Cell-specific Properties of Red Cell and Liver Ferritin from Bullfrog Tadpoles Probed by Phosphorylation in Vitro*

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Cell-specific differences occur in the primary structure of ferritin. For example, red cell and liver ferritin from bullfrog tadpoles differ by 1.5 times in serine content. To determine if cell-specific differences in ferritin primary structure are expressed in the tetraecosomer, which thus might distinguish the proteins in a functional state, phosphorylation in vitro was employed as a probe using [γ-32P]ATP and the catalytic subunit from the cAMP-dependent protein kinase of bovine skeletal muscle. Subunits of both proteins in the tetraecosomers were phosphorylated. Based on tryptic peptide maps, five regions common to both red cell and liver apoferritin were phosphorylated, as confirmed for two peptides by amino acid analyses. [32P]Apoferritin from red cells yielded an additional four 32P-fragments by mapping, at least three of which were unique by amino acid analysis and, in one case, might represent a 32P-Fe complex bound by a fragment of the iron-binding site. One peptide appeared to be unique to liver apoferritin. High concentrations of ATP yielded one additional peptide common to liver and red cell and one red cell-specific peptide in the tryptic peptide maps. The maximum moles of 32P/molecule were 13 ± 4 and 6 ± 2, respectively, for red cell and liver apoferritin, which corresponded within experimental error to the number of 32P-tryptic peptides. The level of phosphorylation was, on the average, not more than 1.5 times in serine levels of phosphorylation, some subunits in the assemblage of 24 appeared to be unavailable as substrates, possibly because of charge repulsion or conformational changes. The possibility that post-translational modifications of ferritin which amplify cell-specific structural features occur in vivo with cytoplasmic components, e.g. protein kinases, is considered in terms of the physiological availability of iron from different iron storage cells and developmental changes in iron storage.

The protein shell of ferritin, which surrounds an iron core of variable size (0-4500 iron atoms as (FeOOH),FeOPO₃H₂), displays cell-specific structural features; the protein shell is composed of 24 subunits of similar size (20 kDa) assembled into a hollow sphere. Although the biological significance of the cell specificity is not currently understood, a possible relationship between variations in apoferritin structure and the iron storage function is indicated by the cell-specific differences in the metabolic availability of ferritin iron, which have been observed in vivo (1, 2). A number of observations suggest that ferritin is coded for by a multigene family (3, 4); the cell specificity of ferritin structure could then result, in part at least, from the expression of ferritin genes in a cell-specific manner. Apparently, analogous combinations of cell-specific variations in structure and expression of multigene families occur for actin, tubulin, and myosin (5-7).

Erythroid cell ferritin appears to provide iron at a relatively rapid rate in vivo (1, 2, 8). Properties of such a cell-specific ferritin may be matched to the rapid rate at which iron is used for heme synthesis during red cell maturation. An unusual period of iron utilization occurs during the hemoglobin switch of early animal development (8, 9). At this time of life, the rate of new red cell formation is more rapid than in the steady state represented by the adult because the entire population of embryonic cells is replaced quickly (within 1-4 weeks, depending on the organism) and because the total number of red cells increases when growth is occurring. In many developing animals, including man, the usual need for iron appears to be met by an unusual form of iron storage: ferritin accumulates in the circulating red cells of the embryo to be mobilized and used in forming the first generation of red cells from the adult cell line (1, 9).

