The chemistry of oxidative deposition of iron(III) in ferritin and apoferritin is poorly understood. This study was undertaken to look for radicals formed as the hydroxous ferric oxide core is developed from Fe(II) and O₂. Radicals were observed indirectly by using the spin-trapping reagent N-tert-butyl-a-phenylnitrone (PBN) at room temperature and directly by measuring ESR spectra of frozen solutions at 77 K. In both instances, radical production was inhibited by the hydroxyl radical scavenging agents dimethyl sulfoxide, thiourea, and mannitol and enhanced by the addition of hydrogen peroxide. These findings strongly suggest that hydroxyl radical, produced from the iron-catalyzed Haber-Weiss reaction, is a by-product of core formation in ferritin and is a precursor to the observed radicals. The yield of ESR-observable and spin-trapped radicals is quite low, being at the micromolar level when millimolar concentrations of ferrous ion are employed. Furthermore, radical production appears to be confined to the interior of the ferritin molecule, where cellular components would be protected from the oxygen-derived toxic effects of iron. It is postulated that hydroxyl radical-mediated oxidative damage to the protein, a process that may contribute to the formation of hemosiderin from ferritin, leads to the observed radicals. By serving as a sink for hydroxyl radical, the protein shell may therefore efficiently minimize damage to other biomolecules in the cell.

The reactions of oxygen radicals with proteins, lipids, and nucleic acids have been the subject of numerous investigations in recent years. These reactions have been implicated in such diverse biological phenomena as inflammation, carcinogen activation, and aging (1–25). The iron-catalyzed Haber-Weiss process is known to be a promoter of oxygen radicals under aerobic conditions. Superoxide (O₂⁻), hydroxyl radical (·OH), and hydrogen peroxide (H₂O₂) are produced in aqueous solutions of ferrous salts by the reactions:

\[
\begin{align*}
\text{Fe}^{2+} + \text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{O}_2^-
\text{2O}_2^- + 2\text{H}^+ & \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{HO}^- + \text{HO}^+ + \text{Fe}^{3+}
\end{align*}
\]

Ferritin, the iron storage protein, is the principal reservoir for iron within the cell (26, 27). Recent studies suggest that Fe(II) passes through the channels to the interior of the protein shell where it is oxidized and stored as an FeOOH core along with nonstoichiometric amounts of phosphate (27). Obviously, the presence of both Fe(II) and dioxygen may result in the production of oxygen radicals through the above reactions.

The role of oxygen radicals in the biochemistry of ferritin has been the subject of several recent studies (8, 9, 16–19, 28). Superoxide may be involved in the mobilization of iron from ferritin (9, 16, 17, 19), and the presence of ferritin iron has been shown to enhance lipid peroxidation (28, 29). To date, all studies concerning the observation of radicals in ferritin have investigated their effects on iron mobilization. The purpose of this study was to determine whether radicals are produced during the oxidative deposition of iron in ferritin. The recent observation of Good's buffer radicals from HEPES, PIPES and EPPS during iron(II) oxidation in the presence of ferritin and molecular oxygen suggest that oxygen radicals are produced when iron is deposited in the protein (30). The present study confirms this finding and provides further insight into the chemistry of radical production in ferritin and the role of ferritin in protecting cellular components from the toxic effects of iron.

**MATERIALS AND METHODS**

Horse spleen ferritin (twice crystallized, cadmium-free, having nominally 2300 atoms of Fe⁺ per protein) was purchased from Miles Scientific or Boehringer Mannheim Biochemicals and exhaustively dialyzed against 0.15 M NaCl, pH 7.5, before use. Superoxide dismutase (bovine erythrocyte, EC 1.15.1.1), catalase (bovine liver twice crystallized, EC 1.11.1.6), and xanthine oxidase (buttermilk, EC 1.2.3.2) were purchased from Sigma; PBN, DMPO, H₂O₂, DTPA, thioglycolic acid, thiourea, and xanthine were from Aldrich, and EDTA, KH₂PO₄, mannitol, Me₂S0, and Fe(NH₄)₂(SO₄)₂·6H₂O were from VWR Scientific. All chemicals were reagent grade or better. Apoferritin was prepared as described previously (31).

