Journal of Biochemistry and Molecular Biology, Vol. 34, No. 4, July 2001, pp. 365-370

JOURNAL OF
Biochemistry and Molecular Biology

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Cooperative Activity of Subunits of Human Ferritin Heteropolymers in *Escherichia coli*

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We constructed a comparative expression system in order to produce recombinant human ferritin homo- and heteropolymers in *Escherichia coli.* Human ferritin H- (*hfH*) and L-chain (*hfL*) genes were expressed without amino acid changes under the control of a *tac* promoter. Ferritin heteropolymers of varying subunit composition were also produced by combining two different expression systems, a bicistronic expression system and a coplasmid expression system. As a result, recombinant H-chain ferritin and ferritin heteropolymers were catalytically active in forming iron core *in vivo*. In particular, the ferritin heteropolymer that is composed of 7% H-subunit and 93% L-subunit was capable of forming an iron core of the protein, while the L-chain ferritin homopolymer was inactive *in vivo*. This result indicates that the two H-subunits (*i.e.*, 7% H-subunit content) are important to keep ferritin active in the cells. In addition, human ferritins were identified as the major iron binding proteins in the transformed cells. Also, the amount of iron bound to the recombinant ferritins was proportional to the H-subunit content in ferritin heteropolymers *in vivo*.

Keywords: Cooperativity, *E. coli*, H- and L-subunits, Heteropolymer, Human ferritin

Introduction

Ferritin is the major intracellular iron storage protein. Its ability to sequester iron gives ferritin the dual function of iron reserve and supply. The other major cellular function is to prevent cells from toxicity that is caused by intracellular free iron (Harrison and Arosio, 1996). All ferritins are composed of 24 subunits in order to give a hollow protein shell (apoferritin) with the capacity to store up to 4500 Fe (III) atoms. There are two main subunits in apoferritin-heavy (known as heart type, or H) and light (known as liver type, or L) chains. Tissue isoferritins have functional differences that may be related to variations in the subunit composition. H-rich ferritins are characteristic of the heart and brain. Also, the ferritins have a relatively low iron content. L-rich ferritins, characteristic of the liver and spleen, have a relatively high iron content. Because of the heterogeneity of tissue ferritins, the functional differences of the two subunits are not clearly defined (Harrison *et al.*, 1990; Harrison and Arosio, 1996).

With the aid of protein engineering, a recombinant H-chain ferritin (H-ferritin) and L-chain ferritin (L-ferritin) was produced. The H-ferritin has a faster iron uptake rate than the L-ferritin *in vitro*, due to its ferroxidase activity (Levi *et al.*, 1988; Cozzi *et al.*, 1990). L-ferritin is more resistant to denaturants than H-ferritin, and assists in core formation (Levi *et al.*, 1992; Santambrogio *et al.*, 1992). In these cases, recombinant ferritin genes were expressed under the control of a λP_L promoter. L-ferritin was modified at the first two amino acids (Levi *et al.*, 1989). Recombinant ferritin heteropolymers, produced from the unfolded H- and L-chains *in vitro*, were also characterized. The increase in the H subunit content (up to 35%) increased the iron uptake rate (Santambrogio *et al.*, 1993).

Much attention has been given to the metal-microbe interactions. These include biomineralization, mineral leaching, and bioremediation (Beveridge, 1989). Bacteria and higher organisms have developed metal resistance using different defense systems, such as exclusion, compartmentalization, mineralization, and synthesis of metal binding proteins (like metallothioneins or phytochelatins). The heterologous expression of the metal binding protein metallothionein has been widely utilized to increase the metal binding capacity and tolerance of bacteria and plants (Mejáre and Bülow, 2001). Metallothioneins are cysteine-rich proteins which have a low molecular weight (Mr = 6-7 kDa), and bind

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6-7 atoms of the metal ion. Ferritins have a higher molecular weight (approx. 450-500 kDa) and show a higher metal binding capacity (4500 Fe atoms/molecule). Comparing its metal binding capacity to molecular weight, the binding capacity of ferritin is higher than that of metallothionein. Therefore, it is interesting to evaluate the metal binding capacity of mammalian ferritin in microbes.

In the present work, human ferritin H- and L-chain genes were expressed without amino acid changes under the control of a *tac* promoter in *Escherichia coli.* The expression system was suitable for studying the binding of the metal ions in the transformed cells. Ferritin heteropolymers were also produced by combining two different expression strategies, *i.e.*, and the bicistronic and coplasmid expression systems. In addition, human ferritins were identified as the major iron-binding proteins in the transformed cells. We also confirmed that human ferritins were biologically active in *E. coli.* Since ferritin related proteins of *E. coli* have a relatively low level of expression, our bacterial system provides a good and simple model in understanding the cellular function of the two subunits.

Materials and Methods

Strains and plasmids The *E. coli* strain, JM109, was used as a host strain. Plasmid pUC8 and pACYC184 (Rose, 1988) were used as cloning vectors. Plasmid pVUCH-1 that contains a *tac* promoter was kindly provided by Dr. S.-H. Oh at Woosuk University. Plasmid pACYC184 has a p15A replication origin and presents a low copy number. Bacto-trypton, Bacto-agar, and yeast extract were purchased from Difco Lab. Restriction enzymes were purchased from Bio-Rad, Pharmacia, and Sigma. The cells transformed with the plasmids pVUHFH, pVUHFL, pVHFH-L, pVHFL-H, pVUHFH/pACHFL and pVUHFL/pACHFH are denoted as strains HFH, HFL, H-L, L-H, H/L and L/H, respectively.

