 CHEMICAL PROPERTIES OF FERRITIN AND THEIR RELATION TO ITS VASODEPRESSOR ACTIVITY *

BY ARRAHAM MAZUR,† IRENE LITT, AND EPHRAIM SHORR
(From the Department of Medicine, Cornell University Medical College and The New York Hospital, New York)

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The studies of Granick and Michaelis (1) on the chemistry of ferritin and its iron-free protein, apoferitin, were concerned with their preparation in crystalline form, the nature of the iron in ferritin, and the rôle of ferritin as an iron storage compound for hemoglobin regeneration. Ultra-centrifuge data obtained by Rothen (2) have shown that ferritin is a mixture of some free apoferitin together with ferritin, the latter in an aggregated state. Apoferitin, however, gives a single uniform sedimenting boundary with a molecular weight for horse spleen apoferitin of 465,000.

Work in our laboratory (3) has led to the identification of ferritin with a hepatic vasodepressor material which appears in the circulation in low but measurable concentrations during the irreversible or hyporeactive stage of hemorrhagic shock in animals, and, together with a vasoexcitor material of kidney origin, in the circulation during experimental renal hypertension in dogs and essential hypertension in man.

The present study is concerned with the further exploration of some chemical properties of ferritin and apoferitin and their relation to the vasodepressor activity of these proteins as measured by the rat mesoappendix test of Zweifach and Chambers (4). Data are presented from experiments on electrophoresis, viscosity, fractionation studies with ammonium sulfate and high speed centrifugation, the effects of crystalline pepsin and trypsin, the effect of pH on the vasodepressor activity, as well as a complete amino acid distribution.

EXPERIMENTAL

Crystalline ferritin and apoferitin were prepared¹ by the method of Granick and Michaelis (5) as modified by the authors (3). Total nitrogen was determined by a modified micro-Kjeldahl method (6), total phosphorus

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by that of Fiske and Subbarow (7), total iron by the Wong method (8), total S according to Zahnd and Clarke (9), and cadmium by the method described by Granick and Michaelis (5). Amino nitrogen was determined by the Van Slyke micromethod (10) and all amino acids with the exception of tryptophan and cystine by the microbiological procedure of Henderson and Snell (11) with \textit{Lactobacillus arabinosus, Leuconostoc mesenteroides,} and \textit{Streptococcus faecalis}.\textsuperscript{2} Tyrosine and tryptophan were determined by the method of Folin and Marenzi (12) adapted to the photocolorimeter, and total cystine by the method of Kassell and Brand (13).

Electrophoretic Behavior of Ferritin—It had been previously reported (14) that the mobilities of crystalline ferritin and apoferritin prepared from horse spleen were identical over a wide pH range, and that these proteins

\textsuperscript{2} We are indebted to Dr. Ernest Borek of the New York State Psychiatric Institute for the specimen of \textit{L. arabinosus} and to Dr. Erwin Brand of the College of Physicians and Surgeons for \textit{L. mesenteroides} and \textit{S. faecalis}.

Fig. 1. Electrophoretic mobilities of horse spleen (▲), human liver (○), and dog liver (●) ferritins at various pH values. All determinations were done in acetate buffer, 0.1 ionic strength.
gave uniform single boundaries on electrophoresis. Fig. 1 illustrates the
data obtained with ferritins from horse spleen, dog liver, and human liver.
Electrophoretic data were obtained by use of a Longsworth-Tiselius ap-
paratus with 0.2 per cent ferritin solutions dialyzed against acetate buffers
of 0.1 ionic strength. All boundaries were uniform and showed no evidence
of contamination with proteins of different mobilities. The isoelectric
points calculated from these data were 4.4 for horse, 5.2 for dog, and 5.5
for human ferritin. These data, together with the immunochemical dif-
fferences previously observed (14) among these species of ferritin, indicate
dissimilarity with respect to surface-charged amino acid groupings. Sev-
eral preliminary observations on horse liver ferritin gave mobility values
identical with those shown for horse spleen ferritin. This finding would
confirm our earlier observations concerning the immunochemical identity
of the ferritins of horse liver and spleen.

