Expression and Structural and Functional Properties of Human Ferritin L-Chain from *Escherichia coli*†

Sonia Levi,*,‡,§ Jochen Salfeld,‖ Francesca Franceschinelli,‡ Anna Cozzi,‡ Marianne H. Dorner,⊥ and Paolo Arosio‡

Department of Biomedical Sciences and Technology, University of Milan, Ospedale San Raffaele, Via Olgettina 60, I-20132 Milano, Italy, Center of Molecular Biology, University of Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG, and Department of Medicine, University of Heidelberg, D-6900 Heidelberg, FRG

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ABSTRACT: The human ferritin L-chain cDNA was cloned into a vector for overproduction in *Escherichia coli*, under the regulation of a λ promoter. The plasmid obtained contains the full L-chain coding region modified at the first two codons. It is able to direct the synthesis of the L-chain which can constitute up to 15% of the total soluble protein of bacterial extract. The L-chains assemble to form a ferritin homopolymer with electrophoretic mobility, molecular weight, thermal stability, spectroscopic, and immunological properties analogous to natural ferritin from human liver (95% L-chain). This recombinant L-ferritin is able to incorporate and retain iron in solution at physiological pH values. At variance with the H-ferritin, the L form does not uptake iron at acidic pH values and does not show detectable ferroxidase activity. It is concluded that ferritin L-chain lacks the ferroxidase site present in the H-chain and that the two chains may have specialized functions in intracellular iron metabolism.

Ferritin is an iron storage protein, present in all eucaryotic cells (Harrison et al., 1980; Theil, 1987), with a major function of storing and detoxifying cellular iron (Drysdale, 1977). Human ferritin is composed of both heavy (H) and light (L) subunits which are encoded by distinct genes (Costanzo et al., 1986; Santoro et al., 1986) on chromosomes 11 and 19, respectively (Cragg et al., 1985). The expression of the two subunits and the corresponding messengers varies in different tissues and during cell differentiation and proliferation (Chou et al., 1986; Cairo et al., 1986; Cayre et al., 1987). H- and L-chains are 182 and 174 amino acids long, respectively; they share a sequence homology of 55% (Dorner et al., 1985; Jain et al., 1985). They appear to fold in similar structures and normally coassemble in various proportions in the same ferritin molecules (Arosio et al., 1978). Human H- and L-chains have different immunological properties which have been characterized with monoclonal antibodies (Luzzago et al., 1986).

Most of the present knowledge on structure/function relationships of ferritin comes from the extensive studies on the readily obtainable horse spleen ferritin, which contains about 90% of L-chain. It has been crystallized and its three-dimensional structure determined with a 2.8-Å resolution (Ford et al., 1984). This protein is formed by the assembly of 24 subunits surrounding a central cavity, where iron is stored as polynuclear hydrous ferric oxide (Ford et al., 1984). The protein shell is pierced by two types of pores, with hydrophobic and hydrophilic properties, respectively, that allow iron to enter and leave the cavity. Ferritin is able in vitro to promote the formation and growth of its iron core from ferrous ions, through a mechanism that has not yet been fully elucidated (Bakker & Boyer, 1986; Harrison et al., 1986, Levi et al., 1988).

The natural ferritins are composed of variable proportions of the two subunits (Arosio et al., 1978), undergo various posttranslational modifications (Theil, 1987), and are therefore not well suited to study the specific structural and functional properties of H- and L-chains. Such a study can be more easily performed on biosynthetic homopolymers produced by genetic engineering. The cDNAs for the human ferritin H- and L-chains have been cloned and sequenced (Costanzo et al., 1984; Santoro et al., 1986; Dorner et al., 1985) and can be used for protein expression. In previous work, we succeeded in expressing human ferritin H-chain in *Escherichia coli* in high yield (Levi et al., 1987). We showed that the recombinant H-ferritin in vitro incorporates as much iron as the natural liver ferritin but that it has iron uptake and ferroxidase kinetics severalfold faster than the liver ferritin (Levi et al., 1988). Here we report the cloning of human L-chain cDNA in a vector for overproduction in *E. coli* and the structural/functional characteristics of the recombinant protein (rLFo)[1] as compared to those of natural human liver ferritin (HLF) and recombinant H (rHF).

Materials and Methods

Strains, Media, and Chemicals. The *E. coli* strain used was 537 (Strebel et al., 1986). All cultures were grown in LB medium, containing ampicillin (100 mg/L) and kanamicin (25 mg/L). In some experiments, FeNTA iron (0.5–1 mM) was added to LB medium during the induction, as in Levi et al. (1988).

Construction of the Plasmid. The plasmid pExHF14 was constructed as follows: the plasmid p6808 (Dorner et al., 1985) was cleaved partially with *Pst*I, and the L-ferritin cDNA was subcloned into the expression vector pEx34b. This expression vector is identical with pEx31b (Strebel et al., 1986) but with the pUC18 backbone missing the *Pst*I restriction site in the ampicillin resistance gene, and was kindly provided by Ewald Beck, Center for Molecular Biology, Heidelberg, FRG. The

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* Address correspondence to this author at the Ospedale San Raffaele, Via Olgettina 60, I-20132, Milano, Italy.

