Reaction of Hydrogen Sulfide with Native Horse Spleen Ferritin

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Introduction

Ferritin is a protein that consists of 24 subunits that self-assemble to form a roughly spherical shell with external and internal diameters of approximately 12 and 8 nm, respectively.[3] Hydrophilic and hydrophobic channels connect the internal cavity with the exterior space. These channels are thought to facilitate the passage of ions into and out from the central cavity of the protein. Different forms of ferritin are found in all five living kingdoms and function variously as iron-storage, -detoxification, -regulation, and -transport proteins.[3-6] Iron is usually bound in the form of an inorganic solid phase of hydrated iron oxyhydroxide or phosphate within the central cavity of the molecule.[7] The composition, size, and structure of the inorganic phase vary depending on the source and history of the ferritin.

Recently it was proposed that the supramolecular protein structure of ferritin could be used as a reaction cage to engineer novel inorganic nanospace structures with structural and optical properties that may find application in catalysis and optoelectronic devices.[8,9] Ferritin has already been used to synthesize discrete inorganic nanospace structures containing iron sulfides, manganese oxides, uranium oxides,[8] and ferrimagnetic iron oxides.[10] It was shown using transmission electron microscopy (TEM) and energy-dispersive X-ray analysis (EDXA) that iron and sulfur were present within ferritin cages after reaction of native ferritin (containing ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$)-like cores) with hydrogen sulfide gas.[8] The aim of the present work is to use Mössbauer spectroscopy (i) to determine the extent of the conversion of ferrihydrite to iron sulfide phases and (ii) to determine the nature of the iron sulfide phase(s) generated.

Materials and Methods

Native horse spleen ferritin solution was obtained from Sigma Chemical Co. Synthetic ferrihydrite was prepared by adding potassium hydroxide solution to iron(III) nitrate solution such that the final pH was between 7 and 8.[11] The precipitate was dialyzed with distilled water for 2 weeks and was then freeze-dried. Hydrogen sulfide gas was prepared by reacting HCl with pyrite (FeS_2).

The ferritin solution (500 μL) was buffered with 100 μL of 0.1 M Trizma base (Sigma) at pH 8.5. A suspension of the synthetic ferrihydrite was prepared by adding 200 mg of synthetic ferrihydrite to 5 mL of 0.1 M Trizma base at pH 8.5. The ferritin solution and the ferrihydrite suspension were then deaerated by bubbling nitrogen gas through for 15 min. Hydrogen sulfide gas was then bubbled through for 90 s. Both the ferritin solution and the ferrihydrite suspension turned black within 20 s, as has been reported previously.[8] This procedure was carried out on nine samples of horse spleen ferritin from two different stock solutions with similar results each time. One sample (0.6 mL) each of the black ferritin solution and the ferrihydrite reaction products were then immediately transferred to 19 mm diameter nylon sample holders and frozen in liquid nitrogen ready for Mössbauer spectroscopic measurements. Mössbauer spectra were recorded and analyzed using α-Fe as a calibration reference as described elsewhere.[12]

Results

Reaction with Ferritin. The reaction of hydrogen sulfide gas with clear reddish brown horse spleen ferritin solution at pH 8.5 produced a black solution with no bulk precipitation. This suggested that most of the black reaction product was bound within ferritin molecules. However, after a few hours, a greenish tinge was noted, and eventually a cloudy white precipitate was also observed.

Mössbauer spectra of the sample of reaction product that was immediately frozen are shown in Figures 1 and 2. Spectral parameters derived from fitting Lorentzian peaks to the data are shown in Table I. Figure 1 shows the Mössbauer spectra recorded over the velocity range −13 to +13 mm/s with the sample at temperatures between 13.5 and 78 K. With a sample temperature of 78 K, the spectrum appears to consist of two doublets. As the temperature is lowered, a sextet of broad peaks due to magnetic hyperfine field (B_{hf}) splitting appears in the wings of the spectrum. At 40 K, this component has $B_{hf} = 45.4 \pm 0.5$ T and continues to grow in intensity at the expense of doublet 1 as the temperature is lowered. By 13.5 K, B_{hf} has increased to 48.2 ± 0.5 T.