Viscosity of Ferritin and Apoferritin—Since the viscosity of a solution
is related to the volume occupied by the solute molecules as well as to
their shape, it was of interest to compare ferritin with apoferritin because
of the presence of much iron in ferritin and the lack of homogeneity of
ferritin in the ultracentrifuge as reported by Rothen (2). Treffers (15)
has observed that the relative fluidity (the reciprocal of the relative vis-
cosity) varies as a linear function of concentration with many proteins.

Fig. 2. Relative fluidities of horse spleen ferritin (●) and apoferritin (○) at vari-
ous concentrations of protein. The determinations were done in 0.1 M phosphate
buffer, pH 7.4, at 25°.

3 We are indebted to Dr. V. du Vigneaud for the use of the electrophoresis appara-
tus.
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The relative viscosity of the protein solutions was measured in a 0.1 M phosphate buffer, pH 7.4, with an Ostwald viscosimeter. Fig. 2 demonstrates that the relative fluidity of ferritin and apoferritin is identical at several different concentrations of protein N and that a linear relationship exists. This would indicate that the iron in ferritin is without effect on the viscosity owing to the protein moiety.

Fractionation of Ferritin by Ammonium Sulfate—In order to demonstrate the relationship between the iron and protein moieties of ferritin, a solution of horse spleen ferritin recrystallized four times was treated successively with varying amounts of a saturated ammonium sulfate solution. The precipitated protein was freed of ammonium sulfate by dialysis and analyzed for Fe, N, and P. The results are shown in Table I. The original ferritin solution, which was homogeneous electrophoretically, contained 1.88 mg. of Fe per mg. of N. The first precipitate obtained at 23 per cent saturation with ammonium sulfate had a Fe:N ratio of 2.20, whereas the Fe:N ratio of the last fraction, precipitated at 40 to 50 per cent saturation of ammonium sulfate, was 0.55. The ratio of Fe:P was fairly constant in all fractions, bearing out the conclusions of Granick and Michaelis (5) as to the probable nature of the iron hydroxide-iron phosphate micelles.

Table I

<table>
<thead>
<tr>
<th>Per cent saturation with (NH₄)₂SO₄</th>
<th>Per cent of original total N in ppt.</th>
<th>Mg. Fe/Mg. N</th>
<th>Molar ratio, Fe:P</th>
<th>Analyses, dry weight basis</th>
<th>Vasodepressor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>Fe</td>
</tr>
<tr>
<td>Original</td>
<td>100</td>
<td>1.88</td>
<td>9</td>
<td>11.0</td>
<td>20.7</td>
</tr>
<tr>
<td>0 -23</td>
<td>18.4</td>
<td>2.20</td>
<td>10</td>
<td>10.2</td>
<td>22.5</td>
</tr>
<tr>
<td>23 -26</td>
<td>21.5</td>
<td>2.09</td>
<td>10</td>
<td>13.6</td>
<td>12.6</td>
</tr>
<tr>
<td>26 -30</td>
<td>38.3</td>
<td>1.83</td>
<td>10</td>
<td>16.2</td>
<td>0</td>
</tr>
<tr>
<td>40 -50</td>
<td>6.0</td>
<td>1.46</td>
<td>10</td>
<td>9.4</td>
<td>5.5</td>
</tr>
<tr>
<td>Apoferritin*</td>
<td>2.3</td>
<td>0.93</td>
<td>9</td>
<td>16.6</td>
<td>0</td>
</tr>
</tbody>
</table>

* Apoferritin was prepared by treating the original ferritin with Na₂S₂O₄ in 1 M acetate buffer, pH 4.6, and dialyzing against \( \alpha, \alpha'-\)dipyridyl according to Granick and Michaelis (5).
All fractions gave identical crystals with CdSO₄. The original solution, as well as those fractions precipitated at 0 to 23, 34.6 to 40, and 40 to 50 per cent saturation with ammonium sulfate, gave identical vasodepressor activities on a nitrogen basis when tested by the rat mesoappendix technique (4).

