Kinetic Studies of Ferrous Ion Oxidation with Crystalline Human Ferroxidase (Ceruloplasmin)*

SHIGEMASA OSAKI

From the Department of Chemistry and the Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306

SUMMARY

The kinetics of the catalysis of Fe(II) oxidation by crystalline human ferroxidase (ceruloplasmin) has been studied under various conditions at 30°C. Two Km values with respect to Fe(II), $K_{m1} = 0.6 \mu M$ and $K_{m2} = 50 \mu M$, were obtained at pH 6.5, at which ferroxidase gave the maximum activity of 550 Fe(II) per ferroxidase per min in an acetate buffer. Competition between the two substrates, p-phenylenediamine and Fe(II), was observed. The stoichiometry of Fe(II), ferroxidase, and oxygen, determined from the experimental data, was 4:1:1.

In 1952, Koechlin (1) reported that the rate of complex formation between ferrous ion and apotransferrin* was enhanced by certain catalytic factors (or factor) present in human plasma. Curzon (2) in 1961, studied $N,N$-dimethyl-$p$-phenylenediamine oxidation by ceruloplasmin (ferroxidase) in the presence of Fe(II) and concluded that Fe(II) is essentially a second substrate in competition with the first substrate, $N,N$-dimethyl-$p$-phenylenediamine, for the active center of the enzyme. No extensive study of the kinetics of Fe(II) oxidation by this enzyme has appeared except for a recent paper from our laboratory (3). In this report, the oxidation of Fe(II) by serum was studied at pH 7.35 and at various oxygen concentrations which approach the physiological conditions of human serum. It was shown that ceruloplasmin was the only enzyme in serum responsible for Fe(II) oxidation and that Fe(II) was the substrate of the greatest activity known for ceruloplasmin. Based on these observations, the designation of the enzyme as serum ferroxidase (ferro-Oxidoreductase, EC 1.12.3) was proposed (3). In view of the possible importance of this activity, a careful study of the kinetic aspects of the ferroxidase activity of this enzyme was undertaken.

EXPERIMENTAL PROCEDURE

Materials

Ferroxidase—Crystalline ferroxidase (ceruloplasmin) was obtained by the method previously described (4) and nonenzymic copper ion was eliminated by Chelex 100 resin (Bio-Rad) treatment (3, 5). A solution of crystalline ferroxidase was ultracentrifugally homogeneous with an $s_{20}$ value of 6.3 S and an absorbance ratio ($A_{280}/A_{600}$) of 22.0 ± 0.3 (3, 6).

Buffer—The enzyme and nonenzymic oxidation of Fe(II) was observed in a reaction mixture containing 0.2 M acetate or 0.0133 M phosphate as the buffer. The stock solution of these buffers, 0.6 M and 0.04 M, respectively, was treated with a Chelex 100 column.

Ferrous Iron—Crystalline ferrous ammonium sulfate hexahydrate (Fe(NH$_4$)$_2$(SO$_4$)$_2$·6H$_2$O; J. T. Baker, Lot 25899) was dissolved in deoxygenated Chelex 100-treated water and used as the substrate (3).

*p-Phenylenediamine-HCl—A concentrated $p$-phenylenediamine HCl (Eastman) solution was treated with a Chelex 100 column to remove contaminating heavy metals (mostly copper and iron) and recrystallized by a method previously described (3). The concentration of the stock solution was 2.76 × 10$^{-4}$ M.

Apotransferrin—Iron-free transferrin, purchased from Lloyd Brothers, Inc., Cincinnati, was dissolved in Chelex 100-treated water at 2.2 × 10$^{-4}$ M and stored in an ice bath. The stability and other physicochemical properties of the apotransferrin solution were reported in previous papers (3, 7).

Deferoxamine—Deferoxamine, $N$-[5,3-[(5-aminopentyl)-hydroxy carbamoyl]propionamido)pentyl] 3 [(5 (N-hydroxyacetamido)pentyl) carbamoyl) propionohydroxamic acid methane sulfonate was kindly donated by Ciba Pharmaceutical Company, New Jersey. This Fe(III)-specific chelator (pk ~ 31) was dissolved in Chelex 100-treated water at 2.2 × 10$^{-4}$ M and stored in an ice bath. The reagent was stable in solution at room temperature (25°C).

