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Kinetics of the Cr^{II}edta Reduction of the Water-Soluble $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ Cluster to the Superreduced Form and Related Studies

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Reduction of the iron–sulfur cluster $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ with 1 equiv of Cr^{II}edta (reduction potential ca. −1.0 V) gives the 7– species in a rapid process, which is too fast to follow with the stopped-flow method. With Cr^{II}edta in large excess a second stage first order in both reactants and giving the superreduced (8–) cluster could be monitored on the stopped-flow time scale. This step is independent of pH in the range 8.0–9.4 investigated, I = 0.10 M (NaCl), with free β-mercaptopropionate ligand (0.05 M) as buffer, and is also independent of buffer concentration (0.10–0.40 M), I = 0.40 M (NaCl). At 20 °C $k = 2.2 \times 10^5$ M^{-1} s^{-1}, and activation parameters are $\Delta H^{\ddagger}$ = 10.3 kcal mol^{-1} and $\Delta S^{\ddagger}$ = 0.2 cal K^{-1} mol^{-1}. UV–visible spectra of the reduced and superreduced forms were obtained, and cyclic voltammetry experiments gave quasi-reversible and reversible waves for the first and second stages respectively, reduction potentials −0.56 and 0.95 V (against SHE), in keeping with the observed redox behavior. Observations on the stability of the 7– and 8– clusters are reported. The Cr(II) complex with the macrocyclic 15-aneN_4 ligand is a weaker reductant and does not reduce the 6– clusters. No superreduced forms of the 8-Fe protein from *Clostridium pasteurianum* or of the HIPIP protein from *Chromatium vinosum* were obtained with use of Cr^{II}edta as reductant.

Holm and colleagues have prepared and characterized a range of analogues of the active site of iron–sulfur proteins,[1-5] where the latter are now known to have key roles in many biological processes.[6,7] The chemistry of these clusters containing a variety of organic mercaptides in place of the cysteine residues of the polypeptide chains has helped immensely in the understanding of the function and behavior of the proteins.[8,9] The preparation (and X-ray crystal structure) of the water-soluble 4-Fe cluster $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ has recently been described,[10] using a modified procedure.[2] Because measured reduction potentials (and redox properties generally) of the Holm analogues in nonaqueous solvents differ considerably from those in aqueous media (the medium in which the proteins can be studied),[11] we thought this cluster an ideal compound to compare redox interconversions in aqueous solution by using simple inorganic complexes as redox partners.

Although chromium(II) is one of the strongest known metal ion reductants, use of $Cr(H_2O)_6^{2+}$ at pH >4 is questionable because of the tendency of the $Cr(H_2O)_6^{3+}$ product to polymerize and at the higher concentrations to precipitate. Instead we here explore the possible use of two more versatile Cr(II) complexes, Cr^{II}edta (edta ≡ ethylenediaminetetraacetate), in which the edta is believed to be quinquedentate with H_2O coordinated in the sixth position, and the Cr(II) complex of the macrocyclic ligand 15-aneN_4 (1,4,8,12-tetraazacyclopentadecane).

Experimental Section

Preparation of Reactants. The iron–sulfur cluster $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ was prepared as a salt with six cations (one tetrabutylammonium, $N(C_4H_9)_4^+$, and five Na^+) and five molecules of *N*-methylpyrrolidinone (C_5H_9NO) of crystallization, by the procedure described by Carrell et al.[10] The purity of the compound was established by comparison of the visible absorption spectrum λ(max) 300 nm (ε 2.0×10^4 M^{-1} cm^{-1}) and λ(sh) 400 nm (ε 1.6×10^4 M^{-1} cm^{-1}), which are in excellent agreement with published values λ(max) 300 nm (ε 1.98×10^4) and λ(sh) 400 nm (ε 1.63×10^4 M^{-1} cm^{-1}).[12] The full spectrum is shown together with product spectra in Figure 1. Over the pH range studied 8.0–9.4, the β-mercaptopropionate used in the cluster, i.e., $HSCH_2CH_2CH_2CO_2^-$ (pK_a = 10.1 in 0.1 M $LiClO_4$[12]), was used as buffer. This buffer was essential in order to suppress the aquation of the coordinated β-mercaptopropionate (hereafter mercaptide). All solutions were made up under rigorous air-free conditions (N_2 gas) by using standard syringe, stainless-steel needle, and serum cap techniques.

