/2}$, the pseudo-first order rate constants were computed from the initial rate of change of absorbance with time (Eq. 2) where

$$(\text{initial slope}/A_1) = k_{\text{obs}} \quad [2]$$

A_1 = initial absorbance and k is in sec^{-1} . 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_{obs} for the disappearance of $\text{Fe}_4\text{S}_4[\text{SCH}_2\text{CH}(\text{CH}_3)_2]_4^{-2}$. 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_{obs} are related only to changes in concentration of lyate species. In Fig. 1, the log of the values of k_{obs} is plotted versus pH for $\text{Fe}_4\text{S}_4[\text{SCH}_2\text{CH}(\text{CH}_3)_2]_4^{-2}$. The line of Fig. 1 was generated from the empirical expression of Eq. 3 where $A = 8.5 \times 10^{-2}$; $B = 7.0 \times 10^{-3}$; $C = 1.0$; $D = 8.1 \times 10^{-6}$; and $E = 8.8 \times 10^{-12}$ and a_{H} is hydrogen

$$k_{\text{obs}} = \frac{Aa_{\text{H}}^3 + Ba_{\text{H}}^2}{Ca_{\text{H}}^2 + Da_{\text{H}} + E} \quad [3]$$

ion activity. Changing the R of $\text{Fe}_4\text{S}_4(\text{SR})_4^{-2}$ from $-\text{CH}_3$ to $-\text{C}(\text{CH}_3)_3$ at pH values of 0.5, 4.1, and 6.0 provided 1.5- to 2-fold changes in k_{obs} . The rate constants as a function of R and pH are given in Table 1. Holm *et al.* have found that ligand and exchange equilibria are also largely independent of the

TABLE 1. Observed rate constants of $\text{Fe}_4\text{S}_4(\text{SR})_4^{-2}$ hydrolysis as a function of alkyl steric bulk

R	pH	k_{obs} (10^{-3} sec^{-1})
$-\text{CH}_3$	0.50	17.8
$-\text{C}(\text{CH}_3)_3$	0.50	19.8
$-\text{CH}_3$	4.20	2.43
$-\text{C}(\text{CH}_3)_3$	4.20	2.76
$-\text{CH}_3$	5.75	0.541
$-\text{C}(\text{CH}_3)_3$	5.75	0.296

FIG. 2. Spectrophotometric titration of the isobutyl tetranuclear cluster [30° , solvent 60/40 (v/v %) *N*-methylpyrrolidinone in H_2O , $\mu = 0.1$].

steric bulk of $-\text{SR}$ (14). Since the pH values employed pertain to positions on the log k_{obs} versus pH-profile of slopes -1 , 0 , and 2 , 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 $\text{S}_{\text{N}}2$ reaction of $\text{Br}^- + \text{RBr} \rightarrow \text{RBr} + \text{Br}^-$, an 18,000-fold difference in rate between methyl and *tert*-butyl substituents is observed (15).

For the $\text{Fe}_4\text{S}_4[(\text{SCH}_2\text{CH}(\text{CH}_3)_2)_4]^{-2}$ substrate, the values of k_{obs} were found to change 2- to 2.5-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 $(\text{CH}_3)_2\text{CHCH}_2\text{SH}$ exceeding the initial concentration of substrate by 20-fold. From this result one may conclude that no portion of the log k_{obs} versus pH profile represents a competitive preequilibrium exchange of lyate species for mercaptan (Eq. 4). At pH

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 dianions $\text{Fe}_4\text{S}_4(\text{SR})_4^{-2}$ represent conjugate bases of what could be rather weak acids (Eq. 5), a spectrophotometric titration of $\text{Fe}_4\text{S}_4[\text{SCH}_2\text{CH}(\text{CH}_3)_2]_4^{-2}$ was carried

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 60/40 NMP- H_2O was tipped into a buffered solution (t_0) 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 $\text{p}K_a$ 3.92.