‡ University of Milan.

§ Recipient of a fellowship from Istituto San Raffaele.

‖ Center of Molecular Biology, University of Heidelberg.

⊥ Department of Medicine, University of Heidelberg.

[1] Abbreviations: HLF, human liver ferritin; rHF, recombinant human H-chain ferritin; rLFo, recombinant human L-chain ferritin with a substitution at the first two N-terminal amino acids from Ser-Ser to Asp-Pro; FeNTA, ferric nitrilotriacetate; SDS, sodium dodecyl sulfate; LB, L-broth; FPLC, fast protein liquid chromatography.

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*Sst*I restriction site overlapping the L-ferritin initiation codon (AT*GAGCTC*) was converted into a *Bam*HI restriction site, resulting in the expression of ferritin L-chain as a fusion protein, as described in Salfeld (1985). The cDNA was subsequently subcloned as a *Bam*HI–*Xba*I restriction fragment into the plasmid pP1c245 (kindly provided by Hans Kuepper, Biogen, SA), having the MS2 coding part of plasmid pP1c24 (Kuepper et al., 1982) replaced by a *Nco*I–*Bam*HI linker. In this step, the *Xba*I restriction site was eliminated. In the resulting plasmid, pExHF14, the sequence around the initiation codon is then CCATGGATCCCCAG..., changing the very N-terminal amino acids from Ser-Ser-Gln... to Asp-Pro-Gln... in the recombinant L-chain.

Expression. It was performed essentially as in Levi et al. (1988). *E. coli* strain 537 transformed with the plasmid pExHF14 was grown at 28 °C until the medium reached an absorbance of 0.7 at 650 nm (2–3 h); then ferritin expression was induced by rapidly shifting the temperature to 42 °C. After 1–5 h, the cells were collected by centrifugation, resuspended in 20 mM Tris-HCl, pH 7.4, containing 0.02% sodium azide and 0.2 mM phenylmethanesulfonyl fluoride (PMSF), and sonicated for 8 min in an ice bath, and the supernatant was clarified by centrifugation at 10000*g* for 10 min. The ferritin in cell extracts was either analyzed as described below or purified.

Purification. We used a modification of the procedure previously used for human tissue (Arosio et al., 1978) and recombinant H-chain ferritins (Levi et al., 1987, 1988). The cell extracts were heated at 75 °C for 10 min and clarified, and ferritin was precipitated with 80% saturated ammonium sulfate. Then the ferritin (5 mL) was first loaded onto a Sephacryl S-200 column (60 × 2.6 cm) equilibrated in 20 mM Tris, pH 7.4, and then on a Sephadex DEAE column which was eluted with a 0–500 mM NaCl gradient in 20 mM Tris, pH 7.4. At this stage, the ferritin was judged pure by electrophoretic analysis (Arosio et al., 1978).

Ferritins from human liver, heart, and the recombinant H were prepared as previously described (Arosio et al., 1978; Levi et al., 1987, 1988). Apoferritins were prepared by incubating the proteins for 18 h in 1% thioglycolic acid in 0.1 M sodium acetate, pH 5.4, in a stoppered test tube, adding excess 2,2′-bipyridine, and dialyzing extensively versus 0.1 M Hepes, pH 7.0 (Bryce & Crichton, 1973).

Analytical Techniques. Protein concentrations were determined by the BCA method (Pierce) calibrated on bovine serum albumin. When in the presence of 0.1 M Hepes buffer, the protein assay method (Bio-Rad) was used. Ferritin iron was determined as in Levi et al. (1988): samples were incubated in 1 M acetic acid, 0.75 M sodium thiosulfite, and 0.5% 2,2′-bipyridine, boiled for 1 h, and clarified, and the absorbance was read at 520 nm. In the conditions used, the method sensitivity was about five Fe atoms per ferritin molecule.

Ferritin concentration was determined by a radioimmunoassay kit (Corning) based on a polyclonal antibody and spleen ferritin standard, or by the enzymo-immunoassay previously described (Luzzago et al., 1986) based on monoclonal antibodies and crystallized human liver ferritin.

Polyacrylamide gel electrophoreses were performed in vertical gel slabs 1.5 mm thick with 25 mM Tris–glycine buffer, pH 8.3 (Arosio et al., 1978). In SDS electrophoresis, we used 10–30% polyacrylamide gradient gels, and in nondenaturing systems, we used 8–22% polyacrylamide gradient gels. Isoelectric focusing runs were done on 0.5-mm-thick horizontal 5% polyacrylamide gel slabs, with 2% Ampholines pH 4–7 (LKB). The gels were stained with Coomassie blue or Prussian blue or used in blotting experiments.