Crystals of $CrCl_2 \cdot 4H_2O$ were prepared by a procedure described[13] and stored under N_2. A stock solution of 0.5 M Cr^{2+} in 0.1 M HCl was obtained by adding HCl to a sample of solid in a round-bottomed flask under N_2 (the solid was loaded in a glovebag). Due to the extreme oxygen sensitivity of Cr^{II}edta the complex was generated in the stopped-flow apparatus by using the following procedure. The drive syringe was first loaded with 1 mL of Cr^{2+} (concentration 0.1 M). This was then pushed back into the storage syringe containing 0.1 M Na_2edta solution (10 mL), already thermostated, together with a small magnetic stirrer so that rapid mixing could be achieved. A further 5 min was allowed for thermostating.

The macrocyclic ligand 15-aneN_4 was purchased from Strem Chemicals. The solution of Cr^{II}(15-aneN_4) was prepared by adding a small volume of 0.5 M Cr^{2+} solution to a solution of the ligand, the concentration of which was 1.5–2.0 times that of the final Cr(II) concentration, i.e., sufficient to give constant spectrum. This solution was then loaded into the stopped-flow apparatus.

Samples of the 8-Fe protein from *Clostridium pasteurianum*[14] and high potential iron–sulfur protein (HIPIP) from *Chromatium vinosum* strain D[15] were isolated by known literature procedures. UV–visible absorbance peak ratios were used as a criterion for purity. The 8-Fe and HIPIP protein solutions were in the oxidized forms and average oxidation states of Fe 2.5 and 2.75, respectively. More extensive studies of other reactions of these proteins and handling procedures adopted will be reported elsewhere.

Kinetic Studies. Reactions were monitored on a Durrum-Gibson stopped-flow spectrophotometer with the Cr(II) reactant (>10-fold) excess. The concentration of $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ was ca. 8×10^{-6} M. First-order rate constants k_{obsd} were obtained from the slopes (×2.303) of plots of absorbance (A) changes at 450 nm, log (A_t –

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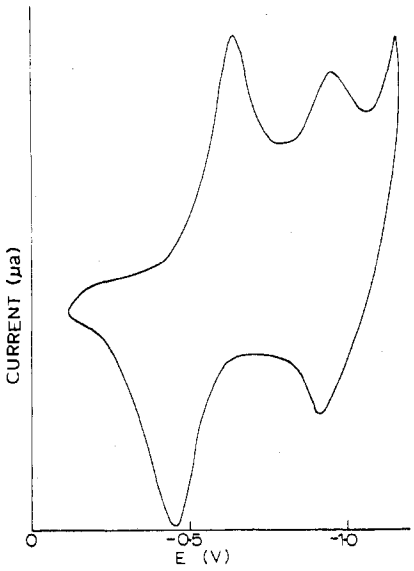

Figure 1. Cyclic voltammogram of a 5×10^{-4} M solution of $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ in 8×10^{-3} M mercaptide buffer, pH 8.5, at a Pt wire electrode recorded at a scan rate of 30 mV s^{-1} and I = 0.10 M ($LiClO_4$).

A_∞), against time. Such plots were linear to at least 50% completion. Rate constants obtained at other wavelengths in the range 300–600 nm were in satisfactory agreement. Once the stopped-flow spectrophometer was loaded with the Cr^{II}edta reactant, rate constants determined over a 5–15-min period (the initial period was required for thermostating) were invariant.

Cyclic Voltammetry. A Chemical Electronics Ltd. (Washington, Co. Durham) potentiostat/signal generator, Type DD50 SU, and J. J. Lloyd X-Y chart recorder PL51 were used. The cell was equipped with platinum working and secondary electrodes, a salt bridge (containing 1.0 M NaCl), and a saturated calomel electrode. Buffer solution (50 mL of 8 mM or 16 mM $HSCH_2CH_2CO_2H$/NaOH at pH 8.5) with I = 0.10 M ($LiClO_4$) was deoxygenated for at least 1 h by using a N_2 stream (Teflon tubing). A weighed amount of analogue to give a 0.5 mM solution was dissolved in ca. 5 mL of deoxygenated solution and then transferred to the bulk solution in the cell.