Room temperature ESR spectra were collected using a quartz flat cell or a liquid nitrogen dewar insert on a Varian E-4 spectrometer interfaced to a Digital Equipment Corp. MINC 11/23 computer. g-Factors were measured on a Varian E-9 spectrometer employing a MicroNow NMR gaussmeter and a Hewlett Packard model 5350A microwave frequency counter.

Unless otherwise stated, all experiments were done under an oxygen atmosphere (pO₂ = 1 atm) with 50 mM phosphate buffer, pH 7.5, previously passed through a column containing the chelating resin Chelex, Sigma dry mesh 50–100. Final reaction volumes were 250 µL. Control experiments to verify that the PBN spin trap was functioning properly were done in the following manner. Xanthine oxidase (0.1

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1 The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; EPPS, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; DTPA, diethylenetriaminepentacetic acid; PBN, N-tert-butyl-a-phenylnitrone; DMPO, 5,5-dimethyl-1-pyrroline-N-oxide; Me₂S0, dimethyl sulfoxide.
unit/ml) was added to 250 µl of phosphate buffer, pH 7.5, containing 50 mM PBN, 0.2 mM xanthine, and 5 mM DTPA. PO₂ = 1 atm. The solution was allowed to mix for 1 min, and the ESR spectrum was then recorded.

In experiments using the Fe²⁺/EDTA/H₂O₂ hydroxyl radical generating system (32), a 20-µl aliquot was taken immediately after mixing equal volumes of 0.2 M Fe(NH₄)₂(SO₄)₂ and 0.4 M Na₂EDTA. This aliquot was added to 250 µl of phosphate buffer containing 50 mM PBN and 10 µl of 3% H₂O₂. The hydroxyl radical scavengers, when used, were present at a concentration of 50 mM (thiourea and mannitol) or 0.14 M (Me₂SO).

Ultrafiltration experiments were performed using an Amicon 3-ml ultrafiltration cell fitted with either a PM10 or a UM05 membrane. Initial sample volume was 600 µl with all components at the same concentrations as mentioned above. After addition of Fe(II), the sample was stirred under an oxygen atmosphere for 2 min. Finally, a positive PO₂ pressure was applied to the cell, 250 µl of ultrafiltrate was collected, and the ESR spectrum was recorded.

The frozen solution experiments were performed with assembled apoferritin present at a concentration of 0.042 mM in 50 mM phosphate buffer, pH 7.5. The sample was purged of oxygen by stirring under moist argon gas for at least 30 min. Iron(II) was then added at a ratio of 5 Fe(II) per ferritin subunit, allowed to mix 10 min, and the ESR spectrum was recorded. Finally, the sample was opened to oxygen and allowed 5 min to oxidize and freeze, and the ESR spectrum was scanned again. Tetramethyl-1-piperidinioxy, free radical was used as an ESR intensity standard.

RESULTS

Control experiments were performed to verify that the PBN spin trap chemistry was functioning properly. To produce superoxide (O₂⁻) and the resultant spin trap adduct PBN-OH (aN = 1.50 mT; aH = 0.27 mT) (Fig. 1A), xanthine oxidase was added to an aerobic solution containing xanthine, PBN, DTPA, and phosphate buffer. Phosphate was used as a buffer since it is a natural constituent of ferritin and of cells. Other buffers commonly employed in biochemistry, namely HEPES and Tris, are known to participate in radical reactions and thus were avoided (30, 33). The experiment was repeated in the presence of superoxide dismutase (380 units/ml), which catalyzes reaction 2, consuming the O₂⁻ in the solution. As expected, no spin-trapped O₂ radical was observed (Fig. 1B).

The Fe²⁺/EDTA/H₂O₂ hydroxyl radical generating system produced the hydroxyl radical spin trap PBN-OH (aN = 1.59 mT; aH = 0.275 mT) (Fig. 1C). The PBN-OH adduct signal was less intense when the hydroxyl radical scavengers thiourea (Fig. 1D) or mannitol (Fig. 1E) were present. The presence of Me₂SO, also an OH radical scavenger, resulted in trapping of the secondary methyl radical produced from the reaction of OH with Me₂SO to form the adduct PBN-CH₃ (Fig. 1F) (34, 35). The results summarized in Fig. 1 demonstrate that the PBN reagent in our hands can be reliably used to probe iron-induced oxygen radical chemistry.