Between 40 and 20 K, a second sextet of broad peaks appears with a lower value of B_{hf} (Table I). The appearance of sextet 2 also appears to be at the expense of doublet 1, since the relative intensity of doublet 2 appears to be invariant with temperature (Table I).

The appearance of two discrete magnetic hyperfine field components from doublet 1 implies that doublet 1 has a more detailed structure. Indeed, at least four doublets were needed to fit the spectrum of the sample at 78 K when recorded over the range −4 to +4 mm/s (Figure 2, Table I).

Reaction with Ferrihydrite. The reaction of hydrogen sulfide gas with the cloudy brown ferrihydrite suspension at pH 8.5 produced a cloudy black suspension (in contrast to the reaction with ferritin solution). A greenish tinge and cloudy white precipitate were also seen after a few hours.

Mössbauer spectra of the sample of reaction product that was immediately frozen are shown in Figure 3. Spectral parameters derived from fitting Lorentzian peaks to the data are shown in Table I. The spectra at 78 and 13.5 K are qualititatively similar to those of the horse spleen ferritin/hydrogen sulfide reaction product. The main quantitative difference between the two sets of spectra is the approximate 50% reduction in spectral contribution from sextet 2 and doublet 2 (Table I). The apparent disappearance of doublet 2 at 13.5 K is probably due to the very small signal being obscured by sextets 1 and 2.

Discussion

The results shown here indicate that the reactions of hydrogen sulfide gas with (i) native horse spleen ferritin solution and (ii) a suspension of ferrihydrite particles at pH 8.5 generate similar

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Table I. Mössbauer Spectral Parameters for the Products of the Reactions of Hydrogen Sulfide Gas with Native Horse Spleen Ferritin Solution and Aqueous Ferrihydrite Suspension at pH 8.5[a]

		doublet 1			doublet 2			sextet 1				sextet 2			
	T, K	δ	ΔE_Q	%A	δ	ΔE_Q	%A	δ	ΔE_Q	B_{hf}	%A	δ	ΔE_Q	B_{hf}	%A
H_2S + ftn	78	0.41	0.76	93	1.30	2.98	7								
±13 mm/s	13.5	0.24	0.61	12	1.45	2.77	7	0.46	−0.08	48.2	53	0.43	0.00	24.8	29
H_2S + fhdt	78	0.42	0.70	97	1.19	2.70	3								
±13 mm/s	13.5	0.05	0.59	2			0	0.44	−0.02	47.2	83	0.38	−0.02	24.0	15

		doublet 1a			doublet 1b			doublet 2a			doublet 2b		
	T, K	δ	ΔE_Q	%A	δ	ΔE_Q	%A	δ	ΔE_Q	%A	δ	ΔE_Q	%A
H_2S + ftn ±4 mm/s	78	0.35	0.73	50	0.59	0.78	41	1.34	3.13	5	1.18	2.84	3

[a] ftn = ferritin solution; fhdt = ferrihydrite suspension. δ is the chemical isomer shift relative to metallic Fe (mm/s), ΔE_Q is the quadrupole splitting (mm/s), B_{hf} is the magnetic hyperfine field splitting (T), and %A is the percentage spectral area of each component. Approximate errors: δ, ±0.02; ΔE_Q, ±0.03; B_{hf}, ±0.5; %A, ±2. Where the value of %A is low (<10%), the errors are somewhat larger.