Western Blotting. Proteins were transferred to nitrocellulose paper as in Towbin et al. (1979) and incubated for 2 h with 20% defatted milk and with peroxidase-labeled anti-ferritin monoclonal antibodies at proper dilution. After the paper was washed, the bound activity was detected by reaction with diaminobenzidine (Santambrogio et al., 1987).

FPLC gel permeation analysis was done on a Superose 6 column (Pharmacia) in 20 mM Tris, pH 7.4, and 0.15 M NaCl by injection of 10-μL samples (1–10 mg/mL) at a flow rate of 0.5 mL/min and detection at 280 nm.

Circular dichroic spectra were measured on a Jasco 500A spectropolarimeter. The CD intensity was calibrated on an aqueous solution of camphorsulfonic acid. Far-UV spectra were taken on apoferritin solutions of 0.05–0.1 mg/mL in 1-mm cells; the mean residue ellipticities were calculated from the mean residue weight of 114.4 for rLFo and HLF. Near-UV spectra were determined on 1 mg/mL apoferritin samples in 10-mm cells, the molecular ellipticities were calculated on a 19 906 molecular weight.

Ferritin Iron Saturation. It was studied as in Levi et al. (1988): purified ferritin samples (0.1 μM) in 0.1 M Hepes, pH 7.0, were incubated for 18 h at 4 °C with various amounts of ferrous ammonium sulfate. After centrifugation at 10000*g*, soluble iron and protein concentrations were determined. As controls, we used solutions without proteins or with 50 μg/mL bovine serum albumin.

Ferritin Iron Uptake Reactions. They were performed as in Levi et al. (1988): ferritin (0.1 μM) in 0.1 M Hepes, pH 7.0 at 30 °C, was added with 0.1 mM ferrous ammonium sulfate and the reaction followed at 310 nm with 600-nm reference in a dual-wavelength Aminco DW-2a spectrophotometer.

Ferritin Ferroxidase Reaction. It was performed as in Bakker and Boyer (1986). Ferritin (0.2 μM) and 50 μM apo-ovotransferrin (Recordati, Milano, Italy) in 0.2 M sodium acetate buffer, pH 6.0, were added to 0.1 mM ferrous ammonium sulfate, and the reaction was followed at 470 nm (reference 600 nm) for at least 3 min in a dual-wavelength spectrophotometer.

Results

Production of Recombinant L-Ferritin. The constructed plasmid pExHF14 encodes a human ferritin L-chain with a substitution of the first two amino acids at the amino-terminal sequence from Ser-Ser to Asp-Pro. This plasmid promotes the accumulation in the induced *E. coli* of a major peptide comigrating with the natural human ferritin L-chain (Figure 1A). Maximum ferritin accumulation was reached after 4 h of induction and accounted for about 15% of total bacterial soluble proteins.

Analysis of Ferritin L-Chain in Bacterial Extracts. Analyses with a radioimmunoassay kit confirmed that human ferritin accounted for 12–15% of total proteins in the induced cell extracts. In addition, the extracts were tested with 10 anti-human ferritin monoclonal antibodies: the anti-H-chain did not show any binding, while the anti-L-chain antibodies produced precipitin lines in double diffusion experiments and stained the ferritin in immunoblotting. In nondenaturing gel electrophoresis, the recombinant ferritin showed the α, β, and δ bands corresponding to the ferritin monomer, dimer, and oligomers (Figure 2, lanes 1 and 2), and, on the front of the gel where the free subunits are expected to run, no immunoreactive bands were ever detected (Figure 2, lanes 3 and 4).

After the cell extracts or the liver tissue homogenates were heated at 75 °C for 10 min, 90% of the immunoreactive ferritin

