Distinctive Properties of Ferritin from the Reuber H-35 Rat Hepatoma

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SUMMARY
Ferritin from Reuber H-35 hepatomas was compared with ferritin from livers of ACI rats, the strain of rats in which the original H-35 hepatoma developed. The isoelectric points of these two proteins are 5.20 ± 0.02 (H-35 ferritin) and 4.95 ± 0.03 (ACI liver ferritin). The amino acid composition of apoferritin prepared from H-35 hepatoma ferritin differs significantly from that of ACI rat liver apoferritin. For the majority of amino acids, the differences are significant at p < 0.01. Ion-exchange chromatography on carboxymethylcellulose at pH 5.5 and 7.2 also gave significantly different results for the two ferritins. These findings, taken together, indicate that the primary sequence of amino acids in some or all protein subunits of the two ferritins (apoferritins) is not the same. However, by electron microscopy the two kinds of ferritin are similar in size, shape, and overall appearance. The sedimentation velocities of the two apoferritins are almost identical (18.6 S for ACI liver, 18.5 S for H-35 hepatoma apoferritin). Subunits of the apoferritins, prepared by treatment with sodium dodecyl sulfate, have identical mobilities on electrophoresis in polyacrylamide gel, presumably because the net charge densities of sodium dodecyl sulfate subunit complexes are due to sulfate.

INTRODUCTION
In a recent report (26), we described the occurrence of an electrophoretically distinct ferritin in certain rat hepatoma cells grown in vitro, in the Novikoff ascites tumor, and in Morris 5123 and Reuber H-35 hepatomas carried s.c. in rats. By contrast, the Reuber H-139 and H-146 rat hepatomas, which are more highly differentiated hepatomas that grow more slowly, produced only an ordinary rat liver ferritin (26).

In this report, we compare several properties of ferritin from H-35 hepatomas with those of ferritin from livers of ACI rats, the strain of rats in which the H-35 hepatoma originated. These 2 types of ferritin differ in isoelectric points, behavior on ion-exchange chromatography, and amino acid composition.

MATERIALS AND METHODS
Animals and Tumors. Female weanling ACI rats were obtained from Microbiological Associates, Bethesda, Md.; housed individually in cages; and given liberal supplies of water and regular Purina pellets. The tumors were transplanted s.c., as described previously (26).

Extraction and Purification of Ferritin and Apoferritin. Highly purified ferritin was prepared from pooled ACI rat livers (about 500 g) and pooled H-35 hepatomas (about 1 kg) from rats loaded with iron, as previously detailed (26). The ferritin thus obtained was purified further by chromatography through CM-cellulose columns. Apoferritin was prepared as previously reported (2). The concentration of purified ferritin or apoferritin in solution was calculated from nitrogen determinations by a microkjeldahl procedure (16). We succeeded in crystallizing the H-35 hepatoma ferritin with CdSO₄ by standard methods previously used with other kinds of ferritin, including rat liver ferritin (11, 27). The various samples of ferritin were checked by immunoprecipitation tests with rabbit antisera specific for rat ferritins (26).

Polyacrylamide Gel Electrophoresis. Electrophoresis in polyacrylamide gels was done as described previously (26).

Isoelectric Focusing. The isoelectric points of samples of ferritin were determined with the use of an LKB 110-ml column (LKB 8101 obtained from LKB Instruments, Inc., Rockville, Md.). For each experiment, samples of 2 to 10 mg of ferritin were used. The concentration of Ampholine (LKB 8141 and LKB 8142) was 1%, and the pH ranged from 3 to 6 or from 3 to 10. During the first 24 hr of each run, the potential was 500 V (constant voltage) and the current was 5 to 7 ma. After 24 hr, the current usually fell to about 1.5 to 2 ma and eventually settled at 1 to 1.5 ma, with the potential remaining 500 V. Each run was continued for at least 24 hr after the current had stabilized. For collection of fractions after completion of electrophoresis, the solution in the column was drained by gravity into test tubes (12 to 24 drops/tube) in a fraction collector. The pH and absorbance at 280 nm were then determined.