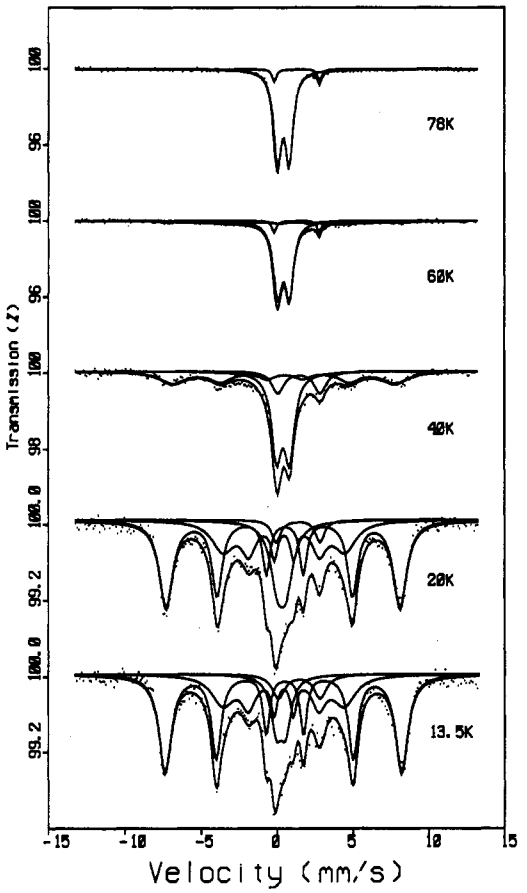

Figure 1. Mössbauer spectra of the products of the reaction of H_2S gas with horse spleen ferritin solution at pH 8.5 recorded over the temperature range 13.5–78 K.

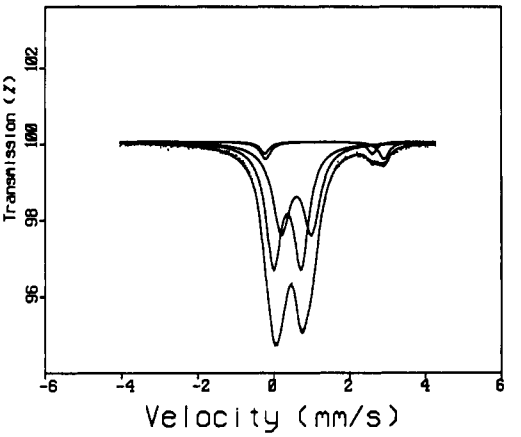

Figure 2. Mössbauer spectrum of the products of the reaction of H_2S gas with horse spleen ferritin solution at pH 8.5 recorded at 78 K.

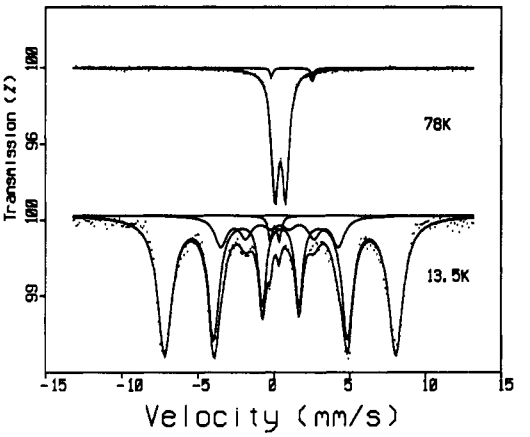

Figure 3. Mössbauer spectra of the products of the reaction of H_2S gas with aqueous ferrihydrite suspension at pH 8.5 recorded at 13.5 and 78 K.

products except that (a) the black reaction product is retained in solution in the case of ferritin (cf. Meldrum *et al.*[8]) and (b) the transformation of the ferritin cores and the ferrihydrite particles to iron sulfide is only partial.

In both the ferritin solution and ferrihydrite suspension reaction products, the appearance of sextet 1 at the expense of doublet 1 in the Mössbauer spectra as the temperature is lowered is characteristic of superparamagnetic nanoscale particles of ferrihydrite.[7] The spectral parameters of these components are also consistent with those of ferrihydrite.[7,13] Thus the Mössbauer spectra indicate that, in these particular samples, 53–65% of the ferritin iron and about 83% of the ferrihydrite iron remained in the ferrihydrite form after reaction with H_2S gas for 90 s. It has yet to be determined what effect experimental conditions such as rate of addition of H_2S, pH, and ionic strength have on the quantitative extent of the reaction.