Results

The cyclic voltammogram of the $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ cluster is shown in Figure 1. A first wave corresponding to a quasi-reversible process involving 6– and 7– species is followed by a second wave involving 7– (reduced) and 8– (superreduced) species in a process which is certainly quasi-reversible and possibly reversible. Measured reduction potentials (against the standard hydrogen electrode) are –0.52 (±0.01) and –0.94 (±0.01) V in the presence of 16 mM mercaptopropionate buffer (four determinations) and –0.58 and –0.97 V in 8 mM buffer (two determinations). The same first-wave potential was obtained when the voltammogram was reversed before the second wave. Average potentials are –0.56 and –0.95 V, respectively.

Spectra of the reduced (7–) and superreduced (8–) forms were obtained point by point from initial and final absorbance readings on stopped-flow traces at a fixed λ (Figure 2). The spectrum of the reduced form was also determined by mixing equivalent amounts of Cr^{II}edta and $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ in an optical cell and monitoring on a conventional scan spectrophotometer. It was found that over periods of a few minutes the 7– cluster aquates appreciably unless 0.7 M mercaptide is present. Final absorbance values for the superreduced form were satisfactorily recorded on the stopped-flow time scale. However over periods of up to 1–2 min the cluster undergoes decomposition. Although the recurring ca. 360-nm feature in the spectra suggests that some 7– species may be retained in the final spectrum, the kinetic data below suggest that this is <10%.

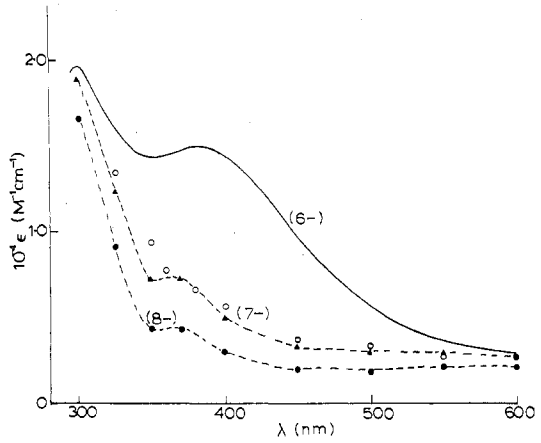

Figure 2. Spectrum of $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ (—) and of the reduced 7– (▲) and superreduced 8– (●) species as recorded from initial and final absorbance values in stopped-flow experiments at pH 8.5 (mercaptide), I = 0.10 M (NaCl), with use of an excess of the Cr^{II}edta reductant (concentrations as in kinetic runs). The 7– species was also obtained as the final product by mixing 1:1 amounts of the two reactants and the spectrum (○) recorded.

Table I. Rate Constants, k_{obsd}, for the Second Stage of the Cr^{II}edta Reduction of $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ (ca. 8×10^{-6} M) at 15 °C, pH 8.5 (Mercaptide Buffer), I = 0.40 M (NaCl), λ 450 nm, and Varying Buffer Concentrations

[buffer],[a] M	10^4[Cr^{II}edta], M	k_{obsd}, s^{-1}
0.1	0.5	16.1
	1.0	29.6
0.2	0.5	15.7
	1.0	24.2
0.4	0.5	13.8
	1.0	29.9

[a] Mercaptopropionate.

Table II. Rate Constants, k_{obsd}, for the Second Stage of the Cr^{II}edta Reduction of $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ (ca. 8×10^{-6} M) at I = 0.10 M (NaCl), λ 450 nm, and pH 8.5 (Except As Stated), in 0.050 M Mercaptide Buffer

temp, °C	10^4[Cr^{II}edta], M	k_{obsd}, s^{-1}
5	0.50	3.0
	4.0	28.0
10	0.50	5.2
	0.50	5.89
	0.50	6.96
	1.0	11.0
	2.0	25.5
	4.0	50.0
	4.0	47.2[a]
	4.0	45.5[b]
	6.0	77.2
15	0.25	4.0
	0.50	9.3
	4.0	66.8
20	0.50	12.2
	4.0	85.4

[a] pH 8.0. [b] pH 9.4.