Fractionation of Ferritin by Centrifugation Rothen's results with the ultracentrifuge led us to fractionate a ferritin solution at 13,000 r.p.m. for 1 hour at 4° in a Servall centrifuge. After centrifugation, an almost colorless, thin layer of solution could be observed at the top. A portion of the top layer was removed by pipette, but some mixing with the dark brown layer underneath it was unavoidable. Similarly, an intermediate portion of the solution was obtained, as well as some from the very bottom of the tube which had settled almost as a pellet. Analyses gave results (Table II) similar to those obtained by chemical fractionation with ammonium sulfate. Again, all fractions were equally active on a nitrogen basis by the rat mesoappendix test for vasodepressor activity.

Effect of Pepsin on Ferritin and Apoferritin—The action of crystalline pepsin on ferritin and apoferritin was followed by the method of Anson (16) on an incubation mixture containing 0.23 mg. of horse spleen ferritin N per ml. together with 0.17 mg. or 0.017 mg. of pepsin N at 35°. Digestion was measured by means of total nitrogen determinations on aliquots of the trichloroacetic acid filtrates and corrected for the appropriate enzyme controls. Table III lists the results of a time-course reaction at pH 2.2. The difference in extent of digestion between ferritin and apoferritin was marked. Because of these results, the extent of peptic hydrolysis was studied at various pH values (Table IV). Enzyme action was allowed to continue for 1 hour at 35° and horse spleen ferritin was compared with

### Table II

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total N per ml. (mg.)</th>
<th>Total Fe per ml. (mg.)</th>
<th>Mg. Fe/Mg. N</th>
<th>Vasodepressor activity $\gamma N \times 10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>5.54</td>
<td>10.43</td>
<td>1.88</td>
<td>5</td>
</tr>
<tr>
<td>Top layer</td>
<td>1.07</td>
<td>1.38</td>
<td>1.28</td>
<td>5</td>
</tr>
<tr>
<td>Intermediate layer</td>
<td>5.11</td>
<td>10.00</td>
<td>1.96</td>
<td>5</td>
</tr>
<tr>
<td>Bottom layer</td>
<td>0.32</td>
<td>0.80</td>
<td>2.49</td>
<td>5</td>
</tr>
</tbody>
</table>
horse spleen apoferritin and a substrate solution prepared from dog hemoglobin according to Anson. At the same time, the extent of denaturation of ferritin and apoferritin at the different reactions was measured in terms of the quantity of insoluble protein precipitated by bringing the pH of the solution to the isoelectric point of this species of ferritin and apoferritin, 4.4. In general, it may be seen that greater digestion takes place at lower

**TABLE III**

**Time-Course of Peptic Hydrolysis of Ferritin and Apoferritin**

The method of Anson (16) was used to estimate peptic hydrolysis, with crystalline pepsin at 35°. Enzymatic action is expressed in terms of per cent of the ferritin nitrogen found in the trichloroacetic acid filtrates. The time-course of digestion was carried out at pH 2.2.

<table>
<thead>
<tr>
<th>Time</th>
<th>Ferritin</th>
<th>Apoferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.17 mg. pepsin N</td>
<td>0.017 mg. pepsin N</td>
</tr>
<tr>
<td>min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>hrs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>14</td>
</tr>
</tbody>
</table>

**TABLE IV**

**Effect of pH on Peptic Hydrolysis and Denaturation of Ferritin and Apoferritin**

The extent of hydrolysis is expressed in terms of per cent of the protein nitrogen found in the trichloroacetic acid filtrates after 30 minutes incubation at 35°, corrected for controls. All proteins were present in a concentration of 0.23 mg. of N per ml., together with 0.017 mg. of pepsin N per ml. The hemoglobin solution was prepared from dog blood by the method of Anson (16). The extent of denaturation is expressed in terms of per cent of the total N insoluble at pH 4.4 (isoelectric point of ferritin) after prior incubation for 1 hour at 35° at each pH. Each precipitate was washed twice with 5 volumes of water before analysis.

<table>
<thead>
<tr>
<th>pH</th>
<th>Ferritin Hydrolysis</th>
<th>Apoferritin Hydrolysis</th>
<th>Hemoglobin Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denaturation</td>
<td>Denaturation</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>67</td>
<td>69</td>
<td>96</td>
</tr>
<tr>
<td>2.0</td>
<td>16</td>
<td>35</td>
<td>79</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>3.0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
pH values. Also, there is an approximate correlation between the amount of digestion and the extent of denaturation.