In order to learn more about cell-specific properties of apoferritin structure, particularly in red cells, apoferritin from bullfrog tadpole red cells and liver was compared, using the previously observed differences in serine content (1) as a guide and phosphorylation with a protein kinase (10) and [32P]ATP as a probe. Bullfrog tadpoles were used because the size and accessibility of the animal at the appropriate developmental stage makes it possible to obtain relatively large numbers of red cells which are embryonic, containing large amounts of ferritin (11, 12). Ferritin obtained from the liver of tadpoles is essentially derived from hepatocytes (1); no qualitative differences have been observed in red cell or liver ferritin between tadpoles and adult frogs, although there is a dramatic (15X) decrease in erythrocyte ferritin content during development (11). Red cell ferritin contains 14.5 serine residues/subunit, compared to 9.7 in liver ferritin (1). The results described show that serine residues are available for phosphorylation in the assembled apoprotein, that more serine residues are phosphorylated in red cell ferritin compared to liver ferritin, and that while several phosphorylatable regions are common to both proteins, several other regions phosphorylated are specific for red cell ferritin. Thus, cell-specific differ-
Ferritin Modification and Cell Specificity

MATERIALS AND METHODS

RESULTS

Extent of Phosphorylation and Characterisation of Residues Phosphorylated in Vitro—Red cell and liver ferritin differ in the number of serine residues per molecule, 364 and 216 per molecule, respectively. Since serine is a potentially modifiable residue in vivo, such a possibility was examined by measuring the extent of enzymatic modification of red cell and liver apoferritin. Phosphorylation was achieved with [γ-32P]ATP and the catalytic subunit of cAMP-dependent bovine skeletal muscle protein kinase. The data in Fig. 1 show that both the liver and red cell proteins may be phosphorylated in vitro. However, only a small number of serine residues appear to be in the correct environment for reactivity. In fact, fewer than 1 residue per subunit was phosphorylated, suggesting an inequivalance of subunits in the assembled structure and/or the inhibition of subsequent phosphorylation by a phosphate group. Phosphorylation did not affect the electrophoretic mobility of the protein under nondenaturing conditions. Red cell apoferritin was more readily (13 ± 4 32P/molecule) phosphorylated than liver apoferritin (6.1 ± 2 32P/molecule) (Fig. 1). Reliable quantitation of protein phosphorylation in the holoprotein was not feasible; ATP was bound to the core, in apparent analogy to previous reports of HPO42− and P2O74− binding (13). Bound ATP was removed incompletely by dialysis against P, and/or ATP, but could be removed by reduction and release of the ferric iron core. However, removal of the iron could also cleave phosphoserine, in analogy to the cleavage of phosphoethers of amino acids in phosvitin by Fe2+ (14).

Ferritin consists of two types of subunits, based on electrophoretic mobility, called H and L; amino acid analyses of ferritin from a number of tissues (3) and preliminary sequence analyses of cloned cDNA fragments (4) suggest that H and L represent families of related peptides. In the case of amphibian red cell and liver ferritins, the apparent subunit sizes are 21 and 19 kDa, and the ratio of H:L is 1:1 and 2:8 in the red cell and liver apoferritins, respectively. The mobility of 32P subunits during electrophoresis in SDS gels differed from the unphosphorylated protein. The apparent size of the 32P subunit was 16.7 kDa, which suggests that phosphorylation impeded unfolding by or binding of SDS. A similar effect has been observed with native ferritin dissociated at low temperatures and/or with insufficient reducing agent (15). Additional minor bands of radioactivity were observed with apparent sizes of 38, 61, and 78 kDa, suggesting incomplete dissociation of subunits (the conditions used were 2% SDS, 1% BME, 100 °C, 5 min, conditions which dissociate native ferritin completely). Since the H:L ratio of the unphosphorylated fraction in phosphorylated ferritin was the same (judged by staining with Coomassie blue) as in the native protein, it appears that subunits that were phosphorylated represented both H and L types. Separation of charged subtypes of apoferritin subunit assemblies by isoelectric focusing showed that phosphorylation did not change the distribution of subtypes (Fig. 2), indicating that all subtypes could serve as substrates; however, heavily phosphorylated molecules tended to aggregate and much 32P was observed near the site of sample application.

