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The Structure of an Iron Core Analog of Ferritin*

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ABSTRACT: The structure of a synthetic ferritin-like polymer of approximate composition $Fe_4O_3(OH)_4(NO_3)_2 \cdot 1.5H_2O$ has been investigated using X-ray scattering from aqueous solution, infrared and Mössbauer spectroscopy, and magnetic susceptibility measurements. The ferric ions are all tetrahedrally coordinated by O^{2-}, OH^-, and H_2O in a corner-sharing bridged structure, with Fe–O–Fe bond angles of 125 ± 30°. The nitrate ions are not coordinated directly to the iron, but act only as counterions to the positively charged polymer spheres. As with natural ferritin, the Mössbauer spectrum of the synthetic iron polymer has a paramagnetic doublet spectrum above 77°K and a magnetic hyperfine-split spectrum at 5°K, while in the range 40–70°K, both spectra appear simultaneously, as expected for superparamagnetic particles. The magnetic susceptibility demonstrates that the 70-Å spheres of the synthetic polymer are antiferrmagnetic with a Néel point above 300°K, and a blocking temperature of 8°K.

Ferritin is an iron-carrying protein known to consist of an inner micelle of hydrated ferric oxide–phosphate, having a maximum diameter of about 75 Å which is surrounded by an outer proteinaceous sheath (apoferritin) having an inner diameter of 75 Å and an outer diameter of approximately 120 Å (Harrison, 1964; Bielig *et al.*, 1966). Though the apoferritin shell may be filled with varying amounts of the iron hydroxide–phosphate, at maximum capacity, the core amounts to about 2000 iron atoms (Fischbach and Anderegg, 1965). The apoferritin is thought to consist of about 20 identical peptide chain subunits, and the iron core has a roughly spherical shape, but with many irregularities apparent in the electron micrographs (Haggis, 1965).

As part of an extensive study of the metabolism of iron in the human body, Saltman and coworkers succeeded in synthesizing a polymeric compound, $[Fe_4O_3(OH)_4(NO_3)_2]_n$, which appears to be a remarkable analog to the iron-containing core of ferritin (Spiro *et al.*, 1966). Its diameter of 70 Å is the same, and when the corresponding ferric citrate micelle is placed in a solution of noncrystalline apoferritin, the synthetic core is surrounded by the protein subunits to give a substance whose gross morphology is very similar to that of ferritin (Pape *et al.*, 1968). The micelles of the ferric citrate polymer have a diameter of 75 Å.

In this paper, we present a study of the structural and magnetic properties of the synthetic iron core polymer, and compare them briefly with the properties of the ferritin micelle. Since ferritin is so important in biological iron chemistry, and since little information is available on the structural properties of its iron core, it was hoped that a study of the synthetic compound might shed light on the structure of the natural material. Moreover, the synthetic iron core polymer is an unusual chemical species, and is of great interest in its own right. Our data includes the radial distribution function for the polymer micelles suspended in water, as determined from X-ray scattering, static magnetic susceptibility measurements on the powder from room temperature to 1.4°K, Mössbauer spectra in the range 5–298°K, and vibrational and electronic spectra.

Experimental Section

The synthetic iron core polymer was prepared by the addition of aqueous $KHCO_3$ to an aqueous $Fe(NO_3)_3$ solution, followed by gel filtration (Spiro *et al.*, 1966). In addition to standard analyses for iron, hydroxide

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ion, and nitrate ion, the water content of the "dry" polymer was estimated from the relative infrared intensities of the 1630- and 830-cm^{-1} bands of H_2O and NO_3^-, respectively. Assuming that the oscillator strength ratio of these bands does not vary with their environment, a calibration plot was first obtained using synthetic mixtures of $Mg(NO_3)_2 \cdot 6H_2O$ and KNO_3, and then checked using $Ni(NO_3)_2 \cdot 6H_2O$. From this plot, the synthetic polymer was shown to have 0.72 H_2O/NO_3^-, and this result, when combined with the analytical data, leads to the polymer composition $FeO_{0.75}(OH)(NO_3)_{0.5}(H_2O)_{0.36}$. There are about 1200 iron ions/70-Å particle of this composition (Spiro *et al.*, 1966).

Solutions of 3.36 *m* for the X-ray-scattering experiments were made by dissolving a weighed amount of the polymer in H_2O with a few drops of HNO_3 added to keep the pH approximately 4. In preliminary experiments, it was found that there was a gradual change in X-ray intensity readings as the experiment proceeded. To obviate this, we prepared the solutions 2 days in advance and kept them in a refrigerator. When treated this way, the solutions were stable enough to obtain satisfactory patterns.

The X-ray patterns were determined using monochromatic Mo $K\alpha$ radiation, with data collected out to $s = 11.5$ ($s = 4\pi/\lambda \sin \theta$, where λ is the wavelength and θ is one-half the scattering angle). The experimental techniques have been previously described (Brady, 1957). Intensities were scaled at each angle 2θ to within 0.5% probable error, and after every ten points were recorded, a check was made at selected reference angles to see that there were no changes in scattered intensity during the course of the experiments.