Spectrophotometric Analysis. Absorption spectra of ferritin and apoferritin from H-35 hepatomas and from ACI rat liver were recorded in the range of 230 to 830 nm in a scanning spectrophotometer. All solutions to be measured

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4The abbreviations used are: CM-cellulose, carboxymethylcellulose; SDS, sodium dodecyl sulfate.

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were exhaustively dialyzed against phosphate buffer (pH 7.2, 0.05 M).

CM-Cellulose Chromatography. The method of CM-cellulose chromatography was modified from that of Drysdale and Munro (9), who showed that CM-cellulose gave considerably higher recovery of ferritin than diethylaminoethyl cellulose. CM-cellulose was obtained from Mann Research Laboratories, Inc., New York, N. Y., as Mannex-CM. The column was prepared and used as described by Drysdale and Munro (9). In every experiment, after ferritin was eluted with the pH 5.5 phosphate buffer the column was still brown. When a phosphate buffer at pH 7.2 (0.10 M) was used subsequently, most of the remaining brown material came off the column. The eluates obtained at pH 5.5 and 7.2 both contained pure ferritin consisting of typical monomer, dimer, and trimer fractions. This was shown by gel electrophoresis, as in previous work (26, 28). A comparison of ACI rat liver ferritin with H-35 hepatoma ferritin, were dialyzed against phosphate buffer (pH 7.2, 0.05 M). Sedimentation velocity runs were done in a double-sector cell in a Spinco Model E ultracentrifuge at 44,000 rpm and 20°. Absorbance at 280 nm was recorded by a photoelectric scanner at 4-min intervals for the solution of H-35 apoferritin (concentration 0.06%). The distance (cm) from the boundary midpoint to the axis of rotation was measured in the tracings of absorbance at 280 nm obtained during the sedimentation runs. The logarithm of this distance was plotted against time.

Electron Microscopy. Sections of crystals, prepared as described in "Results," were examined in a Siemens-Elmiskop 101 electron microscope at 80 kV. Negatively stained preparations of molecules from solutions (22) were also examined, as already described (26).

RESULTS

Electrophoresis. Comparison of H-35 hepatoma ferritin with ACI rat liver ferritin confirmed the distinct mobility of the former. Monomer, dimer, and trimer fractions (α, β, and γ fractions, respectively) of the kind previously noted (26, 28) were present in both H-35 and ACI liver ferritin (Fig. 1). The α, β, and γ fractions of H-35 hepatoma ferritin migrate more rapidly than the corresponding fractions of ACI rat liver ferritin (26). These fractions stained positively for iron and protein. Table 1 shows their relative proportions in H-35 ferritin and in ACI rat liver ferritin after separation in polyacrylamide gels. Apoferritin prepared from H-35 hepatoma ferritin retained the characteristic mobility (26). The fastest (i.e., monomeric or α) fraction of H-35 ferritin was isolated from polyacrylamide gel columns by sectioning and elution in phosphate buffer (pH 7.2, 0.1 M) after electrophoresis; it was then concentrated and checked by electrophoresis in another polyacrylamide gel. This procedure confirmed the characteristic mobility (Fig. 2), and it also revealed redimerization and retrimerization of the monomer, similar to that previously noted with horse ferritin (28).

Isoelectric Focusing. The results of isoelectric focusing of ACI rat liver ferritin and H-35 hepatoma ferritin are shown in Charts 1 and 2, where pH units and absorbance at 280 nm are

<table>
<thead>
<tr>
<th>Ferritin</th>
<th>α (monomer) (%)</th>
<th>β (dimer) (%)</th>
<th>γ (trimer) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-35 hepatoma</td>
<td>90.2</td>
<td>8.9</td>
<td>0.9</td>
</tr>
<tr>
<td>ACI rat liver</td>
<td>80.9</td>
<td>13.7</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Chart 1. Isoelectric focusing of ACI rat liver ferritin in 1% Ampholine. Plot of absorbance at 280 nm and pH units of the collected fractions. Ampholine range, pH 3 to 10.
plotted against tube numbers. The pH gradients are smooth and gradual within the desired pH range, and besides the major peak there are several minor peaks. The probable nature of the minor peaks will be considered in "Discussion." Calculations of the isoelectric points are based on the location of the major peak in each of 3 runs of aliquots from each sample analyzed. As a control, the isoelectric point of the major peak of horse spleen ferritin was checked by the same method. This averaged 4.4 pH units, in close agreement with values previously obtained by other methods (21).