The amino acid residues in apoferritin which served as substrate for phosphorylation were examined after partial hydrolysis in 5.7 N HCl, 100 °C, 2 h. Hydrolysis products were separated by electrophoresis on paper at high voltages or by HPLC. 32P was located by autoradiography or scintillation counting. The results obtained with both red cell and liver apoferritin were essentially the same (Table 1). Serine was the major amino acid that was phosphorylated. The distribution between undigested products, P1, and the apparent background 32P in the regions of other potentially phosphorylatable amino acids is similar to that observed by others (16) for partial hydrolysis of phosphorylated proteins.

Tryptic Peptide Maps of Liver and Red Cell Apoferritin Phosphorylated in Vitro—After phosphorylation by [γ-32P]ATP using the catalytic subunit of bovine skeletal muscle protein kinase, reduction, and alkylation followed by tryptic digestion, soluble phosphorylated peptides were separated by two-dimensional electrophoresis and chromatography, using thin layer cellulose and paper. Comparison of ninhydrin-staining patterns with radioautography on thin layer cellulose

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1 Portions of this paper (including "Materials and Methods," Tables I-III, and Fig. 4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1946, cite author(s), and include a check or money order for $9.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations used are: SDS, sodium dodecyl sulfate; Mes, 2-(N-morpholino)ethanesulfonic acid; HPLC, high performance liquid chromatography; Hepes, 4-(2-hydroxyethyl) imidazole; PIPES, 1-(2-hydroxyethyl) piperazineethanesulfonic acid; BME, β-mercaptoethanol; TPKC, 1-tosylmethyl 2-phenylethyl chloromethyl ketone.

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Fig. 1. Phosphorylation of apoferritin in vitro. Apoferritin, prepared by reduction and chelation of the iron using thioglycolic acid, was phosphorylated with the catalytic subunit of bovine skeletal muscle CAMP-dependent protein kinase and increasing concentrations of [γ-32P]ATP in 21 mM Mes, pH 6.7, 4.75 μg of acetate, and a reaction volume of 50 μl at a concentration of 16.8 μM. Incorporation of 32P was determined on trichloroacetic acid precipitates (see "Materials and Methods"). The data are the average of 5 experiments using 4 different sets of apoferritin preparations. The average maximum level of phosphorylation for red cell and liver apoferritin was 13.1 and 6.1 mol/mol of protein, respectively, where 1 mol of protein is the 460-kDa assembly of 24 subunits. The difference between phosphorylation of the two proteins is significant (p ≤ 0.001; nonparametric rank test (33)). ●—●, red cell apoferritin; ○—○, liver apoferritin.
the location, 5 32P-tryptic peptides from the liver protein were observed in liver apoferritin digests (Fig. 3). Based on were observed in red cell apoferritin and 6 major 32P-peptides and was used for preparative purposes; 9 major 32P-peptides were observed in red cell apoferritin and 6 major 32P-peptides were observed in liver apoferritin digests (Fig. 3). Based on the location, 5 32P-tryptic peptides from the liver protein were also present in the red cell protein; 4 tryptic peptides were red cell unique and 1 was unique to liver. The peptide maps were similar whether the average P/molecule was 0.3 (at 0.13 mM ATP) or 6-13 (at 4-10 mM ATP), suggesting that the site of introduction of the first P/molecule was random among the potential sites of phosphorylation. In one experiment, using maximum levels of phosphorylation (10 mM ATP), 2 new peptides were observed. One was common to the liver and red cell proteins (T-13-2) and one was unique to the red cell protein (T-3). Thus, at high levels of phosphorylation, the total number of 32P-peptides corresponded within experimental error to the number of 32P/molecule, indicating that each potential site reacted only one time although, because of the many subunits, it was present in multiple copies. 32P was also observed at the origin and in trace amounts in variable regions of the map. Peptides T-15-2 and T-17 contained more 32P relative to the other peptides when a high concentration of ATP was used.