Since ferritin, which contains the iron, is more difficult to denature than apoferritin, a comparison was made between the peptic hydrolysis of ferritin with a high iron content and of a specimen with some or all of its iron removed. The ferritin was treated with Na$_2$S$_2$O$_3$ in 1 M acetate buffer, pH 4.6, at room temperature according to the method of Granick and Michaelis (5). After 30 minutes, a portion was removed and dialyzed against running water. The remainder was again treated with Na$_2$S$_2$O$_3$ and $\alpha,\alpha'$-dipyridyl and dialyzed until free of iron, yielding a solution of apoferritin. Table V lists the Fe:N ratio of each fraction and the relative degree of digestion by pepsin. There is a good correlation between iron content and difficulty of peptic hydrolysis.

**Table V**

**Effect of Iron Content on Extent of Peptic Hydrolysis of Ferritin**

Fraction A was prepared from Sample 1287 (horse spleen ferritin) by treatment with Na$_2$S$_2$O$_3$ in 1 M acetate buffer, pH 4.6, for 30 minutes and dialyzed against running water. Fraction C was prepared by allowing the Na$_2$S$_2$O$_3$ to act on Sample 1287 until all the iron had been reduced and removed by dialysis. It represents apoferritin. The extent of hydrolysis at pH 2.2 and 35° is expressed in terms of per cent of the total protein N found in the trichloroacetic acid filtrates.

<table>
<thead>
<tr>
<th>Ferritin sample No.</th>
<th>Mg. Fe</th>
<th>Mg. N</th>
<th>Per cent hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1287</td>
<td>1.55</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>1287, Fraction A</td>
<td>1.04</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>1287, &quot;C&quot;</td>
<td>0.00</td>
<td></td>
<td>82</td>
</tr>
</tbody>
</table>

**Effect of Trypsin on Ferritin and Apoferritin**—Tryptic digestion was carried out with crystalline trypsin containing approximately 50 per cent MgSO$_4$, by the method of Anson (16) with use of the Folin phenol reagent, on aliquots of the trichloroacetic acid filtrates. The protein substrates were pretreated in several different ways in order to discover the effects of these treatments on the effectiveness of trypsin. In the control, the proteins were not pretreated at all but were brought to pH 7.5 for trypsic digestion. In the remaining tubes, the protein was treated with alkali alone (pH > 12), urea alone (final concentration 40 per cent by weight), or urea plus alkali, as recommended by Anson for hemoglobin. After the preliminary treatment, the mixtures were brought to pH 7.5. Each ml. of the digestion mixture contained 1.65 mg. of protein N and 2 mg. of crystalline trypsin-MgSO$_4$. Digestion was carried out at 35° for 30 minutes. The results in Table VI clearly indicate the correlation between tryptic digestion and severity of treatment required for denaturation.
Effect of pH on Vasodepressor Effect of Ferritin—The effect of pH on the denaturation (insolubility at its isoelectric point) of ferritin led to a determination of the effect of pH on the vasodepressor activity of ferritin. The lowest concentration of ferritin (crystallized with CdSO₄) which will give a clearly positive vasodepressor effect by the rat mesoappendix test is equivalent to 0.0005 γ of ferritin N per 0.5 ml. Solutions of ferritin containing 300 γ of ferritin N were incubated at 37° for 1 hour at different reactions, brought to pH 7.4, and diluted for testing to a concentration of 0.0005 γ of N per 0.5 ml. At pH 3.0 or less, the vasodepressor activity was destroyed, but from pH 3.5 to 8.0 it was strongly positive. At a pH greater than 8, the activity was destroyed. This inactivation of vaso-

### Table VI

**Effect of Trypsin on Ferritin and Apoferritin**

The method of Anson (16) was used with crystalline trypsin. Incubation with NaOH alone, urea alone, or urea + NaOH for 30 minutes at room temperature. Tryptic digestion was carried out with 1.68 mg. of protein N per ml. and 4 mg. per ml. of a trypsin preparation containing 50 per cent MgSO₄. All proteins were brought back to pH 7.5 for the enzyme action. The extent of hydrolysis is expressed in terms of per cent of the original Folin phenol color value obtained in the trichloroacetic acid filtrates after digestion for 30 minutes at 35°, corrected for the blank value.