1,10-Phenanthroline—Twice recrystallized 1,10-phenanthroline was used as a specific chelator of Fe(II). The concentration...
of 1,10 phenanthroline in a stock aqueous solution was 3 mm. The solution was stored in a dark container at room temperature.

Methods

Fe(II) Oxidation—Oxygen uptake measurements with the use of a polarographic oxygen electrode or a spectrophotometric measurement following Fe(III)-transferrin formation were used to determine the enzymic activity of ferroxidase. The details of these measurements were extensively described in previous reports (3, 7).

p-Phenylenediamine Oxidation—The absorbance change at 540 nm caused by a colored product (or products) was measured with a Cary model 15 spectrophotometer at 30° (3).

pH Measurement—The pH of each reaction mixture was determined before and after the rate measurements with a Beckman model G pH meter equipped with a microelectrode. No variation in pH was noted over the relatively wide range of pH 5.2 to 7.5 with either 0.2 M acetate or 0.0133 M phosphate buffer.

RESULTS

Linearity in Oxidase Activity and Enzyme Concentration—The principle of enzymic activity measurement is based on the following schematic equation

\[ 2 \text{Fe(II)} + 2 \text{E-Cu(I)} + 2 \text{Fe(III)} + 2 \text{E-Cu(I)} \]

\[ \text{2Fe(III)} + \Delta T_f \rightarrow \text{Fe(II)} + 2 \text{H}^+ \rightarrow \text{Fe(II)} + \text{H}_2 \text{O} \] (absorption at 460 nm)

where \( \Delta T_f \) is the change in absorbance with molar absorbance, \( E_{460} = 2500 \text{ cm}^{-1} \text{ cm}^{-1} \text{ Fe(III)} \times \text{transferrin} \). The electrons are carried from Fe(II) to oxygen through the enzymic copper ion.

The relationship between enzyme concentrations and the rate of product (Fe(III)-transferrin) formation or oxygen uptake was tested. A good correlation between the activity and enzyme concentration was obtained, as shown in Fig. 1. Although the pH in these two experiments (Fig. 1, a and b) is different, it was found that the absorbance change measurement at 460 nm was more sensitive than the \( O_2 \) uptake measurement in detecting enzymic activity. In the former method, the optimum concentration range to achieve the most accurate assay in enzymic activity was found between 10 and 30 nM, whereas approximately 10 times more enzyme was required for \( O_2 \) uptake measurement.

Two Fe(III) per protein molecule.
pH Dependence—The effect of pH on ferroxidase activity was measured with an oxygen electrode (Fig. 2). The initial oxygen concentration in the reaction mixture was kept at 60 μM to minimize nonenzymatic oxidation of the substrate (3). The non-enzymatic Fe(II) oxidation is a second order reaction with respect to Fe(II) and O2 concentration (3). The curves in Fig. 3 represent a corrected enzymic rate. The nonenzymatic rate, which was fairly low up to pH 6.5 in 0.0133 M phosphate and to pH 7.1 in 0.2 M acetate, respectively, was subtracted from the over-all rate at every experimental point.

There are two different pH optima depending on the kind of buffer used. In the phosphate buffer, the observed pH optimum was 5.7 with a slight shoulder between pH 6.5 and 7.0, whereas the enzyme is most active at pH 6.5 in 0.2 M acetate. The Km and Vmax values at various pH values, obtained by oxygen uptake measurement in oxidation of Fe(II) by ferroxidase, are summarized in Table I. This pH dependence curve in Fe(II) oxidation is somewhat different from the pH dependence curves for the activity of p-phenylenediamine or N,N-dimethyl-p-phenylenediamine reported previously (9-11). However, a Fe(II) stimulation curve showed a maximum activating effect on ascorbate oxidation caused by ferroxidase at pH 6.3 to 6.5 (12).

