THE ACID-BASE PROPERTIES AND KINETICS OF DISSOLUTION OF THE Fe_4S_4 CORES OF CHROMATIUM FERREDOXIN AND HIGH POTENTIAL IRON PROTEIN

Richard Maskiewicz, Thomas C. Bruice,* and Robert G. Bartsch

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106, and Department of Chemistry, University of California at San Diego, La Jolla, California 92037.

Received May 5,1975

Summary

The kinetics for the dissolution of Chromatium ferredoxin and High Potential Iron Protein have been compared (H_o = -1.05 to pH = 7). Chromatium ferredoxin hydrolyzes via a mechanism following the kinetics $A^{=} + H^{+} \rightleftarrows AH^{-} \rightarrow BH^{-} \rightarrow C$ at constant pH. Initial absorbances describe a theoretical one proton titration curve corresponding to a pK_a of 2.7 for AH^{-}. Chromatium High Potential Iron Protein shows no titration behavior and hydrolyzes slowly in water. These results confirm the availability of the Fe_4S_4-cores of Chromatium ferredoxin as compared to that of High Potential Iron Protein. Further, these results establish similarities in the hydrolytic chemistry of protein bound Fe_4S_4-cores as compared to synthetic compounds of structure $Fe_4S_4(SR)_4^{=}$.

Materials and Methods

Chromatium ferredoxin (C.Fd.) and Chromatium High Potential Iron Protein (HIPIP) were prepared by a published method (1). The ferredoxin had a purity index (A_{278}/A_{384}) of 2.09, and HIPIP (A_{283}/A_{388}) 2.56.

Rates and absorbances were obtained using a Durrum model D-110 stopped-flow spectrophotometer (under a N_2 atmosphere) with a Biomation model 805 waveform recorder. Titration curves were obtained by plotting initial absorbances of the starting species of the observed biphasic hydrolysis reaction as a function of -log (hydrogen ion activity) i.e. H_o and pH. Initial absorbances were measured 3 msec after completion of stopped flow mixing. All rate processes were followed at 390 nm. Measurements were performed anaerobically under pseudo-first-order conditions ([buffer] > 100 [iron-sulfur protein]) and at an ionic strength of 0.1 (KCl) at all pH's

greater than 1.0. HCl was employed as a buffer between H_0 (-0.45) and pH 3.43. Formate was employed at pH 3.72, acetate at pH 4.63, and buffering was not required to hold the adjusted pH between pH 4.63 and 6.85.

Results and Discussion

Initial absorbances of the iron-sulfur clusters in Chromatium ferredoxin obtained 3 msec after mixing protein and buffer solutions are shown as a function of pH in Figure 1. The points correspond to the theoretical curve of a simple one proton dissociation. The curve of Figure 1 represents the dissociation of an acid of pK_a 2.72. Chromatium HIPIP initial absorbances, shown in the inset in Figure 1, evidence spectral change only below pH 1.0.

As shown in Figure 2, the hydrolytic reaction of the Fe_4S_4 clusters in the ferredoxin showed biphasic behavior ($AH^- \rightarrow BH^- \rightarrow C$, where AH^- represents an initial protonated form of an active site cluster $A^=$, and C represents hydrolysis products). In order to determine the pH-dependence

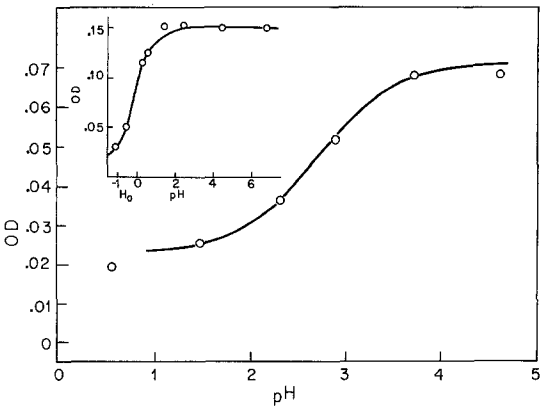


Figure 1. Spectrophotometric acid dissociation curve for the protonated Fe_4S_4 clusters (i.e., AH^-) of Chromatium ferredoxin. The inset represents a spectrophotometric titration of Chromatium High Potential Iron Protein. All optical densities were measured 3 msec after completion of stopped-flow mixing.

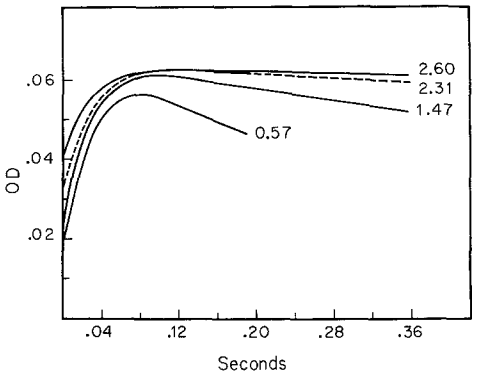


Figure 2. Optical density of Chromatium ferredoxin as a function of time at various pH's. The optical densities at t=∞ were zero at these pH's.