The black color of the reaction products of H_2S gas with ferritin solution and with ferrihydrite suspension is characteristic of several iron sulfide phases. The iron sulfides (Fe_xS_2) range in stoichiometry ($2 \geq x \geq 1$). Both pyrite (FeS_2) and marcasite (FeS_2) are yellowish. They are diamagnetic low-spin iron(II) sulfides with Mössbauer spectral parameters that are very different from those of any of the components in the present study (pyrite at 81 K, δ = 0.40 mm/s, ΔE_Q = 0.62 mm/s; marcasite at 81 K, δ =

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0.37 mm/s, $\Delta E_Q = 0.50$ mm/s).[14] Thus pyrite and marcasite are not present in the reaction products. Troilite (FeS) is an antiferromagnetic mineral which has a Mössbauer spectrum with B_{hf} about 32 T and $\delta = 0.8$–1.0 mm/s at 77 K[15,16] and thus is also not implicated as a reaction product.

All iron sulfides (Fe_xS_2) with $2 > x > 1$ are ferrimagnetic and show one or more magnetic hyperfine-split components in their Mössbauer spectra at low temperatures.[15] These sulfides include the pyrrhotites ($Fe_{11}S_{12}$, $Fe_{10}S_{11}$, Fe_9S_{10}, β-$Fe_{1-x}S$, Fe_7S_8), smythite (Fe_9S_{11}), greigite (Fe_3S_4), and γ-iron sulfide (Fe_2S_3).

The magnetic hyperfine field splitting of sextet 2 is less than those reported for the ferrimagnetic sulfides. However, the fact that this component shows a magnetic splitting at all indicates that the reaction product is more similar to the ferrimagnetic sulfides than to the diamagnetic ones. In addition, the small-particle nature of this component would be expected to reduce B_{hf} from that for the bulk mineral. Thus, this reaction product may be noncrystalline[8] but could be related to the mixed-valence ferrimagnetic sulfides in terms of its magnetic structure.

Doublet 2 has spectral parameters corresponding to high-spin Fe(II). This may correspond to an intermediate form of iron in the reduction of ferrihydrite to form the ferrimagnetic sulfide. Alternatively, it may correspond to a side product of the reaction. The substructure of doublet 2 (doublets 2a and 2b, Figure 2 and Table I) allows for both of these possibilities simultaneously. The parameters do not correspond to those for $FeSO_4 \cdot 7H_2O$ at 78 K ($\delta = 1.35$ mm/s, $\Delta E_Q = 3.35$ mm/s).[17]

Thus H_2S reacts with the ferrihydrite cores of ferritin in solution at pH 8.5 to give products similar to those produced by its reaction with ferrihydrite particles in suspension. In addition, the ferritin reaction products are retained within the protein shell. Only a fraction of the ferrihydrite is partially reduced and converted to a ferrimagnetic iron sulfide. The incompleteness of the conversion suggests that the reaction may only take place at the surface of the ferritin cores. The higher yield of ferrimagnetic sulfide in the ferritin solution reaction may reflect the smaller ferrihydrite core particle size and hence larger surface to volume ratio compared to those of the suspension of ferrihydrite particles.

Ferritin core sizes typically have a mean of about 7 nm with a standard deviation of about 1 nm.[18,19] Each core contains about 3000 Fe atoms. Assuming that the iron is evenly distributed throughout the core, a 40% conversion of ferrihydrite iron to iron sulfide in the ferritin molecule corresponds to a surface layer about 0.5 nm deep being converted. A 0.5-nm layer of ferrihydrite corresponds to a depth of about 1.3 Fe atoms, suggesting that only the first layer of Fe atoms is attacked by the H_2S reaction. Thus the ferritin/H_2S reaction product may be in the form of 6 nm diameter ferrihydrite spheroids convered by a 0.5-nm skin of ferrimagnetic iron sulfide which is in turn encapsulated in a ferritin protein shell which is about 2 nm thick. These multilayered nanoscale structures are held in aqueous suspension by the hydrophilic nature of the external surface of the protein shell.

We are currently extending our measurements to investigate the effect of longer reaction and deoxygenation times, varying pH, and ferritin core size on the reaction products.

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