For polymer solutions of 3.36 *m*, the scattering unit was taken as $Fe_{0.0391}O_{0.0293}(OH)_{0.0391}(NO_3)_{0.0196}(H_2O)_{0.8130}$. In this formula, we note that the ratios Fe^{3+}/O^{2-}, Fe^{3+}/OH^-, and Fe^{3+}/NO_3^- are 0.75, 1, and 0.5, respectively.

$$J = \frac{2r}{\pi}\int\left(\frac{I - \sum f_m{}^2}{f_e{}^2}\right)\sin rs ds \qquad (1)$$

The integrals were evaluated on an IBM 7094 computer. In this integral, I is the measured coherent intensity, $\Sigma f_m{}^2$ is the sum of the scattering factors of the components of the system, $f_e{}^2$ is the "average" scattering per electron, and r is the distance of any atom from an arbitrary origin. From this expression, the radial distribution function (eq 2) was derived.

$$4\pi r^2\rho_m(r)\sum K_m = 4\pi r^2\rho_0\sum K_m + J \qquad (2)$$

In this expression, ρ_0 is the average electron density in the solution, K_m is the effective number of electrons in the m-th atom (Warren *et al.*, 1936; B. E. Warren, personal communication), and $\rho_m(r)$ is the density of m atoms at a distance r about a given atom. The values of K_m used here for the different species were $K_{Fe^{3+}} = 33.2$, $K_{O^{2-}} = 8$, $K_{OH^-} = 8$, and $K_{H_2O} = 7.7$. The incoherent scattering of the H's was substracted from the measured intensity, and their coherent scattering was not included in the calculations.

Magnetic susceptibility measurements were made on the synthetic iron core polymer using a pendulum magnetometer (Bozorth *et al.*, 1956) and the Mössbauer spectra were determined on powders using essentially the spectrometer described previously (Kurkjian and Buchanan, 1964a,b). Infrared spectra were taken using KBr pellets and Nujol mulls.

Results and Discussion

X-Ray Scattering. The combination of X-ray scattering and optical spectroscopy was used successfully by us (Brady *et al.*, 1964) in the past to elucidate the structure of polymeric ferric chloride in aqueous solution. However, in the case of the iron core polymer, the interpretation of the optical spectra is not nearly so clear, and we must therefore regard the results of our X-ray-scattering experiments to be of prime importance, and base our interpretations of the other experiments on it. Even before we consider the X-ray-scattering data, however, there are two other facets of the problem which must be considered.

First, solution of the synthetic iron core polymer in water does not appear to alter significantly the morphology of the particles. The viscosity of aqueous polymer solutions is consistent with the particles being spherical (Allerton *et al.*, 1966), and electron microscopy shows that they are spherical after evaporation of the water (Spiro *et al.*, 1966). Furthermore, it is known from X-ray-scattering experiments that the iron micelle of ferritin remains nearly spherical when dissolved in water (Kleinwachter, 1964). Thus, the X-ray results on aqueous solutions appear pertinent to a discussion of the structure of the solid polymer.

Second, an understanding of the primary structure of the synthetic iron core polymer rests, in part, in determining the role of the nitrate ions in the structure, the two possibilities being that either an oxygen of the nitrate ion is directly coordinated to the iron, or that the nitrate ion is simply charged compensating, and bound to the iron core by hydrogen bonding to H_2O and/or OH. Two experiments suggest that the latter is the case. First, in complexes where the NO_3^- ion is bound to the metal through one of its oxygen atoms, the doubly degenerate band ν_3 at $\sim$1350 cm^{-1} is split into two bands, one at $\sim$1500 cm^{-1} and the other at $\sim$1270 cm^{-1}, and the totally symmetric vibration, ν_1, which is forbidden in the undistorted ion, appears strongly at about 1000 cm^{-1} (Nakamoto, 1963). The infrared spectrum of our polymer shows a strong band, broad but apparently unsplit at 1310 cm^{-1} and a weak one at 1030 cm^{-1}, consistent with only a small perturbation away from the ideal D_{3h} symmetry of the free nitrate ion.

In another experiment, a fresh solution of the iron core polymer in H_2O was treated with nitron acetate reagent so as to precipitate all weakly bound nitrate ions. To the filtrate from this solution (deep brown as was the starting solution) was then added sufficient 1 N NaOH to completely degrade the polymer to insoluble Fe_2O_3, thereby freeing any nitrate ion bound internally by coordination to the iron. Addition of nitron acetate reagent to this second filtrate gave a negative test, demonstrating that all the nitrate ion can be stripped from the polymer without denaturing it, and hence that nitrate is not an integral structural member of the polymer. The fact that apparently similar polymer spheres of different anions can be readily synthesized also suggests that the anions are merely charge compensating, and play no great role in determining the primary structure of the polymer particles. It has not yet been determined whether the phosphate groups of the ferritin iron core micelle are directly coordinated to the iron or merely serve as charge-compensating anions. By analogy with the synthetic material, we would guess the latter.