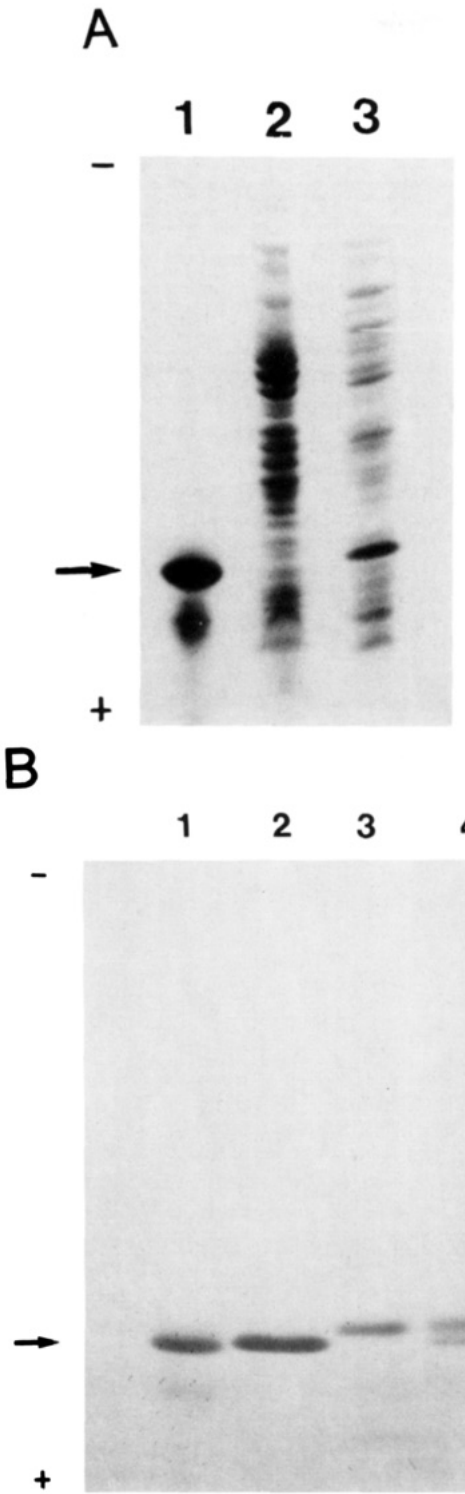

FIGURE 1: (A) Polyacrylamide–SDS gel electrophoresis to monitor L-chain expression, stained with Coomassie blue. Lane 1, purified control HLF (30 μg); lane 2, crude soluble bacterial extract of noninduced *E. coli* cells transformed with plasmid pExHF14 (50 μg of protein); lane 3, soluble extract of the induced transformed cells (50 μg of protein). The position of the L-chain is indicated by the arrow. (B) SDS electrophoresis of the purified proteins (10 μg) stained with Coomassie blue. Lane 1, HLF; lane 2, rLFo; lane 3, rHF; lane 4, human heart ferritin. The arrow indicates the position of the L-chain. The small proportion (about 5%) of H-chain in HLF, evident in the gel, does not appear in the picture.

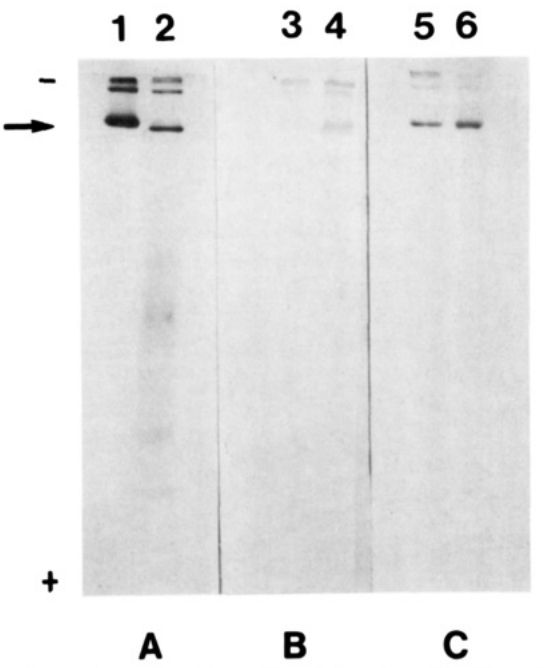

FIGURE 2: Nondenaturing electrophoresis in polyacrylamide gradient gel of purified HLF (10 μg; lanes 1, 3, and 5), of rLFo crude extracts (50 μg of protein; lanes 2 and 4), and of purified rLFo (15 μg; lane 6). Stained with Coomassie blue (panel A), subjected to immunoblotting with peroxidase-labeled anti-L-chain LF03 monoclonal antibody (panel B). (Panel C) Prussian blue stain of purified rLFo and apo-HLF which have been incubated with 1000-fold molar excess of Fe(II) at pH 7 for 3 h. The purified and untreated rLFo is not stained by Prussian blue. The arrow indicates the migration of ferritin monomer.

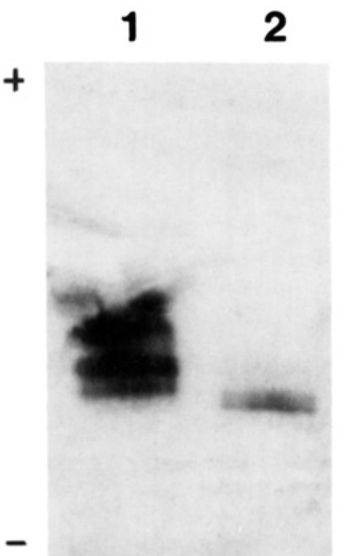

FIGURE 3: Isoelectric focusing in the pH range 4–7 of 30 μg of HLF (lane 1) and rLFo (lane 2) stained with Coomassie blue.

was recovered, as detected by radioimmunoassay.

Analysis of Purified rLFo. The recombinant rLFo was purified following a modification of the procedure normally used for natural ferritins, with a recovery of about 70%. The rLFo appeared pure on nondenaturing and SDS gel electrophoresis (Figure 1B), focused in two close bands on the basic side of the HLF spectrum (Figure 3), and eluted from the Superose 6 column in the same volume as HLF (14.2 mL), slightly behind rHF (13.8 mL).

The purified rLFo and HLF gave parallel calibration plots in an immunoradiometric assay based on anti-L-chain monoclonal antibody, while rLFo gave a signal 15% higher than HLF, in keeping with its higher content of L-chain (not shown).