The calculated results are listed in Table 2. The isoelectric point of H-35 ferritin, pH 4.95 ± 0.03, is significantly lower than that of ACI rat liver ferritin, pH 5.20 ± 0.02. The difference accounts for the faster mobility of H-35 ferritin towards the anode in buffers in which the pH is above both isoelectric points.

Ion-Exchange Chromatography. When eluates obtained at pH 5.5 and 7.2 were concentrated and run in polyacrylamide gels, ferritin was found in both. However, when mixtures of ferritin from H-35 hepatomas and from ACI rat livers were chromatographed at pH 5.5 and the eluates were subsequently subjected to electrophoresis, a change in the proportion of the 2 kinds of ferritin was noted. Because of this observation, gels were traced in a densitometer after staining with Naphthol Blue Black to determine the relative concentrations of the 2 kinds of ferritin.

The ratios of ferritin from H-35 tumors to ferritin from ACI rat livers, eluted at the 2 pH values from a CM-cellulose column, are shown in Table 3. In the pH 5.5 eluate, the ratio H-35:ACI was higher than in the original mixture. By contrast, in the pH 7.2 eluate, the ratio H-35:ACI was lower than in the original mixture. As a control, the isoelectric point of the major peak of horse spleen ferritin was checked by the same method. This averaged 4.4 pH units, in close agreement with values previously obtained by other methods (21).

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Quantitative Amino Acid Analysis. The results of the amino acid analyses of apoferritin prepared from ACI rat liver ferritin and from H-35 hepatoma ferritin are shown in Table 4. For the ACI liver apoferritin, the values represent the means of 3 runs of a 24-hr hydrolysate; for H-35 hepatoma apoferritin, the values represent the means of 7 runs of a 24-hr hydrolysate.

Analytical Ultracentrifugation. Plots of log x versus time are shown in Chart 3. The sedimentation coefficient of ACI rat liver apoferritin was 17.80 S, and that of H-35 hepatoma apoferritin was 17.94 S. After correction for density and viscosity of the solvent, sedimentation coefficients of 18.6 (ACI liver) and 18.5 (H-35 hepatoma) were obtained.

Spectrophotometric Analysis. The absorption spectra of ferritin from H-35 hepatoma and ACI rat liver are shown in Chart 4. Both kinds of ferritin give a plateau of maximal absorption at 280 nm, with subsequent decrease in absorbance.

The values are expressed as moles of amino acid residues per mole of apoferritin (M.W. 450,000) and are averages of 3 and 7 analyses of 24-hr digests for apoferritin from ACI rat liver and from H-35 hepatoma, respectively. The S.D. and p were obtained from computer (Fortran IV) calculations of 1-way analysis of variance with the use of Fisher's F.

### Table 2

<table>
<thead>
<tr>
<th>Source of ferritin</th>
<th>No. of determinations</th>
<th>Isoelectric point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse spleen</td>
<td>3</td>
<td>4.44 ± 0.03</td>
</tr>
<tr>
<td>ACI rat liver</td>
<td>3</td>
<td>5.20 ± 0.02</td>
</tr>
<tr>
<td>H-35 hepatoma</td>
<td>3</td>
<td>4.95 ± 0.03</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

### Table 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Original mixture (FH-35FACI)</th>
<th>Eluate from pH 5.5 buffer, 0.05 M (FH-35FACI)</th>
<th>Eluate from pH 7.2 buffer, 0.10 M (FH-35FACI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62.3:37.6</td>
<td>67.1:29.9</td>
<td>49.2:50.8</td>
</tr>
<tr>
<td>2</td>
<td>60.7:39.3</td>
<td>75.8:24.2</td>
<td>48.5:51.5</td>
</tr>
<tr>
<td>3</td>
<td>65.2:34.7</td>
<td>76.1:23.9</td>
<td>49.4:50.6</td>
</tr>
</tbody>
</table>

* FH-35, H-35 hepatoma ferritin; FACI, ACI rat liver ferritin.