The radial distribution function of the synthetic iron core polymer solution is shown in Figure 1. A well-resolved peak is found at 2.1 Å, followed by a composite peak with a shoulder at about 2.9 Å and a maximum at 3.4–3.5 Å. Other maxima occur at 4.8, 5.5, and 6.3 Å. The region before the first peak shows very little ripple, attesting to the accuracy of the curve fitting. The very weak peak at 1.1 Å is partly due to the intramolecular N–O vector in the NO_3^- ion (1.21 Å), but this contribution is very small, and has been displaced because of the ripple.

We can identify the peak at 2.1 Å as arising from the Fe–X (X = O^{2-}, OH^-, H_2O) interaction, since the ionic radius of O^{2-} is 1.40 Å, that of OH^- is 1.33 Å, and the radius of Fe^{3+} is 0.64 Å. The measured area of the 2.1-Å peak is 75 e^2, and therefore $0.0391 \times 2 \times 33.2 \times 8.0 \times n_1 = 75$, where n_1 is the number of oxygen neighbors about Fe. The value of n_1 is thus 3.6, indicating that on the average there are four oxygen-containing ligands about each Fe^{3+}. In view of the affinity of Fe^{3+} for tetrahedral coordination in oxides, as evidenced by the structures of α-Fe_2O_3, Fe_3O_4, yttrium iron garnet, and iron in silicate glasses, we conclude that the iron in the synthetic iron core polymer is tetrahedrally surrounded by its ligands. Lest there be any doubt about the assignment of the K_m's we point out that if one takes K_m as the number of electrons in the ion, *i.e.*, $Fe^{3+} = 23$ and $O^{2-} = 10$, one obtains a value of $n_1 = 4.1$, again indicating tetrahedral coordination.

Thus, having established the basic close-order structure of the polymer, we proceed to glean as much further information as we can from the radial distribution function. Since the formula of the dry polymer does not allow anywhere near four oxygens per iron (exclusive of the nitrate ions), the first question which arises is whether the tetrahedra share edges or corners. Edge-sharing is immediately ruled out because this would result in a strong peak at 2.4 Å caused by the Fe–Fe interaction which would result therefrom. Also, any tilting of edge-shared tetrahedra would give rise to a peak at a distance less than 2.4 Å, and examination of the curve shows that there is no peak in this region. Thus our second deduction is that the structure is basically a corner-shared tetrahedral one.

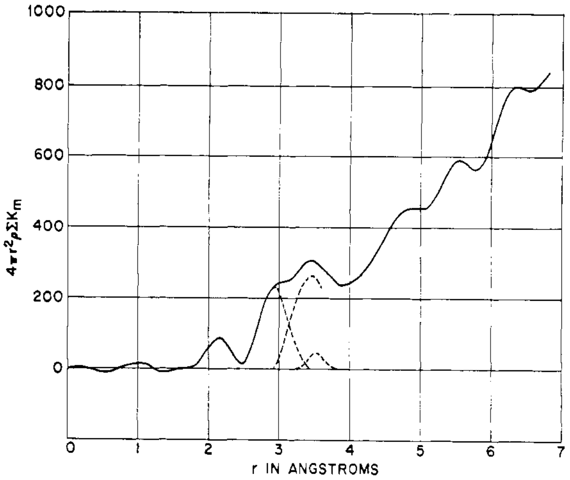

FIGURE 1: The radial distribution function of the synthetic iron core polymer in 10^{-4} M HNO_3.

With reference to the determination of the Fe–Fe distance, it is possible to get an estimate of this from the radial distribution function in the following way. In Figure 1, the region of the curve between 2.5 and 4 Å has been resolved into three peaks by first assuming that the shoulder at approximately 2.9 Å is due to the H_2O–H_2O interaction which occurs in liquid H_2O at 2.92 Å. Centering the peak at this distance and drawing in the right side symmetrically with the left, one obtains a peak area of 215 e^2. The calculated value is 210 e^2, but this remarkable agreement is probably fortuitous since we have made no correction for the different environment of the H_2O's at the surface of the polymer. The important point is however that when the 2.9-Å peak is subtracted from the rest of the curve, there results a peak with a maximum at 3.45 Å. In a tetrahedral structure, the ligand–ligand distance is $\sqrt{(8/3)}a$, where a is the metal–ligand distance. Thus we expect a peak at about 3.4 Å, and it should amount to 30 e^2. As shown in the figure, when this second peak is subtracted, there still remains a clearly resolved peak at about 3.5 Å which we attribute to the Fe–Fe interaction. The measured area of the peak is 300 el^2, indicating an average Fe–Fe coordination of approximately six. Since our structure is one in which the tetrahedra share at most four corners, we would expect at most four neighbors. However, there are still other interaction vectors in the 3.5-Å region. Thus there are second nearest neighbor O–O distances, and if we regard the radial distribution function as the sum of the solute and solvent curves, the H_2O–H_2O peak also contributes substantially to the area in this region (Morgan and Warren, 1938; Danford and Levy, 1962). With these facts in mind, and also because the peak decomposition scheme is so approximate, we feel the analysis correctly predicts the Fe–Fe distance and are not disturbed by the discrepancy in the nearest neighbor numbers.