The purified rLFo is colorless, and chemical analyses of 3 different preparations showed it to contain 5–15 Fe atoms per molecule. In another preparation obtained from *E. coli* in-

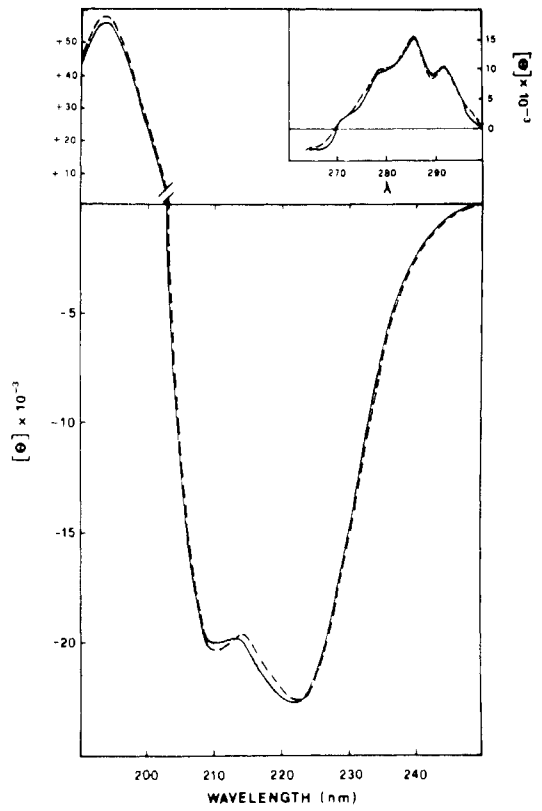

FIGURE 4: Circular dichroism spectra of apoferritins. Solid line, rLFo; dashed line, HLF. Samples in water at pH 7 and room temperature. The far-UV spectra were taken on 0.05 mg/mL apoferritin solutions, 1-mm cell path, and ellipticities calculated on 114.4 mean residue weight. The near-UV spectra (insert) were taken on 1 mg/mL apoferritin solutions, 10-mm cell path, and ellipticities calculated on 19 906 molecular weight.

duced in the presence of 0.5 mM FeNTA, the iron content was not increased (10 Fe atoms/molecule). In contrast, rHF had the typical ferritin amber color and was found to contain 100–130 or 250–300 Fe atoms per molecule when purified from *E. coli* induced without iron addition or in 1 mM FeNTA, respectively.

The UV absorption spectra of the purified rLFo were analogous to the HLF apoferritin spectra with a peak at 280 nm (not shown). Similarly, the circular dichroic spectra of the two ferritins were analogous: in the 190–250-nm range, they showed a positive peak at 194 nm and negative peaks at 210 and 222 nm; in the 250–300-nm range, positive peaks were visible at 280, 286, and 292 nm (Figure 4). The values of molar ellipticity at 222 nm were 22 560 and 22 750 for rLFo and HLF, respectively.

Preliminary crystallization experiments were done with 5% cadmium sulfate, pH 5.5, conditions that selectively crystallize horse spleen and HLF ferritins, but not H-rich ferritins (Arosio et al., 1983). RLFo and HLF gave crystals with cubic symmetry and the same morphology (not shown).

Functional Properties. Functional studies were performed on the purified ferritins treated with thioglycolic acid to remove iron, including rLFo which is essentially iron free. The apoproteins contained less than five Fe atoms per molecule and had a 280/260-nm absorbance ratio above 1.4.

After incubation of apo-rLFo and apo-HLF at pH 7 with a 1000-fold molar excess of iron(II), a stable ferritin iron core is formed, as shown in native gel electrophoresis and Prussian blue stain (Figure 2, lanes 5 and 6). For quantitative analysis, we incubated the apoferritins at pH 7 with various amounts of iron(II) for 24 h, and we evaluated the concentration of

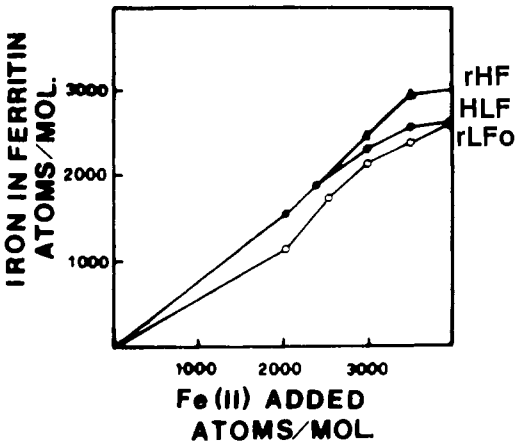

FIGURE 5: Iron incorporation in ferritin molecules. Apoferritin samples (50 μg/mL) in 0.1 M Hepes, pH 7.0, were incubated with various concentrations of ferrous ammonium sulfate for 18 h and centrifuged for 5 min at 10000*g* to precipitate nonferritin polynuclear iron, and the molar ratio between soluble iron and ferritin was measured. In controls without ferritin, the soluble iron was below 20 μM, i.e., accounting for <200 Fe atoms/molecule, in the conditions used.