Iron deposition experiments were performed using 0.017 mM ferritin. 10 µl of 0.2 mM iron(II) was added to 250 µl of the oxygenated solution containing 50 mM PBN, 50 mM phosphate, and ferritin, corresponding to the addition of 470 Fe(II) per protein molecule. The solution was allowed to react for 4 min before measuring the ESR spectrum. A relatively stable PBN-trapped species PBN-R, where R denotes an unknown radical, was observed (Fig. 2A). The ESR signal from PBN-R decayed slowly over the course of several days. The adduct had hyperfine splitting constants of aN = 1.59 mT and aH = 0.39 mT (Fig. 2A) corresponding to neither PBN-OH nor PBN-OOH. In a control experiment, Fe(II) was added to an identical solution lacking ferritin; no trapped species was observed (Fig. 2B). A third experiment using apoferritin likewise at a concentration of 0.017 mM in place of ferritin also yielded a PBN adduct with the same hyperfine splittings as those observed with ferritin but with an ESR signal 36% greater (Fig. 2C).

Ultrafiltration experiments were performed to determine whether the PBN-R adduct is associated with the protein or buffer. In experiments using the Fe²⁺/EDTA/H₂O₂ hydroxyl radical generating system (32), a 20-µl aliquot was taken immediately after mixing equal volumes of 0.2 M Fe(NH₄)₂(SO₄)₂ and 0.4 M Na₂EDTA. This aliquot was added to 250 µl of phosphate buffer containing 50 mM PBN and 10 µl of 3% H₂O₂. The hydroxyl radical scavengers, when used, were present at a concentration of 50 mM (thiourea and mannitol) or 0.14 M (Me₂SO).

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Ultrafiltration experiments were performed to determine whether the PBN-R adduct is associated with the protein or buffer.
is perhaps a small fragment derived from the protein. A PM10 membrane with a molecular weight exclusion limit of 10,000 was employed. The PBN-R ESR signal from the ultrafiltrate indicated that the adduct freely passes through the membrane. When a UM05 membrane (exclusion limit 500) was used, the ratio of intensities between the retentate and the ultrafiltrate was 9:1, indicating partial retention of the adduct. These observations in conjunction with the relatively sharp ESR lines (peak-to-peak line width = 0.46 mT, Fig. 2A) indicate that the PBN-R adduct is a low molecular weight species having a molecular weight of the order 500–1000. Its low concentration (~1.5 μM as judged from the ESR signal amplitude) precluded its further characterization.

Approximately one radical is spin-trapped for every 5000 Fe²⁺ atoms added to the protein (7.7 mM Fe²⁺, 470 Fe²⁺/apoprotein, pO₂ = 1 atm), corresponding on average to one radical per 10 protein molecules. When the amount of added Fe(II) is reduced, the efficiency of radical production increases, reaching a value of one radical per 1300 Fe(II) added (0.77 mM Fe²⁺, 47 Fe²⁺/apoprotein, pO₂ = 1 atm). Further decreases in the amount of Fe(II) added were not possible because the ESR signal became too weak. Decreasing the pO₂ to 0.2 atm (air) caused a 60% reduction in the ESR signal at 470 Fe²⁺/apoprotein. The combined effects of iron concentration and oxygen tension on the ESR signal indicate that radical formation per iron oxidized is favored by low Fe(II)/O₂ ratios.

The change in the PBN-R ESR signal as iron is progressively added in increments of 100 Fe²⁺/apoprotein to the same sample is shown in Fig. 3. The results demonstrate that radical production is most effective in the early stage of iron addition to the apoprotein. Either the putative reactive protein groups are consumed early as the iron is added or the developed core affords protection to the inner surface of the protein from radical damage. The amount of PBN-R formed was found to be independent of the concentration of PBN in the range 12.5–50 mM, implying that 100% efficiency in the trapping of the R' had been achieved.