---

of the rate constants for $AH^- \rightarrow BH^- \rightarrow C$ it was necessary to take into account: (1) the extinction coefficient of $A^=$ exceeds AH^- ($\epsilon_{A^=}/\epsilon_{AH^-} = 3.44$); (2) the absorbance of BH^- as well as the rate constant for conversion of AH^- to BH^- ($k_{AH^- \rightarrow BH^-} = 29\ sec^{-1}$) are pH-independent; and (3) the conversion of BH^- to C exhibits the pH-dependence shown in Figure 3. In order to explain the pH-independence of the absorbance of BH^-, one may assume that the conversion of $AH^- \rightarrow BH^-$ involves a slow conformational change with concomitant intramolecular proton transfer from the protonated Fe_4S_4-core of AH^- to surrounding protein resulting in the reformation of unprotonated Fe_4S_4 cluster in BH^-. This assumption would be in accord with the observation that the absorbance of the species BH^- is similar to that of the native unprotonated protein (i.e., $A^=$). The line of Figure 3, which exhibits limiting slopes of -1, 0, and -2 with increasing pH, was generated from the empirical expression of equation 1

$$k_{obs} = \frac{V(a_H)^3 + W(a_H)^2}{X(a_H)^2 + Y(a_H) + Z} \qquad (1)$$

previously employed to correlate the hydrogen ion concentration with the

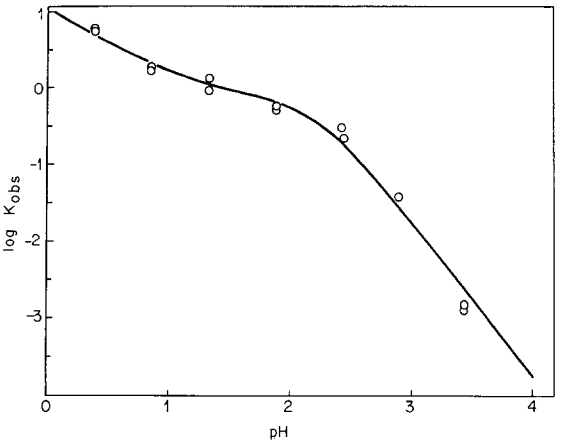


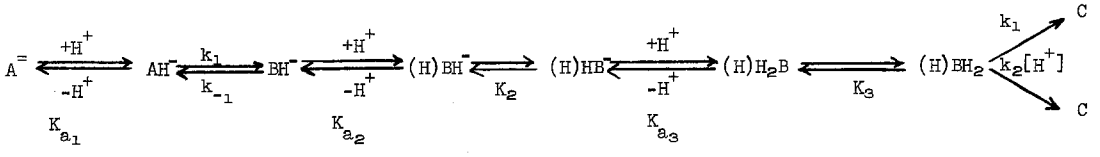
Figure 3. Log k_{obs} vs pH rate profile for the dissolution of the Fe_4S_4 clusters in Chromatium ferredoxin. Reactions were carried out in H_2O at 30°, μ = 0.1. The points are experimental and the line was generated using equation 1 and the constants provided with Scheme I.

---

rate of hydrolysis of synthetic Fe_4S_4 clusters (2). Native HIPIP hydrolyzed at approximately 10^{-4} the rate of the ferredoxin in water, and approximately 10^{-2} as fast in a 70/30 vol. percent DMSO/H_2O solution.

The pH-dependence of the overall hydrolysis reaction ($AH^- \rightarrow BH^- \rightarrow C$) for C.Fd. may be explained via Scheme I. As in the case of the synthetic analogs previously studied by us, any hydrolysis mechanism for $BH^- \rightarrow C$

Scheme I



$K_{a_1} = \frac{[A]^=[H^+]}{[AH]^-} = 1.90 \times 10^{-3}$; $\frac{k_{-1}}{k_1} = \frac{[AH]^-}{[BH]^-}$, $k_1 + k_{-1} = 29\ sec^{-1}$; $K_{a_2} = \frac{[BH]^-[H^+]}{[(H)BH]^-} = 5 \times 10^2$;

$K_2 = \frac{[(H)BH]^-}{[(H)HB]^-} = 1 \times 10^{-2}$; $K_{a_3} = \frac{[(H)HB]^-}{[(H)H_2B]} = 1 \times 10^1$; $K_3 = \frac{[(H)H_2B]}{[(H)BH_2]} = 8 \times 10^{-7}$; $k_1 = 7 \times 10^{-1}$;