If the corner-sharing tetrahedra are connected by 180° Fe–O–Fe bridges, then the Fe–Fe interaction would be expected to appear at 4.2 Å. No peak is found at this distance, showing that the bridge is bent. Thus the proposed Fe–Fe distance of 3.45 Å leads to an Fe–O–Fe angle of 125 ± 30°. The error in the Fe–O–Fe angle is estimated from consideration of the Fe–Fe

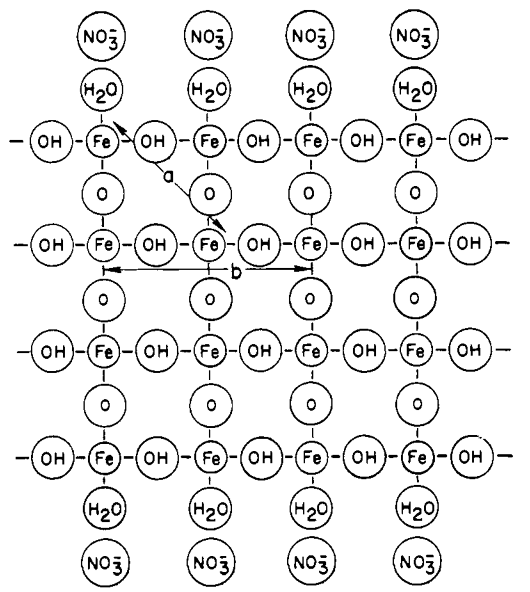

FIGURE 2: A proposal secondary structure of $Fe_4O_3(OH)_4(NO_3)_2 \cdot 1.5H_2O$ shown as a projection on the plane of the paper. The vectors *a* and *b* are the probable Fe–Fe interactions corresponding to the two peaks at 5.6 and 6.4 Å in the radial distribution function. The NO_3^- ions are thought to be hydrogen bonded to the H_2O molecules on the surface.

peak width, and is of the same magnitude as found for the Si–O–Si angle in SiO_2 glasses (B. E. Warren, personal communication). As is mentioned below, the infrared spectrum of the synthetic iron core polymer also supports the bent-bridge geometry.

The next three peaks occur at distances of 4.75, 5.5, and 6.3 Å. Because of their magnitude, we can ascribe the last two to Fe–Fe interactions of various kinds beyond the first neighbor ones, and probable vectors for these peaks are shown in Figure 2. The peak at 4.75 Å is to be expected from the tetrahedral edge H_2O–H_2O interaction in liquid water (Morgan and Warren, 1938; Danford and Levy, 1962).

The stoichiometry of the $FeO_{0.75}(OH)(NO_3)_{0.5}(H_2O)_{0.36}$ polymer and the radial distribution function demand that there be a virtually complete sharing of the O^{2-}, OH^-, and H_2O ligands between iron ions if a tetrahedral coordination is to be achieved. Moreover, our measurement of the density of the dry polymer (2.9–3.0) shows that the structure is a compact one, with few voids and few atoms not multiply bonded. A similar conclusion was drawn in the case of the ferritin micelles. As regards the over-all shapes of the micelles, those of the synthetic iron core polymer appear as spheres in electron micrographs, but those of ferritin, when prepared for electron micrography, have various shapes ranging from spheres, through regular polyhedrons to the most irregular shapes. If we imagine a central tetrahedron connected to four others through O^{2-} and OH^- bridges and each of these then connects to three other tetrahedra, and so on, it is clear that virtually any gross morphological shape can be attained by branching in the proper direction. Such a random picture leads to no special arrangement of the tetrahedra within the micelles. While this is good in a sense, because we have no data on this particular aspect of the structure, it is not meant to imply that we rule out a crystalline arrangement of atoms in the micelle. As a matter of fact, the model which gives the best interpretation of all the experimental facts and at the same time fulfills the requirements of stoichiometry (as written above) and local charge balance is the periodic one shown in Figure 2, where the tetrahedra are shown as projections in the plane of the paper. It can be seen immediately that such a structure has the proper stoichiometry, and with each internal Fe having two O^{2-} and two OH^- ions as ligands and each external Fe having one O^{2-}, two OH^-, and one H_2O, NO_3^- as ligands, the local charges balance exactly. Such a structure is in a sense idealized, however, since two O^{2-} and two OH^-'s can be readily interchanges without upsetting the local charge balance. However, the interchange of O^{2-} and OH^- cannot be done at random without upsetting this balance. The apparent flexibility at the Fe–O–Fe bridges introduce a certain randomness into the system, which reflects itself in the large uncertainity which we report for this angle. In this model, the polymer particle is seen to be a flat ribbon almost 100 times as long as it is wide, and the over-all spherical shape of the particle could possibly come from the gathering of such a chain into a ball *via* occasional bridging cross-links.