Several experiments were also performed using hydroxyl radical scavengers to determine if the hydroxyl radical might be a precursor to the PBN-R adduct. Table I summarizes the effects of 50 mM thiourea and mannitol on the ESR signal amplitude of the PBN-R adduct. Relative to the control, the

![Graph](image)

**Graph:** Accumulated ESR signal (in arbitrary units) of the PBN-R adduct when iron was added in increments of 100 Fe²⁺ per apoferritin to the same solution. Conditions are the same as described in Fig. 2.

**Table I**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>PBN-R radical</th>
<th>Directly observed radical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mannitol ⁹</td>
<td>69</td>
<td>34</td>
</tr>
<tr>
<td>Thiourea ⁶</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Dimethyl sulfoxide ⁹</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Hydrogen peroxide ⁹</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase ⁹</td>
<td>80–90</td>
<td></td>
</tr>
<tr>
<td>Catalase ⁹</td>
<td>91</td>
<td>80–90</td>
</tr>
<tr>
<td>Albumin ⁹</td>
<td></td>
<td>93</td>
</tr>
</tbody>
</table>

*50 mM mannitol or thiourea.
* ⁹ 0.14 M Me₂SO.
* 10 μl of 3% H₂O₂ added to 250-μl sample prior to the addition of iron.
* ³80 units/ml, 0.1 mg of protein/ml.
* ³80 units/ml, 0.01 mg of protein/ml.
* ³0.17 mg/ml.

ESR signal is diminished by 31% in the presence of mannitol and 59% by thiourea, reagents known to be effective 'OH scavengers (36, 37). Furthermore, the addition of 10 μl of 3% H₂O₂ to the reaction mixture enhances the ESR signal of PBN-R 3-fold (Table I) as expected if 'OH radical production occurs by the Fenton reaction 3.

Experiments with superoxide dismutase and catalase were performed to determine whether the formation of the trapped radical is inhibited or facilitated by either of these enzymes. The presence of either enzyme had relatively little effect on the ESR signal compared to the control (Table I), suggesting that any superoxide and/or hydrogen peroxide that might be involved in the formation of R' are inaccessible as substrates to these enzymes.

An analogous series of experiments was performed using DMPO as the spin trap. While the control experiments verified that DMPO trapping chemistry was functioning properly (i.e. DMPO-OOH and DMPO-OH adducts could be formed in the absence of ferritin under conditions similar to Fig. 1), no DMPO spin-trapped radicals were observed upon addition of Fe(II) to ferritin. Similar observations have been reported by Thomas et al. (9) in studies of iron mobilization from ferritin by superoxide. Their experiments, using a xanthine/xanthine oxidase superoxide generating system, resulted in no trapped DMPO-OOH adduct whenever ferritin and DMPO were present initially. The possibility exists that the DMPO may be interacting with the protein or its iron core in some manner. In any case, because of its unreliable behavior with ferritin, we pursued the experiments with DMPO no further.

Experiments were also undertaken to observe radical production directly without the use of spin-trapping reagents. Iron(II) was added anaerobically to a solution of 0.042 apoferritin in phosphate buffer at an iron/subunit ratio of 5:1 (96 Fe²⁺ per protein). The iron was then oxidized by passing O₂ (1 atm) over the stirred sample for 5 min. The ESR spectrum recorded at 77 K showed a single asymmetric line with g = 2.0077 ± 0.0007 (average of four samples) and a peak-to-peak line width of 1.6 mT (Fig. 4A), corresponding to a S = ½ radical concentration of ~2 μM. The efficiency of radical production in this case is also about one radical per 5000 iron(II) oxidized. Reducing the level of added iron to 9.6 Fe(II)/apoprotein increases the yield to one radical per 1500 Fe(II) oxidized. An ESR signal near g = 2 was not observed at room temperature using an aqueous solution flat cell or
when the measurement was carried out at 77 K in the absence of added Fe(II).

Hydroxyl radical scavengers were also used in these studies. The effects of 50 mM mannitol, 50 mM thiourea, and 0.14 M Me2SO are summarized in Table I. Mannitol, thiourea, and Me2SO reduced the ESR signal by 46, 59, and 32%, respectively, relative to the control, whereas addition of 10 μl of 3% H2O2 to the 250-μl sample enhanced the signal by 216%. As with the spin-trapping experiments, these results suggest the involvement of hydroxyl radical produced from the Fenton reaction.