Activity Dependence on Substrate Concentration—The rates of transferrin formation as the result of Fe(II) oxidation at pH 6.5 by ferroxidase at various substrate concentrations (1 to 164 μM Fe(II)) were measured at 460 μM. The observed rates, re-expressed as micromolar concentration of Fe(III)-transferrin per min, were plotted against 1/s in Fig. 3a. The nonenzymic oxidation was found to be negligible, 0.35 μM Fe(III)-transferrin per min at 120 μM Fe(II). A nontypical or diphasic curve was obtained over a 10-fold range of enzyme concentration chosen (210 and 21 mm), yielding two Km values: K1 = 0.6 μM, and K2 = 50 μM. This diphasic nature in enzymic activity was also observed when ascorbate was oxidized in the presence of various amounts of Fe(II) and ferroxidase yielding estimated activation coefficients, K1 = 0.2 μM, K2 = 21 μM, which are the same order of magnitude as Km values mentioned above. (The concentration of Fe(II) which gives 50% of the maximum activation in ascorbate oxidation was estimated from the slope in Fig. 3b). As discussed subsequently in this paper, it is likely that the mechanism of ascorbate oxidation by ferroxidase involves a cyclic reaction through Fe(II) to Fe(III) caused by unavoidable contamination by a trace amount of iron (∼10-18 M (12)), which is approximately one-tenth of the Km value. Recently, Curzon has also mentioned a similar diphasic curve in N,N-dimethyl-p-phenylenediamine oxidation.

Stoichiometry between Fe(II) and O2 in Enzymic and Nonenzymic Oxidation—It has been shown earlier that the oxidation of ascorbate in the presence of crystalline human ferroxidase did not produce hydrogen peroxide (4). In the present study, stoichiometry between Fe(II) added in the reaction mixture and O2 reduced was determined with or without the enzyme at pH 7.35 in air-saturated 0.0133 M phosphate buffer at 30°. The oxidation of ferrous ion was completed within a few minutes with or without enzyme, although the nonenzymic oxidation rate was considerably slower than that with the enzyme. The results presented in Table II indicate that 4 atoms of Fe(II) react with 1 molecule.
of oxygen in both enzymic and nonenzymic oxidation, suggesting that no hydrogen peroxide is formed as a stable product. This conclusion is also supported by the fact that the addition of catalase to the reaction mixture did not affect either the oxidation rate or the stoichiometry between Fe(II) and oxygen (Table II), although it is still possible that the hydrogen peroxide is formed as an intermediate which is rapidly reduced by another electron pair from 2 Fe(II).

**TABLE II**

<table>
<thead>
<tr>
<th>Oxidation</th>
<th>Fe(II) oxidized</th>
<th>O2 reduced</th>
<th>Ratio</th>
<th>Fe(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nenzyemic</td>
<td>60</td>
<td>15</td>
<td>4.0</td>
<td></td>
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<tr>
<td></td>
<td>80</td>
<td>18</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>39</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>60</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Enzymic (255 mg/mL) and</td>
<td>72</td>
<td>18 5a</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>nonenzymic</td>
<td>56</td>
<td>14</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42</td>
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<td>3.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>7.1</td>
<td>3.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.1</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

- No oxygen concentration change was detected by adding catalase after the reaction was over.

The oxidation of p-phenylenediamine by ferroxidase in the presence of approximately 1 mM deferoxamine (Fig. 4) or 1,10-phenanthroline (Fig. 5), which are specific chelators of Fe(III) in the presence of Fe(II) or Fe(III). They proposed a coupled mechanism, involving the substrate, iron, and ferroxidase, to explain a stimulating effect by Fe ion. The present experiments were designed to determine whether p-phenylenediamine and Fe(II) are oxidized by ferroxidase at independent or identical sites.

The results presented in Figs. 4 and 5 clearly indicate the following:

1. A lag phase in absorbance change at 540 μM appears just after introduction of Fe(II) only when deferoxamine has been present in the reaction mixture (Fig. 4a).
2. The more Fe(II) that is added, the more conspicuous the lag phase is (Fig. 4a).
3. At Fe(II) concentrations of more than 21.8 μM, apparently p-phenylenediamine oxidation is completely stopped for about 60 sec together with an appearance of a small absorbance change immediately after the introduction of Fe(II) (Fig. 4a).
4. Without an iron chelator, an immediate increase in the rate of absorbance change at 540 μM is observed by adding Fe(II), as reported in previous papers (2, 13, 14) and as shown in Fig. 5b.