Another feature of the synthetic iron core polymer of possible structural consequence which correlates with the above model is the fact that regardless of the ligand, only a maximum of 50% of the iron within a micelle can be extracted (P. Saltman, unpublished results), just the fraction of iron ions "at the surface" in the model described above. On the other hand, in a spherical micelle of unspecified internal structure containing 1200 iron atoms, 38% of these are on the surface. If the over-all symmetry of the micelle is less than spherical, thereby increasing the fraction of atoms on the surface, it is imaginable that the 50% extractable-iron figure then refers to the fraction of irons on the surface. This connection is hypothetical, however, and we mention it only as a possible guide to further experiments.

Vibrational and Electronic Spectra. The vibrational spectrum of the nitrate ion is discussed above, in the section on nitrate binding. In addition to the nitrate ion bands, the infrared spectrum of the polymer shows a moderately strong band at 700 cm^{-1}, a stronger one at 450 cm^{-1} having a shoulder at 500 cm^{-1}, and a weak band at 270 cm^{-1}. Also, a band at 1600 cm^{-1} attributable to the bending mode of water is clearly evident in the spectrum, even after prolonged drying of the polymer *in vacuo* over P_2O_5. Hewkin and Griffith (1966) have summarized the infrared data on bridged complexes, stating that a sharp band (ν_3) is to be found at approximately 850 cm^{-1} whenever a linear M–O–M bridge is present. Our spectrum contains no such band, and we propose that the features mentioned above arise instead from bent bridges. Thus, in $Cr_2O_7^{2-}$, the bent-bridge stretching frequencies are 772 (ν_3) and 558 cm^{-1} (ν_1) (Stammreich *et al.*, 1958), and for the bent ion $[(NH_3)_5CrOHCr(NH_3)_5]^{5+}$, ν_3 is found at 569 cm^{-1}

TABLE I: The Isomer Shifts, Quadrupole Splittings, and Internal Fields in Ferritin and the Synthetic Iron Core Polymer.

Material (°K)	Isomer Shifts (mm/sec)	Quadrupole Splittings (mm/sec)	K_{eff} (KOe)
Ferritin[a] (30–300)	0.50 ± 0.05	0.60 ± 0.10	
Ferritin[b] (77)	0.47 ± 0.05	0.74 ± 0.04	
Ferritin[b] (4.2)			493 ± 10
Polymer (298)	0.48	0.67	
Polymer (70)			315
Polymer (5)			455

[a] Blaise *et al.* (1965). [b] Boas and Window (1966).

(Hewkin and Griffith, 1966) with ν_1 presumably lower but unreported. Thus, it seems reasonable that our set of bands at 700, 450–500, and 270 cm^{-1} could arise from ν_3 and ν_1 of both oxy- and hydroxy-bent bridges.

The electronic absorption spectrum of the synthetic iron core polymer in aqueous solution shows a broad structureless band centered at 880 mμ (11,400 cm^{-1}). Spectra in ethylene glycol–H$_2$O mixtures (7:3, v/v) at $-100°$ suggest the presence of a second, very much weaker band at 640 mμ (15,600 cm^{-1}). These very weak crystal field excitations are followed by two shoulders at 470 and 280 mμ rising to a maximum at 197 mμ. This last band is undoubtedly due to the $\pi \rightarrow \pi^*$ excitation within the free NO$_3^-$ ion, found ordinarily at 199 mμ, while the 300-mμ band of this ion is much too weak ($f \approx 10^{-7}$) to be seen among the charge transfer bands of the polymer.

As regards the energy of the lowest crystal field band of the synthetic iron core polymer, one can estimate (Stammreich *et al.*, 1958) that for the high-spin Fe^{3+} ion, the 880-mμ wavelength transition corresponds to either Dq = 1100 cm^{-1} in an octahedral site or Dq = -1100 cm^{-1} in a tetrahedral site, the transition in either case being $^6\text{A} \rightarrow {}^4\text{T}_1$. Since Dq = 1100 cm^{-1} is very much to be expected for iron in octahedral oxygen coordination, and since Dq for tetrahedral oxygen coordination should be about $-^4/_9$ that of octahedral, a value of Dq = -1100 cm^{-1} for tetrahedrally coordinated Fe^{3+} is most unusual and unexpected. If our interpretation of the 880-mμ band as derived from the $^6\text{A} \rightarrow {}^4\text{T}_1$ band of tetrahedrally coordinated Fe^{3+} is correct, then we can only conclude that the effect of having chemically different ligands and possible distortions away from tetrahedral symmetry has been to lower its energy appreciably. A similar energy lowering could also result from a shortening of the metal–oxygen bond in the tetrahedral complex as compared with an octahedral one.