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°) with O₂ and containing 40 μM Fe₄S₄[SCH₂CH(CH₃)₂]₄⁻². The sigmoid curve drawn through the points was generated from Eq. 6, where *k*_{O₂} (apparent rate constant for Fe₄S₄[SCH₂CH(CH₃)₂]₄⁻²) = 0.032 sec⁻¹;

$$k_i = \frac{k_{O_2} K_{app}}{K_{app} + a_H} + \frac{k_{O_2}^H a_H}{K_{app} + a_H} \quad [6]$$

*k*_{O₂}^H (apparent rate constant for Fe₄S₄[SCH₂CH(CH₃)₂]₄H⁻) = 0.13 sec⁻¹ and p*K*_{app} = 4.5. Though p*K*_{app} differs from the true p*K*_a 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 p*K*_a of the conjugate acid [Eq. 7, R = CH₂CH(CH₃)₂] is comparable to

that 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 (solvolytic versus ligand exchange, respectively) and establish the acid species to be most susceptible to oxidation by O₂ [*k*_{O₂} for (Box-H)⁻/*k*_{O₂} for (Box)⁻² = 4.06; see *Results*]. Differences in the *E*_{1/2} 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 HIPIP, consideration must be accorded to the control, by the protein moiety, of the p*K*_a of the tetranuclear iron-sulfur cluster, which in turn controls the ratio (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 rationalization 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 thio-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 p*K*_a 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 p*K*_a of the cluster. The scheme of Eq. 8 rather uniquely fits these criteria,

$$K_{a1} = \frac{[\text{Box}^{-2}][\text{H}^+]}{[\text{Box-H}^-]}, \quad K_{a2} = \frac{[\text{A}][\text{H}^+]}{[\text{AH}]},$$

$$K_1 = \frac{[\text{Box-H}^-]}{[\text{A}]}, \quad K_2 = \frac{[\text{AH}]}{[\text{B}]}$$

providing the kinetic derivation of Eq. 9, which is identical in form to the empirical Eq. 6. A mechanism of solvolysis

$$k_{\text{obs}} = \frac{(k_1 + k_2 a_H) a_H^2}{K_1 K_2 K_{a1} K_{a2} + K_2 K_{a2} (K_2 + 1) a_H + (K_2 + 1) a_H^2} \quad [9]$$

which is in accord with (8) and which we believe reasonable follows. The anionic cluster [(Box)⁻²] associates with a proton to provide its conjugate acid [(Box-H)⁻]. The species Box-H⁻ then opens spontaneously and reversibly by scission of two Fe-S bonds which form adjacent edges of the cluster to provide a new compound A. A in turn reversibly associates

with a proton to provide its conjugate acid (AH), which then spontaneously unfolds, in a reversible manner, through scission of two Fe-S bonds which again form two adjacent edges of the ruptured box, yielding species B. The structure of B might be B. Compound B is then destroyed in rate-determining steps by a spontaneous and acid-catalyzed process.

That compounds A and B are not formed by reversible loss of the RS ligand is shown by the fact that inclusion of excess RSH does not appreciably change the rate constants associated with the three portions of the log *k*_{obs} versus pH profile of Fig. 1 (i.e., slope = -1, [*k*_{obs} = *k*₂*a*_H/(*K*₂ + 1)]; slope = 0, [*k*_{obs} = *k*₁/(*K*₂ + 1)]; and slope = -2, [*k*_{obs} = *k*₁*a*_H²/(*K*₁*K*₂*K*_{a1}*K*_{a2})] (Fig. 1). Also, the equilibria involving conversion of Box-H⁻ → A and AH → B cannot involve attack by H₂O (or *N*-methylpyrrolidinone) upon an iron

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 $\text{R} = -\text{CH}_3$ and $-\text{C}(\text{CH}_3)_3$ would be in accord with our suggestion of spontaneous opening of the tetranuclear cluster through Fe-S bond scission (i.e., $\text{Box-H}^- \rightarrow \text{A}$ and $\text{AH} \rightarrow \text{B}$). The ratio of the rate constants for degradation of B can be determined to be $k_1/k_2 = 0.082$.