First-order rate constants, k_{obsd}, corresponding to the Cr^{II}edta reduction of the $Fe_4S_4(SCH_2CH_2CO_2)_4^{7-}$ cluster were independent of the concentration of mercaptide buffer 0.10, 0.20, and 0.40 M, I = 0.40 M (NaCl) (Table I). All other runs were with 0.05 M buffer, I = 0.10 M (NaCl) (Table II). No dependence of rate constants on pH 8.0–9.4 was observed. A linear, i.e., first-order, dependence of k_{obsd} against [Cr^{II}edta] (Figure 3) was observed. Hence from the temperature dependence (5–20 °C) of second-order rate constants, activation

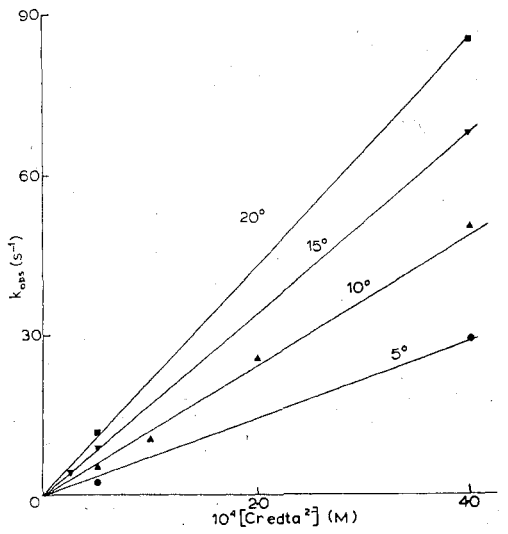

Figure 3. Dependence of first-order rate constants, k_{obsd}, on concentration of Cr^{II}edta; pH 8.5 (0.05 M mercaptide buffer); λ 450 nm; I = 0.10 M (NaCl).

parameters were $\Delta H^{\ddagger}$ = 10.3 ± 2.0 kcal mol^{-1} and $\Delta S^{\ddagger}$ = 0.2 ± 6.9 cal K^{-1} mol^{-1}.

No reduction of the $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ cluster by the Cr^{II}(15-aneN$_4$) complex was observed. Reaction of the oxidized forms of the 8-Fe and HIPIP proteins with excess Cr^{II}edta resulted in one-electron reduction only of the Fe_4S_4 clusters.

Discussion

The present study describes redox interconversions of the water-soluble $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$ cluster. With Cr^{II}edta as reductant it has been shown that the reaction sequence is as in (1) and (2), where (2) but not (1) could be monitored

$$Fe_4S_4(SR)_4^{6-} + Cr(II) \xrightarrow{\text{very fast}} Fe_4S_4(SR)_4^{7-} + Cr(III) \quad (1)$$

$$Fe_4S_4(SR)_4^{7-} + Cr(II) \xrightarrow{\text{fast}} Fe_4S_4(SR)_4^{8-} + Cr(III) \quad (2)$$

on the stopped-flow time scale. The 6– cluster is known to be singly protonated at pH 6 (pK_a = 7.4).[12] With the mercaptide as the buffer the pH range 8.0–9.4 was investigated, and over this range (2) was found to be free from protonation–deprotonation effects. Reaction 2 was investigated under pseudo-first-order conditions with the Cr(II) in large >10-fold excess. For a 12-fold variation in Cr(II) the data conformed to the simple rate law (3). At 20 °C the rate constant k is

$$\text{rate} = k[Cr(II)][Fe_4S_4(SR)_4^{7-}] \quad (3)$$

2.2×10^5 M^{-1} s^{-1} with activation parameters $\Delta H^{\ddagger}$ = 10.3 kcal mol^{-1} and $\Delta S^{\ddagger}$ = 0.2 cal K^{-1} mol^{-1}. Changes in solvation attendant on the reaction of 2– and 7– species might have been expected to give a more negative $\Delta S^{\ddagger}$ term. The 7– charge is however spread out over a large reactant, and the small value probably stems from this and the unusual structure of the cluster and attendant solvation.