Mössbauer Spectra and Magnetic Susceptibility. At the temperature of liquid nitrogen and above, ferritin has a Mössbauer spectrum consisting of a quadrupole split line having an isomer shift with respect to stainless steel of 0.47–50 mm/sec and a quadrupole splitting of 0.60–0.74 mm/sec (Table I). Qualitatively, the Mössbauer spectrum of the synthetic iron core polymer is similar to that of ferritin. At room temperature, the spectrum consists of a fairly symmetrical quadrupole-split doublet, with parameters much like that of ferritin (isomer shift = 0.48 mm/sec, quadrupole splitting = 0.67 mm/sec). Several investigators have proposed a more or less empirical correlation in oxide systems between the isomer shift, the internal magnetic field, H_{eff}, in magnetic materials, and the coordination of the Fe^{3+} ion (Kurkjian and Sigety, 1964; Van Loef, 1966). Though the isomer shift value found here for the synthetic iron core polymer is more nearly characteristic of octahedral coordination, the H_{eff} is more nearly characteristic of tetrahedral coordination. However, since such correlations can be far from exact and most certainly will be a function of the type of ligand involved, we must admit that this aspect of the Mössbauer spectrum neither confirms nor disproves the results of the X-ray-scattering experiment.

The Mössbauer spectra of both ferritin and the synthetic iron core polymer show a rather unusual feature, called "superparamagnetism," which is thought to be characteristic of very fine magnetic particles (Jacobs and Bean, 1963). In both ferritin and the synthetic iron core polymer at low temperatures, a six-line magnetic hyperfine spectrum is observed, indicative of magnetic ordering. As the temperature of these superparamagnetic substances is raised, their hyperfine spectra are *gradually* replaced by the paramagnetic, quadrupole-split doublet spectrum. This behavior differs from that of a bulk magnetic material, in which the change to the paramagnetic spectrum occurs within a very narrow temperature interval, *i.e.*, at the Curie or Néel point. The gradual transition observed for superparamagnetic substances has been explained in the following manner. In the finely divided state, the anisotropy energy of the magnetization is proportional to the particle volume, so that at a given temperature, T, the net magnetization of each particle of volume, V, will fluctuate with a relaxation time given by