Since the basicity of the isolated thiol functions which form either the ligands or the alternate $>\text{S}$ corners of the $\text{Fe}_4\text{S}_4(\text{SR})_4^{-2}$ species could not account for the determined pK_a of $(\text{Box-H})^-$, it must be assumed that the proton associates with one of the six faces of $[\text{Box}]^{-2}$. 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 Ru^{II} in the acid-catalyzed equation of $\text{Ru}(\text{NH}_3)_6^{+2}$. The transition states for the hydrolytic steps $\text{Box-H}^- \rightarrow \text{A}$ and $\text{AH} \rightarrow \text{B}$ 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.

This work was supported by a grant from the National Institutes of Health. R.J. is a Postdoctoral Fellow in the Department of Chemistry, University of California at Santa Barbara.

- Orme-Johnson, W. H. (1973) *Annu. Rev. Biochem.* **42**, 159-204.
- Palmer, G. & Britzinger, H. (1972) in *Electron and Coupled Energy Transfer in Biological Systems*, eds. King, T. E. & Klingenberg, M. (Marcel Dekker, New York), Vol. 1, Part B, pp. 379-476.
- Carter, C. W., Jr., Kraut, J., Freer, S. T., Alden, R. A., Sieker, L. C., Adman, E. & Jensen, L. H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3526-3529.
- Cammack, R. (1973) *Biochem. Biophys. Res. Commun.* **54**, 548-554.
- Sweeney, W. V., Bearden, A. J. & Rabinowitz, J. C. (1974) *Biochem. Biophys. Res. Commun.* **59**, 188-194.
- Herskovitz, T., Averill, B. A., Holm, R. H., Ibers, J. A., Phillips, W. D. & Weiher, J. F. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2437-2441.
- Averill, B. A., Herskovitz, T., Holm, R. H. & Ibers, J. A. (1973) *J. Amer. Chem. Soc.* **95**, 3523-3534.
- Que, L., Bobrick, M. A., Ibers, J. A. & Holm, R. H. (1974) *J. Amer. Chem. Soc.* **96**, 4168-4178.
- Holm, R. H., Phillips, W. D., Averill, B. A., Mayerle, J. J. & Herskovitz, T. (1974) *J. Amer. Chem. Soc.* **96**, 2109-2117.
- Holm, R. H., Averill, B. A., Herskovitz, T., Frankel, R. B., Gray, H. B., Siiman, O. & Grunthaner, F. J. (1974) *J. Amer. Chem. Soc.* **96**, 2644-2646.
- De Pamphilis, B. V., Averill, B. A., Herskovitz, T., Que, L. & Holm, R. H. (1974) *J. Amer. Chem. Soc.* **96**, 4159-4167.
- Van Tamelen, E. E., Gladysz, J. A. & Brulet, C. R. (1974) *J. Amer. Chem. Soc.* **96**, 3020-3021.
- Lahti, M. & Kankaanpera, A. (1970) *Acta Chem. Scand.* **24**, 706-708.
- Que, L., Bobrick, M. A., Ibers, J. A. & Holm, R. H. (1974) *J. Amer. Chem. Soc.* **96**, 4168-4178.
- Ingold, C. K. (1969) in *Structure and Mechanism in Organic Chemistry* (Cornell University Press, Ithaca), p. 555.
- Bruice, T. C. & Benkovic, S. J. (1966) in *Bioorganic Mechanisms* (W. A. Benjamin, Inc., New York), Vol I, chap. 1.
- Kennedy, S. C., Rauner, R. & Gawron, O. (1972) *Biochem. Biophys. Res. Commun.* **47**, 740.
- Ford, P. C., Kuempel, J. R. & Taube, H. (1968) *Inorg. Chem.* **7**, 1976-1983.