The kinetics was studied in the presence of 0.10 M mercaptide, where in separate experiments it was shown that a variation of 0.10→0.40 M in concentration of buffer had no effect on rate or final absorbance. It was therefore concluded that the four mercaptide ligands remained attached to the 6–, 7–, and 8– species until completion of the redox processes 1 and 2. This was confirmed by the cyclic voltammogram when with mercaptide concentration as low as 8 mM reversibility was observed by using a 30 mV s^{-1} scan speed. No evidence was obtained for the involvement of $(SCH_2CH_2CO_2)_2^{2-}$ in the redox cycle.[16] In kinetic studies the 8– superreduced species decomposed within 1–2 min of completion of a run, and it was not possible to introduce an oxidant and reoxidize. For a similar reason it was not possible to test whether the Cr(II) reduction (2) was by an inner-sphere mechanism with initial retention of the Cr(III) product. This would in any case have been a difficult experiment with Cr^{II}edta because of the known anomalous lability off the Cr^{III}edta complex.[17,18] Had the 8– cluster been stable, then experiments with Cr^{II}med3a (med3a ≡ ethylenediamine-*N*-methyl-*N*,*N*′,*N*′-triacetate) as a reductant, where the Cr(III) product is known to be inert,[17] would have been appropriate.

In separate studies we attempted to investigate oxidation of the 7– cluster with Co(III) complexes $Co(en)_3^{3+}$ and $Co(dmg)_2(PhNH_2)_2^{+}$ (dmg ≡ dimethylglyoximate). The mercaptide concentration had to be increased to 0.7 M to stabilize the 7– cluster to aquation over the ca. 10-min period required for thermostating. Under these extreme conditions it was difficult to obtain satisfactory reproducible results. A number of side reactions appear to contribute, and we did not attempt to extend these studies further.

The redox behavior in (1) and (2) is consistent with thermodynamic driving forces. Thus the reduction potentials for the 6–, 7– and 7–, 8– couples from the cyclic voltammogram (Figure 1) of −0.56 V and −0.95 V are in excellent agreement with values −0.58 V and −0.93 V determined previously by polarography.[12] The Cr^{II}edta complex is known to have a reduction potential close to −1.0 V.[19] The rapidity of both (1) and (2) is consistent with efficient electron-transfer interconvertibility of the Fe_4S_4 cluster oxidation states, i.e., high self-exchange rate constants, probably stemming from minimal atom reorganization within the cluster arrangement. Surprisingly with the Cr^{II}(macrocyclic) complex (reduction potential −0.58 V)[20] no reduction of the 6– cluster was observed even when a large 10^4-fold excess of the reductant was used. A possible explanation is that the mercaptide buffer complexes in the axial positions and that the reduction potential is thus less favorable.

Finally Cr^{II}edta reduces the Fe_4S_4 clusters of the 8-Fe protein (average oxidation state 2.5) to the normal reduced state (2.25) but no further. Similarly with the HIPIP protein the normal one-electron reduction (2.75 → 2.50) is observed with Cr^{II}edta, but again no superreduced form is generated. Only when proteins are in an unfolded state in ≥40% Me_2SO can superreduced ferredoxin and HIPIP forms be obtained by electrochemical means.[11]

Two other water-soluble clusters $Fe_4S_4(SCH_2CH_2OH)_4^{2-}$ and Fe_4S_4(peptide)^2 have been reported,[11] and comparisons with redox behavior of these species would be of further interest. Although formation of a superreduced species is not of physiological relevance in the redox cycle of the proteins, it is nevertheless of interest to compare the behavior of proteins and analogues. The polypeptide chain appears to have a considerable protective influence which prevents reduction to the 2.0 oxidation state in the case of the 8-Fe protein and to the two lowest oxidation states in the case of the HIPIP protein.

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Registry No. $Fe_4S_4(SCH_2CH_2CO_2)_4^{6-}$, 56997-14-9; Cr(II)–edta, 12558-56-4; $Fe_4S_4(SCH_2CH_2CO_2)_4^{7-}$, 74185-19-6; $Fe_4S_4(SCH_2CH_2CO_2)_4^{8-}$, 74081-84-8.

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