$k_2 = 1 \times 10^1$

must recognize at least two proton dissociation equilibria and at least one acid catalyzed rate determining step. One of the two acid-base equilibria must pertain to the established preequilibrium protonation of the iron-sulfur cluster, and the other to that of an intermediate. An additional restraint is that any mechanistic scheme which is devised must not directly reflect the true pK_a in the derived log k_{obs} *vs* pH profile. Scheme I satisfies these criteria and provides for the derivation of equation (2). Since $k_{AH^-} \rightarrow BH^-$ is large compared to $k_{BH^-} \rightarrow C$ at all pH's,

$$k_{obs} = \frac{(k_1 + k_2 a_H) a_H^3}{(K_3 + 1) a_H^3 + K_3 K_{a_3} (K_2 + 1) a_H^2 + K_2 K_3 K_{a_2} K_{a_3} (\frac{k_{-1}}{k_1} + 1) a_H + \frac{k_{-1}}{k_1} K_2 K_3 K_{a_1} K_{a_2} K_{a_3}} \quad ($$

equation (2) can be reduced to equation (3). This is of the same form as

$$k_{obs} = \frac{k_1 + k_2 a_H) a_H^2}{(K_3 + 1) a_H^2 + K_3 K_{a_3} (K_2 + 1) a_H + K_2 K_3 K_{a_2} K_{a_3}} \quad ($$

empirical equation (1) employed to fit the profile of Figure 3. Scheme I entails initial protonation of one or both of the Fe_4S_4 clusters to give the species AH^- (for which the titration curve of Figure 1 was obtained). The species AH^- is converted *via* a conformational change to BH^- in a reaction which is otherwise pH-independent. A subsequent equilibrium protonation results in the conversion of BH^- to a more hydrolytically labile species $(H)BH^-$. The species $(H)BH^-$ is then converted to $(H)HB^-$ in an equilibrium reaction which involves the formation of an -SH moiety. Species $(H)HB^-$ then undergoes an equilibrium protonation to yield $(H)H_2B$ which proceeds in an equilibrium reaction to $(H)BH_2$ with formation of an additional -SH moiety. The rate determining steps in the hydrolysis of the cluster involves spontaneous (k_1) and specific acid catalyzed ($k_2[H^+]$) reactions of $(H)BH_2$. A detailed discussion of the mechanism will be provided elsewhere. The constants provided in Scheme I were obtained in the following manner. The constant K_{a_1} was obtained from the titration curve of Figure 1 and $k_1 + k_{-1}$

were obtained as a sum expressed as $k_{AH^-} \rightarrow BH^-$ which was employed in the fit of the $AH^- \rightarrow BH^- \rightarrow C$ kinetics of the overall hydrolysis reaction as shown in Figure 2. The remaining constants were obtained as values which provide a line fitting the experimental points of Figure 3 employing equation (3).

The present investigation establishes that protein bound Fe_4S_4 clusters possess acid dissociation constants as do their synthetic analogs. Thus, a pK_a of 2.72 for C.Fd. may be compared with one of 3.92 determined for the alkyl substituted analog, $[Fe_4S_4(SCH_2CH(CH_3)_2)_4]^{2-}$ (solv. 60/40 vol. percent N-methylpyrrolidinone/H_2O) (2). The observation of a single titration curve for C.Fd., which contains two Fe_4S_4 clusters, implies either very similar cluster environments or the unavailability of one of the clusters. The first case is preferred since both clusters are rapidly hydrolyzed. The unavailability of the Fe_4S_4 cluster of HIPIP for either protonation (over the pH range 0 to 7), or hydrolysis relative to that of C.Fd., is in accord with the established inaccessibility of the iron-sulfur cluster in native HIPIP toward reducing agents in water and with x-ray crystallographic indication of a protective peptide sheath surrounding the Fe_4-S_4 cluster (3). As in the case of reduction of HIPIP (4), its hydrolysis is accelerated on transfer from water to DMSO/H_2O. This is presumably due to a conformational change.

ACKNOWLEDGEMENT

This work was supported by a grant to T.C.B. from the National Institutes of Health.

References

1. Bartsch, R. G. (1971) in Methods in Enzymology, Vol. XXII, Part A, A. San Pietro, ed., pp. 644-649, Academic Press, New York.

2. Bruice, T. C., Maskiewicz, R., and Job, R. (1975) Proc. Natl. Acad. Sci. USA, 72, 231-234.

3. Carter, C. W. Jr., Kraut, J., Freer, S. T., Xuong, N., Alden, R. A., and Bartsch, R. G. (1974) J. Biol. Chem., 249, 4212-4225.

4. Cammack, R. (1973) Biochem. Biophys. Res. Commun., 54, 548-554.