$$\tau = \tau_0 \exp\left(\frac{KV}{kT}\right) \qquad (3)$$

where K is the anisotropy constant of the material. If

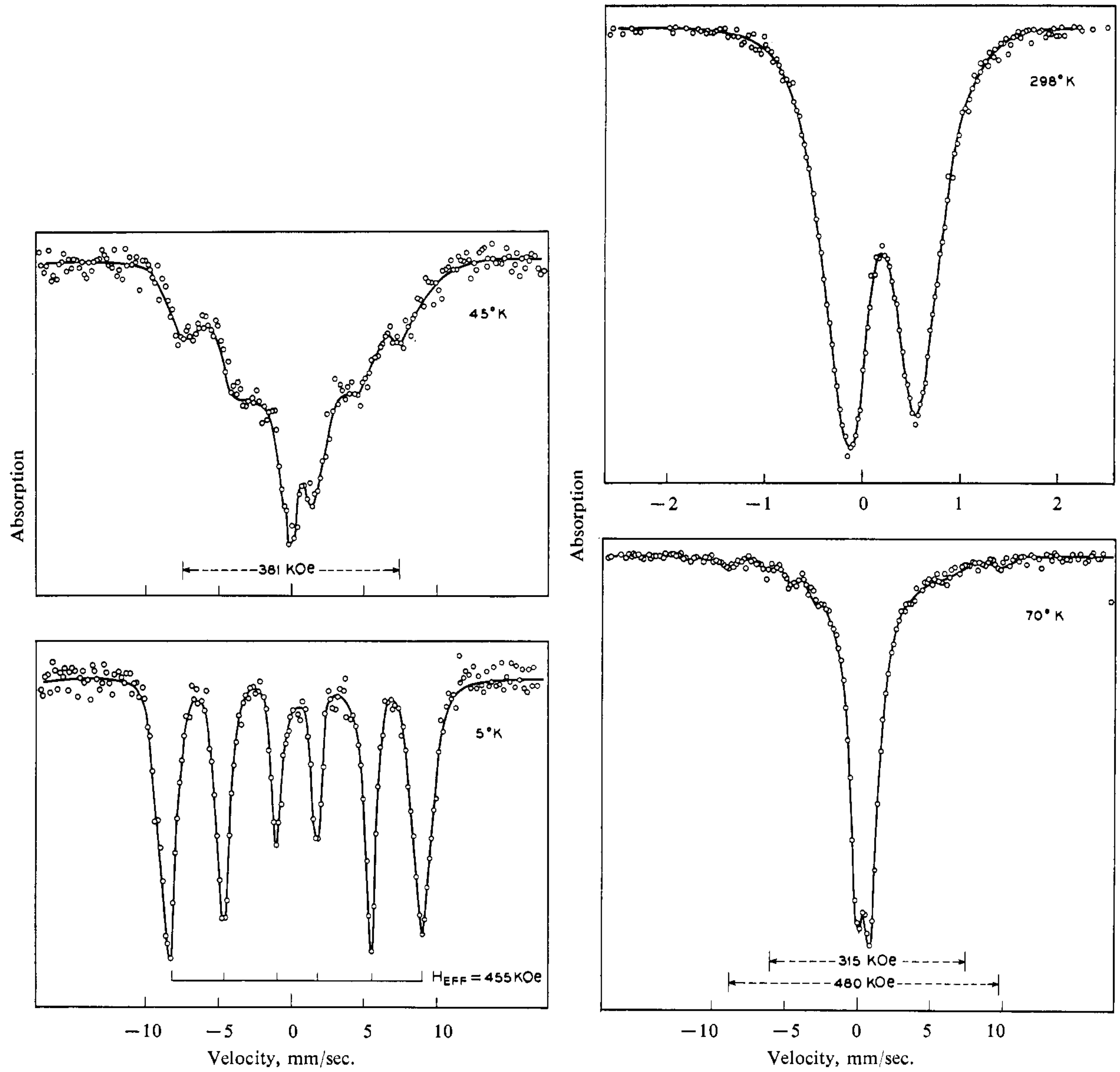

FIGURE 3: The Mössbauer spectrum of the synthetic iron core polymer powder at several temperatures.

the relaxation time, τ, is shorter than the Larmor precession time for the first excited state of the ^{57}Fe nucleus (2.5×10^{-8} sec), then the hyperfine spectrum is destroyed and the Mössbauer spectrum will simply be that of a paramagnetic material. As the temperature is lowered and the relaxation time becomes long compared with 10^{-8} sec, the hyperfine spectrum becomes apparent. However, the presence of a distribution of particle volumes results in a distribution of relaxation times, according to eq 3, and leads to the coexistence of the hyperfine spectrum and paramagnetic spectra over a relatively large temperature range. Moreover, the observation of an hyperfine spectrum or a paramagnetic doublet spectrum will depend upon the particle size, shape, and magnetic anisotropy, and will be essentially independent of the bulk magnetic ordering temperature, provided the particles are below it.

The Mössbauer spectra obtained with the synthetic iron core polymer as absorber in the hyperfine spectrum–paramagnetic doublet transition region are shown in Figure 3. As was found for ferritin (Blaise *et al.*, 1965; Boas and Window, 1966) at 5°K, the spectrum of the polymer consists solely of a six-line hyperfine spectrum. As the temperature is increased, a paramagnetic doublet spectrum appears at the expense of the hyperfine spectrum, and both coexist in the temperature interval 40–70°K. Because the disappearance of the hyperfine spectrum six-line spectrum is due to a relaxation process, it is accompanied by line broadening. In ferritin, the coexistence region is reported as 20–40°K, but the line broadening is not nearly so obvious in this case (Blaise *et al.*, 1965; Boas and Window, 1966).

The Mössbauer measurements reported here were carried out in somewhat more detail than in the ferritin studies, and consequently two important differences have emerged. First, it was found that in addition to a decrease in the intensity of the hyperfine spectrum as the temperature is raised, the magnitude of H_{eff} also decreases, as measured by the separation of the lines in the hyperfine spectrum. Though the precision of the measurement of H_{eff} is rather poor because of its decreasing intensity and line broadening, an estimate of the "bulk" Néel or Curie temperature can be made by fitting the data to a Brillouin curve for $S = {}^5/_2$ (Van der Woude and Dekker, 1966). The predicted value is approximately 100°K. The second important difference is that at 70°K, where the hyperfine spectrum has an H_{eff} of 315 kOe and has almost completely disappeared, a second hyperfine spectrum is resolved having an H_{eff} of 480 kOe. The existence of two hyperfine fields might also be implied by the rather severe broadening of the outer lines at 5°K. At the moment, we ascribe the second hyperfine spectrum pattern to 5% of a second iron species in our samples, but cannot say whether it is a real "impurity" or just a slightly different polymer.