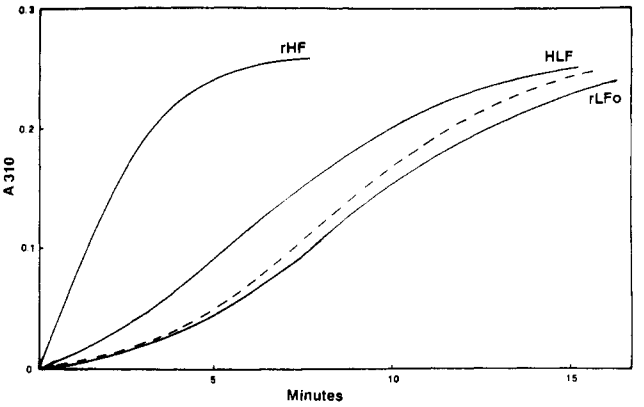

FIGURE 6: Progression plots of iron uptake reactions of 0.1 μM apoferritins in 0.1 M Hepes, pH 7.0, and 0.1 mM ferrous ammonium sulfate, 30 °C. The development of amber iron color was followed at 310 nm. The dashed line is control in the absence of proteins.

Table I: Initial Velocities of Iron Uptake at Various pH Values (μM Fe/min)[a]

pH value	control	rLFo	HLF	rHF
5.0	0	0.2	0.4	2.4
5.5	0	0.6	0.8	3.2
6.0	0	0.4	1.2	9.2
6.5	0.4	0.4	2.0	21.6
7.0	3.6	4.4	8.4	46.8

[a] Reaction conditions: 0.1 mM ferrous ammonium sulfate, 0.1 μM apoferritins, 30 °C; buffer: 0.1 M Hepes in the pH range 6–7 and 0.1 M Mes for pH 5–5.5. Monitoring at 310 nm; calculations based on a molar extinction value of 2475. Means of triplicate determinations.

soluble protein and iron. In these conditions, rLFo and HLF reached essentially the same saturation level (Figure 5), which was analogous to the one previously reported for rHF (Levi et al., 1988).

The iron uptake kinetics, in the presence of oxygen and with a 1000-fold molar excess of Fe(II), were followed at 310 nm (Bryce & Crichton, 1973). At pH 7.0, rLFo had detectable activity with an uptake rate analogous to that of spontaneous iron autoxidation, and slower than that of HLF and rHF (Figure 6). At lower pH values, the activity of all samples decreased, and, in particular, below pH 6.5 rLFo and autoxidation activities were undetectable (Table I). Prussian blue staining of the ferritins electrophoresed after a 3-h incubation

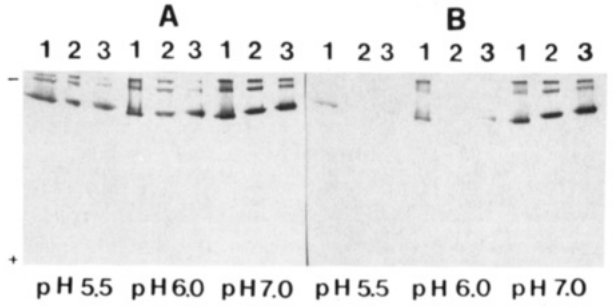

FIGURE 7: Nondenaturing gradient gel electrophoresis of the apoferritins after incubation for 3 h with 1000 molar excess of ferrous iron at various pH values, stained with Coomassie blue (panel A) and with Prussian blue (panel B). Lanes 1, 20 μg of HLF; lanes 2, 20 μg of rLFo; lanes 3, 30 μg of rHF.

Table II: Ferroxidase Activities (μM Fe/min)[a]

control	rLFo	HLF	rHF
0.2	0.3	1.1	4.7

[a] Conditions: 0.2 M sodium acetate, pH 6.0, 0.1 μM ferrous ammonium sulfate, 4 mg/mL apo-ovotransferrin, and 0.2 μM apoferritins. The formation of transferrin Fe(III) was followed at 470 nm, and the rate was measured in the first 3 min of reaction. Calculations based on a molar extinction of 2300. Means of three determinations.

in the uptake medium confirmed that rLFo at pH 6.0 and below does not incorporate iron (Figure 7).

Ferritin ferroxidase activity was studied at pH 6.0 using apotransferrin as the final Fe(III) acceptor (Bakker & Boyer, 1986). The activity of rLFo was not detectable; HLF activity was significant and about 20% that of rHF (Table II).

All these experiments indicate that rLFo, at variance with H-chain containing HLF and rHF, at acidic pH values does not show any reactivity with iron.