Three major 32P-peptides common to liver and red cell apoferritin tryptic digests (T-7, T-9, and T-12) and three 32P-peptides unique to tryptic digests of red cell apoferritin were selected for analysis of amino acid composition. The selection was based on apparent concentration (intensity in autoradiograms) and facility in recovering sufficient amounts. Although all 6 peptides contained lysine, other peptides, e.g. T-18 from red cell apoferritin tryptic digests, were arginine peptides but contained lysine as isolated (0.5/Arg) and were not further analyzed.

The similarity or identity of phosphorylated regions of both liver and red cell apoferritin are represented by peptides T-7 and T-12, as confirmed by amino acid analysis (Table III). Both peptides contained 1 serine, 1 threonine, and 19-21 amino acids/lysine. Valine appeared to be absent in T-7 and isoleucine in T-12. Comparison of T-9 from red cell and liver was not possible because of the low yields of the liver fragment. The red cell peptide T-9 was the only phosphorylated peptide analyzed which contained methionine. T-9 was similar in size and hydrophobicity to T-7 and T-12, but contained valine, isoleucine, and tyrosine (Table II).

The unique red cell apoferritin peptides in the maps (T-13, T-15-1, T-15-2) were also distinct in amino acid composition from those common to liver and red cell. For example, T-13 contained a very high concentration of hydrophobic residues (63%). There were no clusters of serine residues, which could account for the extra serine in red cell ferritin as compared to liver (1); the tetraeicosa peptide contained 1 serine and 2 threonine residues. T-15-1 and T-15-2 are small fragments, 11 and 8 residues in length, deficient in hydrophobic amino

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**Fig. 2.** Heterogeneity of surface charge of red cell and liver ferritin. Chemically prepared apoferritin was fractionated by isoelectric focusing (pH 5-8) as described (1) and stained with Coomassie blue; other experiments have shown that all the protein bands stain for iron in the holoprotein. There was no difference between the control and phosphorylated proteins; the photograph is representative of five independent experiments. Left, liver ferritin holoprotein; right, red cell ferritin holoprotein.

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**Fig. 3.** Tryptic peptide maps of red cell and liver apoferritin phosphorylated in vitro. Freshly prepared apoferritin was phosphorylated with [32P]ATP and the catalytic subunit of CAMP-dependent protein kinase from bovine skeletal muscle (see Fig. 1 and “Materials and Methods”). After reduction, carboxyamidomethylation, and digestion with trypsin, the soluble peptides were separated by electrophoresis and chromatography on paper (see “Materials and Methods”). 32P-peptides were located by autoradiography. Peptides, indicated by dotted lines, were observed at high levels of phosphorylation (9.8 mM ATP; 13 and 6 P/molecule for red cell and liver proteins, respectively), compared to the usual concentrations used, 0.13 mM ATP and 0.3 and 0.2 P/molecule for red cell and liver proteins, respectively. Peptides 15-2 and 17 on the red cell and liver ferritin maps, respectively, were more intense when high concentrations of ATP were used. Ø represents the origin. Top, red cell ferritin; bottom, liver ferritin.
acids and enriched (53 and 80%) in amino acids with carboxyl- and/or nitrogen-containing side chains. In addition, none of the usual phosphorylatable residues (serine, threonine, tyrosine) were recovered. Whether, for example, serine-phosphate was particularly sensitive to destruction, or whether T-15-1 and T-15-2 bound $^{32}$P ($^{32}$P]ATP) tightly enough to survive reduction, alkylation, and trypic digestion is not clear.