According to our interpretation of the Mössbauer spectra of the synthetic iron core polymer, the individual particles must be either ferromagnetic or antiferromagnetic at or below about 70°K, in order to give hyperfine-split spectra at this temperature. If they are antiferromagnetic, then the thermally fluctuating magnetization is thought to arise from the imperfect cancellation of sublattice magnetizations, as must occur in particles of very small dimensions (Néel, 1962). Our measurement of the static magnetic susceptibility of the synthetic iron core polymer (Figure 4) shows what superficially appears to be Curie–Weiss behavior, with a Néel point at 8°K, and a slope corresponding to 3.1 BM/Fe^{3+} after correction for temperature-independent paramagnetism. Similar effective magnetic moments have been reported by Schoffa (1965), for ferritin, and by Schugar *et al.* (1967) for solutions of the synthetic iron core polymer, and were interpreted by them as showing depressed magnetic moments due to dimer spin pairing. However, as Blaise *et al.* (1965) have shown, such a measurement on a superparamagnetic material is of very little value unless combined with a thorough magnetic field dependence study of the magnetization. Thus, Blaise *et al.* arrived at a moment of 5.08 BM/iron in their comprehensive study of ferritin, to be compared with only 3.85 BM obtained by Schoffa from a plot like that of Figure 4. The low value of the apparent moment does show, however, that the particles of both ferritin and of the synthetic iron core polymer are antiferromagnetic when behaving as superparamagnets.

Since the particles of synthetic iron core polymer are antiferromagnetic at 70°K, but have no Néel point between 70° and 300°K as evidenced by the static susceptibility shown in Figure 4, it would appear that the transition from simple paramagnetic behavior to superparamagnetic behavior (*i.e.*, the bulk Néel point) must lie above 300°K. This is at variance with the estimate of 100°K made from the extrapolated collapse of the internal field, and we have no explanation for this discrepancy. Since the particles are already antiferromagnetic far above the apparent Néel point of 8°K, this temperature must instead correspond to the blocking temperature at which the relaxation time of the magnetization fluctuation is longer than the measurement times, *i.e.*, a few seconds. A similar abrupt decrease in magnetization is found in ferritin at 20°K.

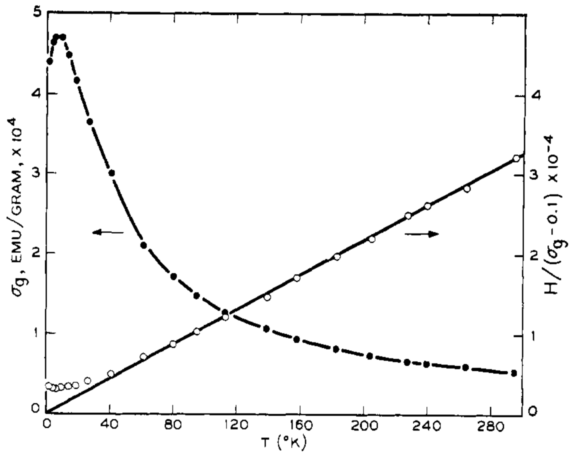

FIGURE 4: The magnetization and susceptibility *vs.* temperature curves for the synthetic iron core polymer. The magnetic field, *H*, was 14,300 gauss.

Conclusions

A comparison of our experimental data on the synthetic iron core polymer with that in the literature for natural ferritin shows that they are similar but not identical materials. Since the X-ray-scattering experiments show that the synthetic material contains Fe^{3+} in tetrahedral coordination, and since the Mössbauer spectra above 70°K of the synthetic and natural materials are so much alike, one concludes tentatively that the Fe^{3+} of ferritin is also tetrahedrally coordinated. On this basis the differences between the synthetic iron core polymer and ferritin probably arise from differences in secondary structure.

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Reductive Alkylation of Amino Groups in Proteins*

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ABSTRACT: When protein solutions are treated with low concentrations of simple aliphatic aldehydes or ketones and small amounts of sodium borohydride, amino groups are converted in high yield into the corresponding mono- or dialkylamino derivatives. Conditions for optimum reaction have been determined using gas–liquid partition chromatography to follow the reductive alkylation of butylamine in aqueous solution. The reaction is strongly pH dependent. At pH 9.0 and 0°, only amino groups of proteins are modified. The principal product of the reductive methylation of proteins with formaldehyde was identified as ϵ-*N*,*N*-dimethyllysine; with acetaldehyde or acetone only the corresponding monoalkylated lysines were formed. The mild conditions and low concentrations of sodium borohydride used did not result in the reductive cleavage of disulfide bonds of lysozyme, insulin, ribonuclease, turkey ovomucoid, human serum transferrin, α-chymotrypsin, or chymotrypsinogen.

Reductive methylation of ribonuclease produced an enzymatically inactive product with less than a single remaining unmodified lysine residue. Reductive alkylation of proteins occurs with minimal changes in gross physical properties as determined by studies of ultraviolet absorption spectra, sedimentation, and optical rotatory parameters.

In recent years, there has been a growing interest in the roles of specific amino acid side chains in the properties of various biologically active proteins. Toward this end, chemical modification of side chains has been widely used with generally good results. There remains a great need, however, for reagents which may be used under mild conditions and yet are specific for particular types of side chains.

Two methods for the modification of amino groups, amidination (Hunter and Ludwig, 1962) and guanidination (Schütte, 1943; Chervenka and Wilcox, 1956), partially satisfy the above needs. In addition, by main-

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