The presence of superoxide dismutase or catalase reduced the signal by only 10–20%, the results being quite variable (Table I). Human serum albumin used as a control also reduced the ESR signal, in this instance by 7%. Given the relatively high concentration of protein used in these experiments, it seems likely that the small reduction in the ESR signal in the presence of superoxide dismutase or catalase is largely a nonspecific effect, possibly due to competition between these proteins and apoferritin for added iron(II).

The order of addition of iron(II) and dioxygen to the protein solution is important. The frozen solution ESR signal is twice as intense when the iron is added to the apoferritin solution first followed by the introduction of oxygen compared to the reverse addition. Prior binding of the Fe(II) to the protein apparently is required for optimum production of the radical signal.

The radical signal reaches its maximum intensity within 0.5 min of exposure of the Fe(II)-apoferitin solution to O2 and then decays in three phases (Fig. 5 and inset). About 13% of the ESR signal decays in the first phase with a half-life of approximately 5 min. In the second and third phases, the signal decays with half-lives of about 32 min and 5 h, respectively, corresponding to changes in the ESR amplitude of 54% and 10%. At 15 h, a residual signal 25% of the original remains (Fig. 4B). These results indicate that several radical species with similar g-factors contribute to the observed ESR signal.

The measured g-factor of the residual signal is likewise 2.0077. A g = 2.0077 signal was also observed when ferritin was used in place of apoferritin at the same protein concentration (0.042 mM); however, the signal was twice as great, a result suggesting that the presence of the iron core facilitates radical production in this instance. The signal was also generated when water rather than phosphate buffer was used, indicating that the presence of phosphate is not required for production of the radical.

DISCUSSION

The above results indicate that radicals are formed during iron deposition in ferritin. The fact that the PBN-R adduct is observed with both ferritin and apoferritin and not in the absence of protein suggests that the trapped species is derived from the protein itself and not simply from the iron core. Diminution of the ESR signal by the use of known hydroxyl radical scavengers and enhancement of the signal by the addition of H2O2 strongly suggests the involvement of hydroxyl radical in both the formation of the observed PBN-R adduct (Fig. 2A) and the radicals observed directly (Fig. 4A). While it is difficult to conclusively show that the radicals observed here arise from the protein, our data along with the growing body of knowledge on radical damage to proteins and on ferritin in particular, argue for such an interpretation.

Recent studies have shown that oxygen radicals (hydroxyl radical in particular) are important in proteolytic degradation of proteins (4–7). These studies conclude that there is a direct and quantitative link between oxygen radical damage and susceptibility of proteins to proteolysis. In principle, iron(II) under aerobic conditions is capable of producing superoxide, H2O2, and hydroxyl radicals, as shown in Equations 1–3. The formation of the core in ferritin may result in a significant production of O2· and H2O2 and ultimately, 'OH.

Addition of superoxide dismutase and catalase to remove O2· and H2O2, respectively, failed to inhibit the formation of the PEN-R radical adduct; these enzymes also had little effect on the formation on the radical observed directly (Table I). Previous kinetic studies have shown that neither enzyme affects the rate of iron deposition in ferritin or the stoichiometry of iron oxidation by molecular oxygen (38–40). Oxi-
Radical Production in Ferritin

The production of Fe(II) probably occurs primarily at interior sites on the protein and on the surface of the growing iron core where the ferritin shell would shield the putative radical products of iron deposition, namely O\textsubscript{2}., H\textsubscript{2}O\textsubscript{2}, and OH\textsuperscript{.} from the enzymes. Hydroxyl radical scavengers, such as thiourea, mannitol, and Me\textsubscript{3}SO, are small molecules capable of penetrating the protein and are expected to reduce the ESR signal of the radical products, as is observed (Table I). Thus, radical production appears to be largely or exclusively confined to the interior of ferritin, an important property of the protein which affords protection to cellular components from the toxic effects of iron.