### Table 4

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>ACI rat liver</th>
<th>H-35 hepatoma</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>422.8 ± 3.8</td>
<td>428.7 ± 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>Threonine</td>
<td>174.4 ± 0.9</td>
<td>181.7 ± 3.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serine</td>
<td>195.3 ± 1.2</td>
<td>258.1 ± 3.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>604.2 ± 8.0</td>
<td>539.3 ± 7.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proline</td>
<td>93.0 ± 0.2</td>
<td>130.6 ± 4.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glycine</td>
<td>277.7 ± 7.9</td>
<td>280.8 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>391.1 ± 3.4</td>
<td>329.7 ± 4.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>23.3 ± 0.8</td>
<td>54.5 ± 0.8</td>
<td>&lt;0.01</td>
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<tr>
<td>Valine</td>
<td>185.0 ± 1.8</td>
<td>204.0 ± 3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Methionine</td>
<td>14.5 ± 0.3</td>
<td>56.9 ± 2.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>50.3 ± 0.5</td>
<td>117.5 ± 3.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Leucine</td>
<td>616.1 ± 1.3</td>
<td>496.9 ± 4.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>100.9 ± 0.8</td>
<td>118.3 ± 1.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>196.7 ± 1.8</td>
<td>197.5 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Histidine</td>
<td>155.6 ± 2.3</td>
<td>166.7 ± 5.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lysine</td>
<td>26.2 ± 2.9</td>
<td>269.0 ± 4.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Arginine</td>
<td>265.5 ± 4.3</td>
<td>196.0 ± 5.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
* NS, not significant.
ferritin from rat hepatoma

0.84
0.83
0.82
0.81
0.80
0.79

I I
12 20 28 36
44 52
t (min)

Chart 3. Ultracentrifugation of apoferritin prepared from ACI rat liver ferritin (---o) and from H-35 hepatoma ferritin (--). x, 50% point on the boundaries of apoferritin with buffer in centimeters from axis of rotation; t, time.

absorption between 230 and 280 nm, and above 280 nm the absorbance slowly declines. At 770 nm, there is another maximum. The latter was absent from the curves obtained with the 2 apoferritins. It may be due to the ferric hydroxide micelles in the cores of ferritin molecules.

StudieS of Subunits. With the method described by Smith-Johannsen and Drysdale (30), each kind of ferritin was incubated with 0.25% SDS at 60°C for 30 min. The solutions were cooled, and samples of 20 μl were used for electrophoresis in 6.5% polyacrylamide gels (pH 6.6) that did not contain SDS. The results are shown in Fig. 3. No electrophoretic difference between the SDS-complexed subunits of ACI rat liver ferritin and those of H-35 hepatoma ferritin was detected.

Crystals of H-35 Hepatoma Ferritin and Electron Microscopy. By light microscopy, crystals of H-35 hepatoma ferritin and ACI rat liver ferritin did not differ notably. Most crystals had slightly rounded corners, but some were well-developed octahedra similar to those of horse spleen ferritin and other ferritins (11). To check this, crystals were prepared for electron microscopy. They were suspended in a minimal volume of 5% cadmium sulfate solution and then exposed to formaldehyde vapor for 5 min. Subsequently, they were suspended in 2% liquid agar (50°C), which was allowed to solidify. Blocks, 1 mm in greatest dimension, were cut, dehydrated, and embedded in Epon 812 epoxy resin according to routine procedures (26). Thin sections were examined in an electron microscope. Lattices similar to those of crystalline horse spleen ferritin were visualized (Figs. 4 and 5). Variation in the intensity of ferric hydroxide cores of the ferritin molecules was evident, presumably due to variation in the amount of iron. Hexagonal stacking patterns were seen in sections of crystals of H-35 tumor ferritin and of ACI rat liver ferritin. In negatively contrasted monolayers, spread from aqueous solutions on carbon-coated specimen grids, molecules of H-35 tumor ferritin were indistinguishable from those of ACI liver ferritin.