5. However, this activation is diminished by adding either Desferrioxamine or apotransferrin to the reaction mixture, restoring the original oxidation rate. Further addition of Fe(II) in the

![Fig. 4. The effects of Fe(II) on p-phenylenediamine (pPD) oxidation by ferroxidase in the presence of deferoxamine (DFA).](http://www.jbc.org/)

**Fig. 4.** The effects of Fe(II) on p-phenylenediamine (pPD) oxidation by ferroxidase in the presence of deferoxamine (DFA). a, the time course of absorbance change at 540 μM (p-phenylenediamine oxidation). Each line represents an independent experiment. The reaction was initiated by adding ferroxidase (E) to 0.05 μM in a reaction mixture containing 9 μM p-phenylenediamine and 977 μM deferoxamine in 0.2 M acetate, pH 5.2. Total volume of the reaction mixture was 0.55 ml. Various amounts of Fe(II) dissolved in 200 μl of water were rapidly injected in the reaction mixture at the point indicated by the arrow. A sufficient mixing was achieved by this technique. The numbers on the right are the final concentrations of iron caused by the injection to the reaction mixture. The final volume of the reaction mixture became 0.85 ml. The time course of absorbance change presented at the bottom of the figure is for the same reaction mixture, but without p-phenylenediamine. b, time course of absorbance change at 430 μM (Fe(III)-deferoxamine complex formation) with or without the enzyme. The reaction mixture contained 27 μM Fe(II), 0.20 μM ferroxidase, and 55 μM deferoxamine in 0.2 M acetate, pH 5.2.
reaction mixture containing Desferrioxamine causes a lag phase in absorbance change (Fig. 5b).

Anaerobic Reduction of $A_{410}$ of Ferroxidase by Ascorbate with or without Iron Chelator—The effects of Fe(III) or Fe(II) chelators on reduction of blue color of ferroxidase by ascorbate were studied at 30° with the use of an anaerobic cuvette with a 1-cm optical light path (15, 16). The fractions of absorbance at 610 nm which remained were plotted semilogarithmically against time as shown in Fig. 6. It was clearly shown that 1,10-phenanthroline or apotransferrin reduced the rate of decolorization of 16.5 $\mu$M ferroxidase. For example, the half-life of the blue color in the presence of 300 $\mu$M 1,10-phenanthroline is 1200 sec, 6 times longer than the 200-sec control which does not contain any chelator. An exposure of the reaction mixture to the air resulted in an immediate restoration of $A_{610}$, suggesting no direct effect of 1,10-phenanthroline on ferroxidase. The apotransferrin affects the decolorization rate in the same way as 1,10-phenanthroline does. The blue color was also restored if exposed to the air. However, addition of 100 $\mu$M Fe(II) to the reaction mixture results in an instantaneous reduction of the $A_{410}$ to 0 even in the presence of oxygen, indicating a rapid reduction of ferroxidase by this substance.

Stoichiometry between Fe(II) and Reduced Ferroxidase—Decolorization of ferroxidase by adding Fe(II) was studied in an anaerobic condition at 30°. The reaction mixture initially contained 16.5 $\mu$M ferroxidase and 55 $\mu$M apotransferrin, which served to trap formed Fe(III) as the result of the oxidation of Fe(I1) by the enzyme, in 0.2 M acetate buffer, pH 6.0. The total volume of the mixture was 2.00 ml. The enzyme solution was injected through a rubber stopper with 5 to 10 $\mu$L of 5 mM Fe(II) dissolved in deoxygenated water. An immediate absorbance change at 610 nm was observed. The results obtained are summarized in Table III. The amount of ferroxidase decolorized was computed from a millimolar absorbance of 10.9 reported previously (17). The mean value for the experimentally obtained Fe(II)-enzyme ratio was 3.93, within 2% of the expected value of 4.0.

**DISCUSSION**

The oxidation of Fe(II) by ferroxidase has a unique kinetic feature in its dependence on the substrate concentration. A di-

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**Fig. 6.** The fraction of remaining absorbance at 610 nm was plotted semilogarithmically against time. Decolorization of 16.5 $\mu$M ferroxidase, dissolved in 0.2 M acetate buffer (pH 6.0) by 100 $\mu$M ascorbate, was followed spectrophotometrically at 610 nm in an anaerobic cuvette with 1-cm optical light path at 30°. ○, no chelator added; △, + 3.0 $\mu$M 1,10-phenanthroline; □, + 20 $\mu$M apotransferrin; ●, + 300 $\mu$M 1,10-phenanthroline. Fe(II) added at the point indicated gave a final concentration of 100 $\mu$M.