<table>
<thead>
<tr>
<th>Treatment prior to addition of trypsin</th>
<th>Per cent of original Folin color value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ferritin</td>
</tr>
<tr>
<td>None</td>
<td>20</td>
</tr>
<tr>
<td>NaOH</td>
<td>28</td>
</tr>
<tr>
<td>Urea</td>
<td>25</td>
</tr>
<tr>
<td>NaOH + urea</td>
<td>56</td>
</tr>
</tbody>
</table>

depressor activity appears to correspond roughly in the acid region with the extent of denaturation, but is more sensitive to alkaline treatment than is indicated by the extent of denaturation in that pH region.

**Distribution of Amino Acids in Ferritin and Apoferritin**—There are two references in the literature by Tria (17) and Kuhn et al. (18), concerning the amino acid content of ferritin. The values reported are completely at variance with each other. In light of the purity tests applied to our crystalline ferritin preparations, the reported presence in ferritin by Kuhn et al. of a nucleoprotein, and a denial of this latter finding by Granick and Michaelis (5), a complete analysis of ferritin was undertaken. In the present study, horse spleen ferritin recrystallized four times was reprecipitated four times at 50 per cent saturation with ammonium sulfate and freed of inorganic ions by dialysis. This solution was used for the prepara-
tion of crystalline apoferritin, which was treated in a similar manner. Both proteins were homogeneous electrophoretically. Apoferritin was used for the complete analysis rather than ferritin, to avoid difficulties from the iron, which interferes with many of the analyses. However, five amino acids were determined in ferritin for comparison. Most of the amino acids were determined microbiologically, by use of the uniform medium of Henderson and Snell (11). Tyrosine and tryptophan (12) and cystine (13) were determined chemically. Serine was not determined. Values for the amino acid nitrogen distribution are presented in Table VII. In all, 98 per cent of the total N of apoferritin was accounted for by the usual

### Table VII

**Distribution of Amino Acid Nitrogen of Apoferritin and Ferritin**

The amino acid content of these proteins was determined, for the most part, by the microbiological procedure of Henderson and Snell (11). Chemical methods were used for tyrosine, tryptophan, and cystine. The serine content was not determined. The results are expressed as the per cent of the total nitrogen accounted for by each amino acid.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Apoferritin*</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm. per 100 gm. protein</td>
<td>per cent of total N</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>17.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Humin N</td>
<td>6.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Glutamic</td>
<td>7.8</td>
<td>9.2</td>
</tr>
<tr>
<td>Aspartic</td>
<td>9.1</td>
<td>18.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*The apoferritin contained, in per cent on a dry weight basis, 16.2 N, 0.0 Fe, 0.05 P, 0.16 Cd, 0.89 total S. Cystine and methionine accounted for 98 per cent of the total S present. The amino N was 5.0 per cent of the total N.

†The value of 2.2 was obtained by the chemical method (12), 2.4 by the microbiological method (11).
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amino acids, free ammonia, and humin; serine was not determined. The free ammonia corresponds to that which would be liberated during hydrolysis if all of the glutamic acid existed as glutamine in the protein. The total S was accounted for to the extent of 98 per cent as total cystine plus methionine. The relative amounts of acidic and basic amino acids correspond to a protein with an acid isoelectric point. The amino acid analyses of ferritin agree with the values for apoferritin very well, and it is difficult to conceive of any nucleic acid fragments in ferritin as reported by Kuhn et al. The value for arginine, calculated in terms of per cent of apoferritin, as reported by Tria is 5.7 per cent, by Kuhn 16 per cent, and by Archibald (19), by the arginase method, 8.3 per cent. Our value calculated on the same basis is 9.1 per cent.