DISCUSSION

The electrophoretic mobilities of various kinds of ferritin, including those here considered, are independent of the amounts of iron in the molecules (12, 17, 26). Our data indicate structural differences between H-35 hepatoma ferritin and ACI rat liver ferritin. Results of electron microscopy and of sedimentation velocity analyses support the impression that the macromolecular architecture of the 2 kinds of ferritin here considered approximates that of horse spleen ferritin, for which extensive data are available [reviewed by Harrison (13)]. For horse spleen ferritin, the existence of subunits in the quaternary apoferritin shells of the molecules has been substantiated (13, 15, 23). Judging from electron micrographs of negatively stained molecules (26), we believe that H-35 tumor ferritin and ACI rat liver ferritin have subunits, similar in size to those of horse spleen ferritin, in their shells. The data here given indicate that some or all of the subunits in H-35 apoferritin differ in primary structure from the subunits in ACI rat liver apoferritin. This inference is based on (a) the difference in isoelectric points, (b) the differences in amino acid composition, and (c) the difference in behavior during CM-cellulose ion-exchange chromatography.

It has been shown by Cann and Goad (4, 5) and by Cann (3) that a single, homogeneous macromolecule can interact with small constituents of buffer, including ampholytes such as those used to obtain the pH gradient necessary for isoelectric focusing. Such interaction can account for the presence of supernumerary bands or peaks of various proteins in zone and moving-boundary electrophoresis, respectively. The theoretical basis of this phenomenon has been analyzed in detail in a recent monograph by Cann (3). With both H-35 hepatoma ferritin and ACI rat liver ferritin, a minor band (Charts 1 and 2, peak) developed on either side of the principal band of ferritin in the Ampholine gradient. We have observed this also when isoelectric focusing was done in polyacrylamide gels. However, these bands (peaks) did not develop during ordinary gel electrophoresis in polyacrylamide gel, in cellulose acetate...
strip electrophoresis, or in free electrophoresis. Therefore, these bands are unlikely to represent distinct species of ferritin. They are probably due to the interaction of ferritin with constituents of the pH gradient used for isoelectric focusing.

Material obtained from each of the 2 ferritins by treatment with SDS had the same electrophoretic mobility in polyacrylamide gels. Shapiro et al. (29) have shown that when proteins complexed with SDS are subjected to electrophoresis in polyacrylamide gels the distance of migration is a linear function of the molecular weight of the proteins in the range between 15,000 and 90,000 daltons. On this basis, one would not expect to see a difference in electrophoretic mobilities of tertiary subunits (molecular weight about 22,500 daltons) of the 2 apoferritins after complexing with SDS. Evidently, differences between net charge densities of proteins in this size range are obscured by the distribution of sulfate (of SDS) over the external surfaces. That treatment of apoferritin or ferritin with SDS can dissociate subunits was previously observed by others who worked with horse spleen ferritin or apoferritin (15, 30). Available data make it appear likely that these subunits number either 20 or 24 (6, 13, 23).

Since the sedimentation coefficients of H-35 hepatoma apoferritin, ACI rat liver apoferritin, and horse spleen ferritin are approximately the same and the size and shape of these 3 kinds of apoferritin are also approximately the same, we suggest that the subunits liberated from the 3 kinds of apoferritin by SDS are also similar in size, shape, and molecular weight (about 22,500 daltons). It has not been shown, however, that all subunits in a given apoferritin molecule must have the same primary structure.