**TABLE III**

<table>
<thead>
<tr>
<th>Fe(II) added</th>
<th>Ferroxidase decolorized $^a$</th>
<th>Ferroxidase-Fe(II) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 $\mu$M</td>
<td>6</td>
<td>4.2</td>
</tr>
<tr>
<td>25 $\mu$M</td>
<td>6.5</td>
<td>3.9</td>
</tr>
<tr>
<td>12.5 $\mu$M</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>30 $\mu$M</td>
<td>5.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>

$^a$ $A_{410} = 10.9$ (millimolar extinction coefficient), which was calculated from the data published (17), was used to compute the concentration change by decolorization.

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**Fig. 5.** p-Phenylenediamine (pPD) oxidation by ferroxidase (E) with and without iron chelator. a: A, the reaction mixture contained 9 mM p-phenylenediamine, 0.35 $\mu$M ferroxidase, and 920 $\mu$M 1,10-phenanthroline (10-10-P) in 0.2 M acetate, pH 5.2. B, the same reaction mixture but without p-phenylenediamine. Fe(II) was added at the point indicated by the arrow, giving a final iron concentration of 47 $\mu$M. Instantaneous absorbance change is caused by Fe(II)-1,10-phenanthroline complex formation. pPD, p-phenylenediamine; E, ferroxidase. b: A, 9 mM p-phenylenediamine + 0.14 $\mu$M ferroxidase in 0.2 M acetate, pH 5.2. Fe(II) or Desferrioxamine was added at the point indicated by the arrow. E, 9 mM p-phenylenediamine + 0.14 $\mu$M ferroxidase in 0.2 M acetate, pH 6.5. Fe(II) or apotransferrin was added to reaction mixture at the point indicated by the arrow. ATf, apotransferrin; DFA, deferoxamine.
Phasic curve obtained with v plotted against v/s (Fig. 3a) gave two $K_n$ values ($K_{m_1} = 0.6 \mu M$, $K_{m_2} = 50 \mu M$) which differ from each other by almost two orders of magnitude. This very low $K_{m_1}$ value, 0.6 $\mu M$, could not be clearly observed experimentally in oxygen uptake measurements, since the lowest substrate concentration measured was 10 $\mu M$. Therefore, the data listed in Table I represent overall values. A similar property was also observed in an activation experiment by Fe(II) in ascorbate oxidation by ferroxidase (see Fig. 3), which is now considered to be a totally iron-dependent oxidation.4 It should be emphasized here that the extremely low value of $K_{m_1}$, 0.6 $\mu M$, enables ferroxidase, in the presence of trace amounts of iron ($\sim 10^{-4} M$), to oxidize certain substances, such as ascorbate,4 catechol,4 and hydroquinone,4 which are not true substrates but can react with Fe(III) in a cyclic reaction.

The deductions drawn from the results obtained can be summarized as follows.

1. Without iron, p-phenylenediamine is directly oxidized by the enzyme-bound copper, but only in a slower reaction.

   \[
   \text{p-Phenylenediamine} + \text{E-Cu(I)} \rightarrow \text{E-Cu(II)} + \text{p-phenylenediamine radical}
   \]  

2. With iron present, not only does the slower reaction described above proceed partially, but a faster reaction also takes place. The ratio of these two different reactions depends on the concentration of each competing substrate, p-phenylenediamine and Fe(II).

   \[
   \text{Fe(II)} + \text{E-Cu(II)} \rightarrow \text{Fe(III)} + \text{E-Cu(I)}
   \]  

3. 1,10-Phenanthroline chelates Fe(II) and thus prevents Fe(II) from reacting with the enzyme. Therefore, Reaction 3 does not take place. Hence, neither activation nor lag phase will be expected.