Discussion

We show that *E. coli* can direct the synthesis of human ferritin L-chain in amounts that are high and comparable to those obtained with human H-chain ferritin: up to 15% of total soluble proteins (Levi et al., 1987). This finding indicates that foreign ferritins are efficiently expressed and not rapidly degraded by the host *E. coli*, probably as a consequence of the remarkable stability of this protein. The two recombinant H- and L-ferritins show a number of similarities: all the expressed subunits are found in the full assembled ferritin molecules which form dimers and oligomers, are stable at 75 °C, and can be purified with good recovery with the procedures normally used for tissue ferritins. A comparison between rLFo (100% L-chain), rHF (100% H-chain), and natural HLF (about 95% L) provides data on the structural and functional properties of the two chains and on the possible differences between recombinant and natural ferritins.

Immunological and Structural Aspects. All the monoclonal antibodies previously elicited by HLF and specific for natural L-chain (Luzzago et al., 1986) recognize both rLFo and HLF ferritins to a similar extent, proving an immunochemical analogy between the natural and the recombinant L-chains.

Gel filtration, electrophoretic, spectroscopic, and crystallization data were analogous for rLFo and HLF, suggesting a structural similarity which was confirmed by the CD analyses. The CD spectra of the two proteins in the far- and near-UV regions were nearly identical, and in good agreement with previous studies on human and horse ferritins (Leach et al., 1976; Listowsky et al., 1972). The high molar ellipticity values at 222 nm are an index of correct folding of the subunits, while the positive ellipticity values in the near-UV region have been interpreted as an index of correct ferritin assembly (Leach et al., 1976; Stefanini et al., 1982).

Only by isoelectric focusing could we detect major differences between natural and recombinant L-ferritins: rLFo focused in two close bands, indicating homogeneity of the sample, while HLF showed the typical complex profile (Arosio et al., 1978). The comparison suggests that the large number of isoferritin bands in HLF is due to the minor content of H-chain and to posttranslational modifications not occurring in the bacterial host.

rLFo, despite not having the N-terminal amino group blocked, focused in the same position as the natural L homopolymer (Arosio et al., 1977), in which the amino group is acetylated. This finding can be explained by the substitution of the acetyl-Ser-Ser- initial sequence of the natural L into the Asp-Pro- of rLFo: the carboxyl group of the aspartic acid neutralizes the positive charge of the free amino group, leaving this sequence, which is exposed on the protein outer surface, with no net charge in both L subunits.

Functional Aspects. rLFo purifies as a colorless ferritin while rHF is an amber color ferritin. Spectral and chemical analyses confirmed the virtual absence of iron in rLFo even when expressed by bacteria exposed to relatively high iron concentrations (0.5 mM), at variance with rHF, which accumulates bacterial iron in proportion to its concentration in the medium.

In the in vitro studies at natural pH, rLFo can incorporate iron to an extent analogous to natural HLF and rHF ferritins. Thus, rLFo is a functional ferritin. However, the iron uptake kinetics of rLFo are slower than those of HLF or rHF, and always similar to the iron autoxidation rates of the controls without protein. In addition, in the pH range 5–6, rLFo does not take up and incorporate iron, and in this pH range, iron autoxidation is absent (Levi et al., 1988). Similarly, in the ferroxidase reaction, which is conducted at pH 6.0, rLFo has no detectable activity. Interestingly, all the kinetics of ferritin–iron interactions at acidic pH clearly differentiate the three ferritins analyzed: rHF has high, HLF low but detectable, and rLFo no activity. This finding suggests that the capacity to incorporate iron in these conditions is related to the presence of the H-chain (Levi et al., 1988). However, present data do not rule out the possibility of posttranslational modifications affecting functional activity of the natural ferritins (Mertz & Theil, 1983).

The steps of oxidation, penetration, and hydrolysis/polynucleation are necessary for the in vitro formation of the ferric iron core from Fe(II) in ferritin. In a previous work, we provided evidence that, in conditions of reduced/absent iron autoxidation, iron uptake starts by its interacting with a ferroxidase site located on or near the surface of the H-chain, suggesting oxidation as the first step for iron incorporation (Levi et al., 1988). Here we show that rLFo lacks the ferroxidase site but, in some conditions, is still able to form a stable core. This reveals that L-apoferritin even without an apparent kinetic advantage is still able to address iron into the inner cavity for core formation. Possibly this occurs because the resulting ferrihydrite product of the ferritin core is thermodynamically more stable than the amorphous polynuclear ferric hydroxide formed in the absence of ferritin.

Thus, present data strongly suggest that ferritin may take up iron with at least two distinct mechanisms: one driven by H-chain ferroxidase activity, which has been previously described (Levi et al., 1988), and the other one driven by iron autoxidation which occurs in the absence of H-chain. In this

latter and slower mechanism, the iron penetration may precede the oxidation/polynucleation steps, as previously suggested for horse ferritin (Roherer et al., 1987). The biological relevance of the H-driven mechanism is indicated by previous findings that the human ferritin without iron is devoid of H subunit (Arosio et al., 1977) and by the present data which indicate that rHF, but not rLFo, takes up bacterial iron in vivo. This further suggests that the ferritins expressed in *E. coli* may provide us with a relatively simple model to study in vivo ferritin iron uptake.