The identities of the radicals observed in this study are unknown. A survey of the data base on spin-trapped radicals (41) failed to produce a reasonable match with the spectral parameters of PBN-R (Fig. 2A). The g-factor (2.0077) for the radical observed directly (Fig. 4A) is most consistent with a nitrogen- or possibly an oxygen-centered radical (sulfur-centered radicals have g-factors near 2.02) (42, 43). Whether the spin-trapped radical and the directly observed radical are the same species or arise from the same radical fragmentation pathway has not been established by the present work. They appear to be different species, however, since production of the directly observed radicals is greater with ferritin than apoferritin, whereas the opposite is true with the spin-trapped radical. The evidence supports the involvement of the hydroxyl radical in both instances.

In previous work, a signal at g = 2.011, presumably due to a radical, was observed when Fe(II) was oxidized in the presence of apoferritin in HEPES buffer (31). This signal is distinctly different from the one reported here (Fig. 4A) and is only observed when HEPES buffer is employed. It may be a decomposition product of the previously described HEPES radical produced during iron oxidation (30) or a reaction product of the HEPES radical with the protein. In any event, it is clear that the radical chemistry of iron deposition in ferritin is complex and involves several radical species which are the products of activated oxygen.

Hemosiderin has long been postulated as being derived from ferritin. Hemosiderin is thought to be formed from the aggregation and the partial degradation of the protein shell by lysosomal enzymes (28, 44–49). A recent study of the relationship between ferritin and hemosiderin concluded that hemosiderin peptides are derived from ferritin peptides and that free radical catalyzed reactions are likely involved in the transformation (28). Hemosiderin is known to have smaller iron cores, and its protein shell is less complete than that of ferritin. While both proteins release iron and promote lipid peroxidation and hydroxyl radical formation, hemosiderin is consistently and significantly less effective than ferritin under conditions likely to be encountered in vivo (28). Furthermore, studies of the amino acid sequence of both ferritin and hemosiderin show that hemosiderin has a relatively low abundance of thiol and aromatic residues, residues that are likely to react with the hydroxyl radical (28, 44, 50). Therefore, transformation of ferritin to hemosiderin may in part involve free radical mediated oxidation of the protein.

The results of the present study lend support to this hypothesis. Clearly, radicals are produced during iron deposition in ferritin and certain of these radicals or radical precursors (O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2}) coupled with the presence of Fe(II) within the shell of ferritin, may result in the formation of hydroxyl radicals. Nearby amino acids may then be oxidized and fragmented, resulting in the radical observed directly with the protein (Fig. 4A) and the spin-trapped radical (Fig. 2A). The observation that the PBN-R adduct passes through the PM10 ultrafiltration membrane with a size exclusion limit of 10,000 but is partially retained with a UM05 membrane (exclusion limit 500) is consistent with the fragmentation hypothesis.

In our experiments, the observed efficiency of radical production, relative to the amount of iron(II) employed, is quite low but increases as the Fe(II)/pO\textsubscript{2} ratio decreases. Previous studies have shown that the stoichiometry of Fe(II) oxidation by O\textsubscript{2} in ferritin is variable, depending on the conditions (39, 40, 51). At millimolar concentrations of Fe(II), comparable to the levels used in our experiments, a stoichiometry near the theoretical limit of 4 Fe(II)/O\textsubscript{2} is obtained (39, 40). Under such conditions, essentially complete reduction of O\textsubscript{2} to H\textsubscript{2}O occurs and little radical production is expected, as is observed here. However, at lower Fe\textsuperscript{2+} concentrations, the stoichiometry becomes ~1.5 Fe(III)/O\textsubscript{2} (40), a situation where partially reduced oxygen species such as O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} would be in abundance, leading to increased radical production and damage to the protein. Our observation of increased radical production as the iron concentration is lowered is consistent with this idea.

It is unclear to what extent radical production in the cell contributes to hemosiderin formation since the concentrations of Fe(II) and O\textsubscript{2} and their flux in the microenvironment of the protein are unknown. In our experiments, other radicals may well have been formed which cannot be trapped by PBN or observed directly by ESR. Thus, the concentration of radicals reported here is probably a lower limit to their true value. One radical was detected per 10 ferritin molecules. However, the yield may be much higher in vivo where the levels of ferritin and hemosiderin are comparable (52).

REFERENCES


\textsuperscript{1} It is possible, however, that at higher concentrations of Fe(II), radical recombination reactions become important, accounting for the apparent reduction in efficiency of radical production under these conditions.
Radical Production in Ferritin