Iron release from Ferritin and red cell apoferritin—Information about the influence of the apoferritin shell on iron uptake and release is limited. In fact, it was at one time proposed that the protein shell simply coated the iron core. More recently, the influence of the protein shell of ferritin on the initial rate of iron uptake (17, 18) and release (17-19) has begun to be investigated. In addition to effects on the initial events in iron release, the maximum amount of iron released appears to be influenced by the coating around the core (Table III and Fig. 4). For example, Imferon is a complex of an iron polymer surrounded by chains of dextran in which the iron environment is very similar to that in the ferritin core (20). In spite of the similarity of the core, 90 ± 7% of the iron is released as diffusible Fe$^{3+}$ in 2.5 min, compared to 22% for fresh horse spleen ferritin. Apoferritin, prepared from aged tissue, stored for 6 months at 4°C in 20 mM K phosphate, pH 6.8, or commercial preparations of horse spleen ferritin resulted in a preparation able to release essentially all the iron in the core, albeit more slowly than Imferon; for example, only 60% of the iron was released in 2.5 min (Fig. 4). The only observable difference between horse spleen preparations, which released only part of the iron, and all of the iron was the presence, in fresh preparations, of components with pl 4.8-5.1, compared to 4.8-4.9 in the aged preparations. Such changes in surface charge may reflect deamidation of Gln and/or Asn and consequent conformational changes which enhance iron release.

In order to assess the effect of a controlled modification of apoferritin, the difference in the extent of phosphorylation by ATP and protein kinase of amphibian red cell and liver apoferritin was exploited. The holoproteins were phosphorylated and the extent of iron release was determined and compared to nonphosphorylated samples. As mentioned above, it was not possible to accurately measure the extent of phosphorylation of the protein shell because of $^{32}$P binding by the core itself. Furthermore, it was not possible to measure protein phosphorylation on a sample from which the iron had been removed, since removal of the core involves conditions which cleave serine-phosphate (14). Nevertheless, in four experiments over a 2-year period with different preparations of enzyme and protein, treatment of red cell ferritin with protein kinase and $^{[\gamma-32P]}$ATP increased iron release significantly (% Fe released: 32.4 ± 3.0 compared to 14.1 ± 5.8) with no effect on liver ferritin (% Fe released: 13.9 ± 3.9 compared to 9.1 ± 4.0). In one experiment, as much as 56% of the iron was released from the phosphorylated red cell protein. However, recent attempts to repeat the experiments have shown no effect of phosphorylation in vitro on iron release from either protein. Whether the inconsistency is due to the enzyme preparations or properties of the isolated protein, e.g. endogenous levels of phosphorylation, is not yet known.

**DISCUSSION**

Since the protein shell of ferritin may influence both the incorporation of iron (17, 21, 22) and release of iron (17-19; Table I) in vitro, cell-specific differences in the apoprotein shell might also be expected to influence iron storage in vivo, as previously suggested (1, 2). Cell-specific differences in the protein which have been observed include amino acid composition, surface charge, and subunit size (reviewed in Ref. 3). Additional cell-specific differences might be introduced or primary structural differences might be amplified by post-translational modifications.

An example of a recently observed post-translational modification is the presence of natural submit cross-links in low iron fractions of ferritin from horse and sheep spleen (17). Another example is the glycosylation of a subunit of human serum ferritin (22). The results in Figs. 1 and 3 show that apoferritin may also be modified by phosphorylation of serine, at least in vitro. Each subunit was not equivalent in the assembled structure since, on the average, fewer than 24 residues in the assembled molecule were phosphorylated. Resistance to complete phosphorylation of all possible sites may result from repulsion by phosphorylated residues nearby or from conformational changes. That conformational changes occur is indicated by the fact that highly phosphorylated apoferritin can aggregate, as judged by isoelectric focusing. A similar finding has been obtained with iron-phosvitin, a protein naturally rich in serine-phosphate (23).