Gabuzda and Gardner (10) noted the presence of 2 electrophoretically different ferritins in rabbit bone marrow. More recently, Yamada and Gabuzda (31) reported that 1 of these 2 kinds of rabbit bone marrow ferritin did not occur in normal marrow but occurred only under pathological conditions. Alfrey et al. (1) and Linder-Horowitz (Linder) et al. (19, 20) have reported the occurrence of “isoferritins” in normal tissues in man and rats. We were unable to substantiate some of these findings (24–26), but we have found (18) that ferritin extracted from pig livers differs electrophoretically from ferritin obtained from pig siderocytes, again under pathological circumstances. Further substantiation of the presence of isoferritins in normal tissues is desirable. The recent report of Drysdale (8) indicating the occurrence of “microheterogeneity” in horse spleen ferritin revealed by isoelectric focusing in columns of polyacrylamide gels also needs further substantiation, first because of the aforementioned interactions of ampholytes with proteins and peptides (Refs. 3 to 5, Chart 2) and, second because the isoelectric focusing was done in polyacrylamide gels rather than in “free” solvent gradients.

There are highly differentiated hepatomas which, unlike the less well-differentiated H-35 hepatoma, the Morris 5123 hepatoma, and the Novikoff tumor, have thus far yielded only ordinary rat liver ferritin (26). This suggests that the state of differentiation of neoplastic cells is related to the quality of ferritin produced. The same can be said for results obtained earlier with human cell lines grown in vitro (23–25). We do not know on what basis such changes may occur and in what form cells possess information for the synthesis of alternative kinds of ferritin. At present, it appears likely that a cell will produce only 1 kind of ferritin at a time. The fact that 2 kinds have on occasion been isolated from a tumor (26) suggests that divergent cells were extracted. The genetic control of ferritin synthesis remains to be discovered, but since even fungi (7) can produce ferritin the time when this will be accomplished may not be far off.

ACKNOWLEDGMENTS

The excellent technical assistance of Mr. Gerald Moppett and Miss Pamela Wade is gratefully acknowledged.

REFERENCES


Fig. 1. Comparison of ferritin from H-35 hepatoma (H 35) and ACI rat liver (ACI) after electrophoresis in 6.5% polyacrylamide gel at pH 6.6 in 0.5-cm columns. An initial current of 8.3 ma/tube was used for 1 to 4 min, and then a current of 4.1 ma/tube was used for the rest of the run (~40 min). Stained with Naphthol Blue Black. Several fractions are visible for each kind of ferritin: the broad band of monomer followed by dimers, trimers, and in the case of ACI ferritin by additional bands, presumed to represent higher oligomers. The monomer band of H-35 hepatoma ferritin has migrated faster towards the anode than the corresponding band of ACI rat liver ferritin in mixtures and alone. A fast-migrating dimer band of the H-35 ferritin is also clearly visible. +, anode.

Fig. 2. Comparison of monomer fraction of H-35 hepatoma ferritin with whole H-35 hepatoma ferritin and with ACI rat liver ferritin. Electrophoresis in 6.5% polyacrylamide gel at pH 6.6, as described in legend for Fig. 1. Stained with Naphthol Blue Black. 1, ferritin from ACI rat liver; 2, mixture of ACI rat liver ferritin and of monomer fraction of H-35 hepatoma ferritin; 3, monomer fraction of H-35 hepatoma ferritin; 4, whole H-35 hepatoma ferritin; 5, mixture of whole H-35 hepatoma ferritin and H-35 monomer fraction; 6, blank control; +, anode.

Fig. 3. Comparison of SDS-complexed subunits of ferritin from ACI rat liver (ACI) and from H-35 hepatoma (H 35) in 6.5% polyacrylamide gel at pH 6.6. An initial current of 8.3 ma/tube was maintained for 2 min, and then a current of 4 ma/tube was maintained for 13 min. The gels did not contain SDS at the outset. Stained with Naphthol Blue Black. SDS, sodium dodecyl sulfate subunits. +, anode.
Fig. 4. Electron micrograph of sectioned crystal of ferritin from H-35 hepatoma. × 420,000.
Fig. 5. Electron micrograph of sectioned crystal of ACI rat liver ferritin. × 420,000.
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