DISCUSSION

The results of the chemical studies emphasize the unique character of ferritin, a protein with over 20 per cent by weight of iron, which is present in many tissues in the dog (14), such as spleen, liver, bone marrow, kidney cortex and, to a smaller extent, in testes and skeletal and cardiac muscle. The protein is absent from normal circulating plasma or red cells. Those data which concern the effect of surface groups, such as electrophoretic mobility, viscosity, and immunochemical reactions, suggest the probability that the iron in ferritin is present in the molecule in such a manner that it does not affect the surface properties of ferritin directly. The ease with which the animal can call on this iron for purposes of iron transport and hemoglobin synthesis (1) suggests an enzymatic reaction which would release the iron from its relatively tight linkage in ferritin.

The variability of the Fe:N ratio in fractions easily obtainable from crystalline ferritin by gentle methods, such as (NH₄)₂SO₄ fractionation or high speed centrifugation, indicates again the aggregated state of ferritin and the fact that the iron or phosphorus can have no specific linkage with any part of the protein moiety such as some particular amino acid. This finding, together with the results of the amino acid analyses, confirms the conclusion that ferritin is not, nor does it contain, a nucleoprotein. The amino acid distribution in ferritin is quite different from that in hemoglobin, and, although the iron of ferritin may be used for hemoglobin synthesis, the protein moiety cannot be used per se. The vasodepressor activity of ferritin is associated with the protein moiety of ferritin, since all fractions with variable Fe:N ratios, as well as apoferritin which contains no iron, are equally active in the rat mesoappendix test. These facts do not, however, rule out the possible role of iron in the vasodepressor activity of ferritin, since the injection of apoferritin or a ferritin with a low
iron content may be accompanied in vivo by a combination with iron to form the active material.

The inability of pepsin or trypsin to digest ferritin, unless it is denatured, agrees with similar findings in the literature (20–22) concerning these two enzymes. Tria and Torboli (23) reported that ferritin was not hydrolyzed by trypsin, although after denaturation with HCl it was hydrolyzed (24). That ferritin is more difficult to denature by acid than apoferritin suggests a protective function for the iron. It should also be pointed out that during the course of its preparation ferritin solutions may be heated to 80° without denaturation. The effect of acid on the denaturation process is associated with a corresponding destruction of the vasodepressor activity of ferritin. On the other hand, treatment of ferritin at pH 9 also inactivates the vasodepressor activity of ferritin, although this treatment in the absence of urea does not denature ferritin to any extent. The alkaline inactivation appears to be concerned more with some chemically sensitive grouping in the protein molecule, or with some disturbance in the protein structure similar to denaturation, without an accompanying insolubility of ferritin at its isoelectric point.

The authors are indebted to Mr. Richard Oliver-Smith for chemical assistance and to Vera Bergman and Ruth Jacob for the rat assays.

SUMMARY

Electrophoretic data gave isoelectric points of 4.4 for horse spleen ferritin and apoferritin, 5.2 for dog liver ferritin, and 5.4 for human liver ferritin.

Viscosity studies, together with the electrophoretic and immunochromical behavior of ferritin, indicate that the iron in ferritin is without effect on the surface-reactive groups of the protein molecule as measured by these methods.

Crystalline pepsin and trypsin will digest ferritin if the protein is first denatured. The iron in ferritin protects the protein to some extent from acid denaturation.

The amino acid distribution of ferritin precludes the presence of any nucleoprotein. All of the sulfur is accounted for by cystine plus methionine. The amino acid distribution is quite different from that of hemoglobin.

The vasodepressor activity of ferritin is dependent on the protein moiety of the molecule, but the data do not preclude the possible rôle of iron. Various fractions obtained either with ammonium sulfate or by high speed centrifugation, although they contain different amounts of iron, are crystallizable with CdSO₄ and have the same vasodepressor activities when meas-
Chemical Properties of Ferritin

...ured by the rat mesoappendix test. The vasodepressor activity of ferritin is destroyed by a pH more acid than 3.5 or more alkaline than 8.0. In the acid region, this inactivation corresponds approximately with the extent of denaturation, but in the alkaline region the presence of some chemically sensitive grouping on the protein molecule is suggested.

BIBLIOGRAPHY

CHEMICAL PROPERTIES OF FERRITIN AND THEIR RELATION TO ITS VASODEPRESSOR ACTIVITY
Abraham Mazur, Irene Litt and Ephraim Shorr


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