Cell-specific differences in apoferritin from different cell types and/or species are superimposed upon a high degree of homology, as indicated by amino acid sequence (24) and immunological cross-reactivity (25-28). Examination of tryptic peptide maps of phosphorylated red cell and liver apoferritin suggests that the homology extends to certain sites of potential phosphorylation on the surface of the molecule (Fig. 3); four of the peptides have the same sequence in both liver and red cell maps. The similarity was supported for two of them (T-7 and T-12) by amino acid analysis. Sites specific to ferritin from red cells were also observed. For example, four red cell ferritin-specific peptides (T-13, T-15-1, T-15-2, T-18) were readily phosphorylated; two additional specific sites were phosphorylated at high ATP concentrations (Fig. 3). The amino acid composition of all three phosphorylated fragments analyzed was unusual. For example, T-13 is very hydrophobic (63%), suggesting either that the phosphorylation site may be accessible to the enzyme through one of the crevices (29), since hydrophobic regions would not be expected on the surface, or that the site is at a bend in the peptide chain at the surface of the molecule, interrupting hydrophobic regions interacting within the chain. T-15-1 and T-15-2 are hydrophilic (Table IV) and no potentially phosphorylated residues were recovered, with the possible exception of histidine; however, it is unlikely that $^{32}$P]histidine would have survived electrophoresis at pH 4.1 (60), and thus would not be observed in the autoradiograms. Serine, for example, if present, could have been easily observed in the hydrolysate since lysine was

**Table IV**

| Properties of selected tryptic peptides from phosphorylated red cell and liver bullfrog apoferritin | Potential P-amino acids | Distribution of residues (%) |
|---|---|---|
| Ser | Thr | Tyr | His | Hydrophobic | N-side chain | -COOH side chain | Total residues |
| Unique to red cell apoferritin | | | | |
| T-13 | 1 | 2 | 0 | 1 | 63 | 5 | 27 | 24 |
| T-15-1 | 0 | 0 | 0 | 1 | 37 | 19 | 44 | 11 |
| T-15-2 | 0 | 0 | 3 | 0 | 22 | 31 | 48 | 8 |
| Common to liver and red cell apoferritin | | | | |
| T-7 | 1 | 1 | 0 | 0 | 40 | 9 | 42 | 21 |
| T-12 | 1 | 1 | 0 | 4.4 | 46 | 9 | 37 | 19 |
| T-9 | 1 | 1 | 1 | 1 | 51 | 7 | 37 | 21 |

(Red cell protein only)

**Total protein**

Red cell | 15 | 7 | 5 | 6 | 40 | 21 | 25 |
Liver | 9 | 5 | 8 | 8 | 37 | 17 | 30 |
present at concentrations as high as 4 nmol and the limit of detection of serine in the system used was at least 0.2 nmol. The low recovery of hydrophobic amino acids suggests the possibility that incomplete hydrolysis might account for the absence of serine in the hydrolysate if it were linked to a hydrophobic amino acid. However, no unusual ninhydrin-reactive material was observed during analysis. An attractive alternative hypothesis depends on the hydrophilic nature of the sites believed to be involved in iron binding. If T-15-1 and 15-2 were part of the sites (31), iron adventitiously acquired from the environment by the fragment could also be bound to $[^{32P}]$ATP, carrying the radioactivity along during isolation; no special attempt was made to render reagents iron-free. The stability of Fe-ATP complexes is illustrated by their recovery in red cell lysates (32). The region proposed to be part of the iron-binding site in horse spleen apoferritin (24, 31) and conserved in the L-chain of human and horse spleen apoferritin would produce a lysine-containing hexapeptide containing alanine, leucine, and three glutamate residues. An apparently homologous fragment from a minor (H subunit?) component of the human spleen molecule contains a histidine substitution for leucine which then becomes similar in composition to red cell peptide 15-2 (Table II), supporting the notion that T-15-2 could be derived from the iron-binding region.

The comparison of phosphorylation of red cell and liver apoferritin described here shows that small differences in primary structure can be reflected in the assembled multimer. The origin of the extra serine residues in red cell apoferritin, whether clustered in an extension of the liver apoferritin sequence or dispersed throughout the subunit, was not apparent from the experiments, but may become clear from analysis of cloned cDNA fragments now in progress. What is clear is that whatever the distribution, serine in red cell ferritin can be uniquely modified (phosphorylated) post-translationally by a physiologically available reagent (general protein kinases). Such cell-specific differences in the structure of red cell and liver ferritin may be related to the cell-specific differences in availability of iron stores observed in vivo (1, 2) and may explain the iron storage function of the erythrocyte in embryos when the rate of iron consumption is high.