4. If apotransferrin or Desferrioxamine is present in the reaction mixture, Fe(III), which is a product of enzymic oxidation, will be trapped by chelation. Thus, Reaction 4 is prevented. But Reaction 3 proceeds as long as Fe(II) is present. In such a case, p-phenylenediamine is oxidized only in the slower reaction described in Reactions 1 and 2, with the enzyme partially available to this substrate in competition with the other substrate, Fe(II). If the concentration of Fe(II) is sufficient to occupy the enzyme exclusively, no p-phenylenediamine oxidation will take place.

In 1961, Curzon studied $N,N$-dimethyl-p-phenylenediamine oxidation by a coupled iron-beroulplasmin (ferroxidase) oxidation system.2 The higher the substrate, the lower was the activation in color formation in the presence of constant amount of Fe(II). Thus, he concluded that “variation of iron activation with $N,N$ dimethyl p-phenylenediamine concentration is explicable in terms of substrates competing for the enzyme” (2). This observation by Curzon supports the data that Fe(II) is a better substrate than $N,N$-dimethyl-p-phenylenediamine with a lower $K_n$ and higher activity. In the iron-ferroxidase-coupled oxidation, iron, changing between two oxidation states (Fe(II) and Fe(III)), is oxidized by the enzyme and reduced by the other substrate. Thus, a clear-cut inhibition cannot be observed, unless a change in the oxidation state of Fe iron is prevented. The specific Fe(III) chelators such as Desferrioxamine or apotransferrin have been used for this purpose. Competition between the two substrates, Fe(II) and p-phenylenediamine, is clearly shown in Fig. 4c. The results also indicate that Fe(II) has a very high affinity for the enzyme, contrary to a recent report by Peisach and Levine (14), in which they stated that “in the presence of p-phenylenediamine, Fe(II) oxidation does not occur.” However, a small amount of Fe(II) ($\sim 10 \mu M$) must be enzymically oxidized even in the presence of a very high concentration (9 mM) of the other substrate because of 100% inhibition in p-phenylenediamine oxidation by adding a competing substrate, Fe(II). It must be concluded that Fe(II) is oxidized at the same site as p-phenylenediamine.

It has been reported previously by Blumberg et al. (18), Broman et al. (19), and Kasper, Deutsch, and Beinert (20), and believed generally, that 50% of $8$ (4 Cu(I) + 4 Cu(II)) ferroxidase copper atoms are Cu(II). These 4 Cu(II) atoms are reduced to Cu(I) by accepting the electrons from substrates. Therefore, theoretically, ferroxidase can accept 4 electrons from its substrates. The optical absorption at 610 nm of ferroxidase and its electron spin resonance signal are proportional to Cu(II) in the enzyme (18, 19). Because only one electron change is expected for oxidation of Fe(II) to Fe(III) and the enzyme has a very low $K_{m_1}$ value for Fe(II) oxidation, Fe(II) was utilized to titrate the capacity for electron acceptance by the enzyme. The data presented in Table III clearly show that 4 Fe(II) atoms or 4 electrons are required to complete the reduction of 1 ferroxidase molecule. From the data shown in Table II and from that of our previous report (3), one can conclude that the stoichiometry among three components, Fe(II), ferroxidase, and oxygen molecule, is 4 : 1 : 1.

McDermott (12) has shown that it is difficult to reduce the contaminating iron level in a reaction mixture below the $10^{-4} M$ range. As also mentioned, the ascorbate oxidation by ferroxidase is now believed to depend on the iron-coupled system. Inhibitory effects of iron chelators on the rate of decolorization of the enzyme with ascorbate and certain other agents are to be expected unless iron is eliminated. The time course of the absorbance change at 610 nm, shown in Fig. 6, gives strong support for this deduction.

The molecular activity of the ascorbate oxidation by ferroxidase in the presence of iron was reported to be 276 at pH 6.5 (6), whereas the molecular activity for Fe(II) as a substrate estimated from the data presented in Fig. 3a was 550 at pH 6.5. This represents a good agreement since the ascorbate can donate 2 electrons in reducing Fe(III) to Fe(II).

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4 J. A. McDermott, S. Osaki, and E. Frieden, unpublished data.
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REFERENCES

Kinetic Studies of Ferrous Ion Oxidation with Crystalline Human Ferroxidase (Ceruloplasmin)
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