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REFERENCES

Arosio, P., Yokota, M., & Drysdale, J. W. (1977) *Br. J. Haematol. 36*, 199–207.

Arosio, P., Adelman, T. G., & Drysdale, J. W. (1978) *J. Biol. Chem. 253*, 4451–4458.

Arosio, P., Gatti, G., & Bolognesi, M. (1983) *Biochim. Biophys. Acta 744*, 230–232.

Bakker, G. R., & Boyer, R. F. (1986) *J. Biol. Chem. 261*, 13182–13185.

Bryce, C. F. A., & Crichton, R. R. (1973) *Biochem. J. 133*, 301–309.

Cairo, G., Vezzoni, P., Bardella, L., Schiaffonati, L., Rappocciolo, E., Levi, S., Arosio, P., & Bernelli-Zazzera, A. (1986) *Biochem. Biophys. Res. Commun. 139*, 652–657.

Cayre, Y., Raynal, M. C., Darzynkiewicz, Z., & Dorner, M. H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7619–7623.

Chou, C. C., Gatti, R. A., Fuller, M. L., Concannon, P., Wong, A., Chada, S., Davis, R. C., & Salser, W. A. (1986) *Mol. Cell. Biol. 6*, 566–573.

Costanzo, F., Santoro, C., Colantuoni, V., Bensi, G., Raugei, G., Romano, V., & Cortese, R. (1984) *EMBO J. 3*, 23–27.

Costanzo, F., Colombo, M., Staempflis, S., Santoro, C., Marone, M., Frank, R., Delius, H., & Cortese, R. (1986) *Nucleic Acids Res. 14*, 721–736.

Cragg, S. J., Drysdale, J. W., & Worwood, M. (1985) *Hum. Genet. 71*, 108–112.

Dorner, M. H., Salfeld, J., Will, H., Leibold, E. A., Vass, J. K., & Munro, H. N. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3139–3143.

Drysdale, J. W. (1977) *Ciba Found. Symp. 51*, 41–57.

Ford, G. C., Harrison, P. M., Rice, D. W., Smith, J. M. A., Treffry, A., White, J. L., & Yariv, J. (1984) *Philos. Trans. R. Soc. London, B 304*, 551–565.

Harrison, P. M., Clegg, G. A., & May, K. (1980) in *Iron in biochemistry and medicine II* (Jacobs, A., & Worwood, M., Eds.) pp 131–162, Academic Press, Amsterdam.

Harrison, P. M., Treffry, A., Lilley, T. H. (1986) *J. Inorg. Biochem. 27*, 287–293.

Jain, K. S., Barrett, K. J., Boyd, D., Favreau, M. F., Crampton, J., & Drysdale, J. W. (1985) *J. Biol. Chem. 260*, 11762–11768.

Kuepper, H., Delamarter, J., Otto, B., & Schaller, H. (1982) in *Proceedings of the 4th International Symposium on Genetics of Industrial Microorganisms* (Ikeda, Y., & Beppu, T., Eds.) Kodansha Ltd., Tokyo.

Leach, B. S., May, M. E., & Fish, W. W. (1976) *J. Biol. Chem. 251*, 3856–3861.

Levi, S., Cesareni, G., Arosio, P., Lorenzetti, R., Soria, M., Sollazzo, M., Albertini, A., & Cortese, R. (1987) *Gene 51*, 267–272.

Levi, S., Luzzago, A., Cesareni, G., Cozzi, A., Franceschinelli, F., Albertini, A., & Arosio, P. (1988) *J. Biol. Chem. 263*, 18086–18092.

Listowsky, I., Blauer, G., Englard, S., & Betheil, J. J. (1972) *Biochemistry 11*, 2176–2182.

Luzzago, A., Arosio, P., Iacobello, C., Ruggeri, G., Capucci, L., Brocchi, E., De Simone, F., & Gamba, D. (1986) *Biochim. Biophys. Acta 872*, 61–71.

Mertz, J. R., & Theil, E. C. (1983) *J. Biol. Chem. 258*, 11719–11726.

Rohrer, J. S., Joo, M. S., Dartyge, E., Sayers, D. E., Fontaine, A., & Theil, E. C. (1987) *J. Biol. Chem. 262*, 13385–13387.

Salfeld, J. (1985) Doctoral thesis, University of Heidelberg, FRG.

Santambrogio, P., Cozzi, A., Levi, S., & Arosio, P. (1987) *Br. J. Haematol. 65*, 235–237.

Santoro, C., Marone, M., Ferrone, M., Costanzo, F., Colombo, M., Minganti, C., Cortese, R., & Silengo, L. (1986) *Nucleic Acids Res. 14*, 2863–2876.

Stefanini, S., Chiancone, E., Arosio, P., Finazzi-Agro', A., & Antonini, E. (1982) *Biochemistry 21*, 2293–2299.

Strebel, K., Beck, E., Strohmaier, K., & Schaller, H. (1986) *J. Virol. 57*, 983–991.

Theil, E. C. (1987) *Annu. Rev. Biochem. 56*, 289–316.

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.