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Ferritin Modification and Cell Specificity

**YJPPElCNl**

**TO**

**CELL-SPPCIFIC PROPERTIES OF REO CELL**

**NO**

**LlVER FERRITIN FROM**

**FULLFRa**

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By Koji Ihara, Kunio Ueguchi, Clyde I. Young, and Elizabeth C. Tkeil

Table 1

| Aar      | 3.1 | 7.4 | 2.7 | 5.5 | 1.5 | 1.5 |
|----------|-----|-----|-----|-----|-----|-----|
| Glu      | 0.63 | 0.57 | 1.2 | 3.8 | 2.7 | 2.5 |
| Ser      | 1.2 | 1.1 | 1.7 | 1.6 | 1.5 | 1.9 |
| Pro       | 0.48 | 0.27 | 0.99 | 0.86 | 0.80 | 0.61 |
| Phos       | 2.4 | 2.8 | 2.3 | 1.2 | 1.0 | 0.9 |
| Hsp       | 0.94 | 0.47 | 0.22 | (0.08 | (0.22 | (0.18 |
| Lys       | 1.5 | 0.72 | 1.7 | 1.9 | 1.9 | 1.8 |
| Arg       | 1.5 | 0.81 | 1.0 | 1.0 | 0.8 | 0.8 |
| n.d.       | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

Table 2

**Iron release from ferritin and other components**

Ferritin was prepared by heating tissue homogenate in 4 volumes of 1 M KCl to 70°C for 10 min, chilling, removing the supernatant solution after sedimentation at 18,000 g for 40 min. After the supernatant was discarded, the residue was kept at 4°C. The absence of protein degradation was indicated by the absence of fragments below 51 kDa. Iron was released after reduction with 2.5 mM FMNH₂ at 25°C, pH 7.2, generated from 2.5 M NaOH·H₂O and light in 0.2 M imidazole buffer, as the amount of Fe(II)-bipyridyl complex (see Methods). Iron and protein concentrations were measured colorimetrically (see Methods).

| Maximum % Iron released | For-Protein |
|-------------------------|-------------|
| Horse spleen (fresh)    | 40 ± 12%    | 0.39 |
| Horse spleen (stored)   | 90 ± 0.3%   | 0.85 |
| Red blood cell (in vitro)| 90 ± 0.5%   | 0.56 |

Table 3

**Iron released from ferritin in cells**

Iron (0.15 μM) was then added to the reaction mixture described in Table 2. Duplicate samples were taken at 0, 1, 5, 10, 15, and 30 min, and assayed as described in Methods.

**Fig. 4.** Iron release from ferritin in cells. (A) (120 μM) was incubated in the reaction mixture described in Table 2. The iron released was measured by the iron uptake of 3H-labeled ferritin into the desired cell line. (B) (120 μM) was incubated in the reaction mixture described in Table 2. The iron released was measured by the iron uptake of 3H-labeled ferritin into the desired cell line.

**Table 4**

**Distribution of 3H** after partial acid hydrolysis of phosphatidyl ethanolamine

Red cell apoferritin

| 3H%         | 3H%         |
|-------------|-------------|
| Unphosphorylated protein | 42.0 ± 6.2  |
| Phosphorylase        | 31.6 ± 6.1  |
| Phosphorylase        | 13.2 ± 1.4  |
| Phosphorylase        | 12.3 ± 1.2  |
| Phosphorylase        | 40.1 ± 6.1  |
| Phosphorylase        | 46.0 ± 7.2  |
Cell-specific properties of red cell and liver ferritin from bullfrog tadpoles probed by phosphorylation in vitro.

K Ihara, K Maeguchi, C T Young and E C Theil

J. Biol. Chem. 1984, 259:278-283.

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