Construction of the expression plasmids In order to produce ferritin homo- and heteropolymers in *E. coli*, a comparative expression system was constructed (Fig. 1). The plasmid pVUCH-1 was used to construct the expression plasmid for the human ferritin H- and L-chain gene. The human ferritin H-chain gene *(hfH)* fragment, containing EcoR I/Hind III restriction sites, was obtained from PCR and inserted into pVUCH-1. The resulting plasmid was designated as pVUHFH. For the human ferritin L-chain gene (*hfL*), plasmid pVUHFL was also constructed by inserting the EcoR I/ Sma I fragment of the PCR product. For the bicistronic expression system, the plasmids were constructed by cloning the two-subunit genes in tandem into pVUCH-1. This yielded two constructs: one with an order of a *hf*H-*hf*L structural gene and the other with an order of a *hf*L-*hf*H structural gene. The resulting plasmids were named pVHFH-L and pVHFL-H. In addition, plasmids pACHFH and pACHFL were constructed for the coplasmid expression system into pACYC184 by inserting *hfH* and *hfL* genes, respectively. The expression plasmids were inducible with isopropyl-β-D-thiogalactopyranoside (IPTG). The correct coding region was verified by nucleotide sequencing according to the chain-termination method.

Expression of ferritin heteropolymers The *E. coli* JM109 cells were transformed with the recombinant plasmids including pVUHFH, pVUHFL, pVHFH-L, pVHFL-H, pVUHFH/pACHFL and pVUHFL/pACHFH. After the cells were cultured overnight at 37°C in a LB medium that contained ampicillin (50 μg/ml) or chloramphenicol (34 μg/ml), 0.1 mM IPTG was added for induction and further incubated for 4 h. The cells were harvested and the crude cell extracts were examined using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and 7.5% polyacrylamide gel electrophoresis in non-denaturing gels. Samples for SDS-PAGE were heated at 100°C for 10 min in the SDS gel-loading buffer. Partially purified samples that were obtained by heat denaturation at 75°C for 10 min were also heated at 100°C (10 min) for SDS-PAGE. The production of ferritin subunits was measured by densitometry (Molecular Dynamics PD-120, USA). The total amount of ferritin produced by each strain was analyzed as described previously (Chung *et al.*, 1998).

Western blotting Rabbit antiserum raised against the human liver ferritin (85% L-chain and 15% H-chain) was obtained from the Sigma Chemical Co. The immunological detection of proteins on nitrocellulose was performed using the method described by Burnette (1981).

Identification of iron binding protein The transformed *E. coli* cells were precultured overnight at 37°C in 5 ml of a LB medium that contained ampicillin or chloramphenicol as an appropriate selection marker. It was then transferred to 300 ml of a LB medium that contained 2 mM Fe-nitrilotriacetic acid (NTA) and cultured at 37°C for 4 h. Ferritin was induced by the addition of 0.1 mM IPTG and further incubated for 4 h. The cells were harvested and washed in 20 mM Tris-HCl (pH 7.4). Aliquots of the crude cell extracts were applied to gel-filtration on Sephacryl S-300 (2.6 × 100 cm). The eluents were analyzed for iron using the atomic absorption spectrometer (SpectrAA-880, Varian).

Results and Discussion

Construction of expression vectors In order to produce recombinant human ferritins of varying subunit composition *in vivo*, a comparative expression system for *hfH* and *hfL* was constructed (Fig. 1). This system guided the expression of ferritin homo- and heteropolymers in the cell. Both *hfH* and *hfL* were expressed by using a monocistronic expression system, respectively. For the production of the ferritin heteropolymer, both *hfH* and *hfL* were first expressed by using a bicistronic expression system, in which the *hfH* gene was inserted into the upstream and downstream regions of the *hfL* gene. From this, two constructs were obtained in the order of the structural genes, *hfH-hfL* and *hfL-hfH.* Next, each gene was subcloned into compatible plasmids to utilize a coplasmid expression system.

Expression of ferritin H- and L-subunits in *E. coli* Cells harboring the expression plasmids for *hfH* and *hfL* were induced. The crude cell extracts were then analyzed on 15%

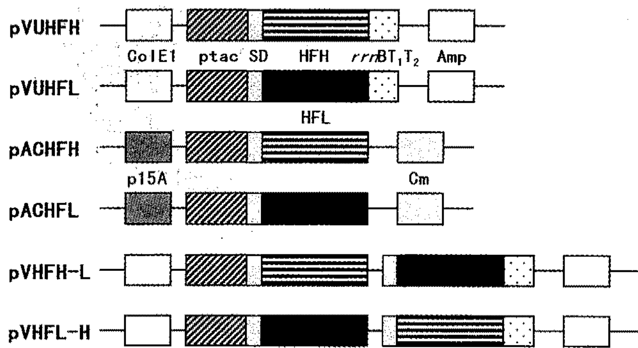

Fig. 1. Schematic diagram of the plasmids constructed. Line represents the plasmid DNA and the boxes represent the gene or their corresponding functional domains.

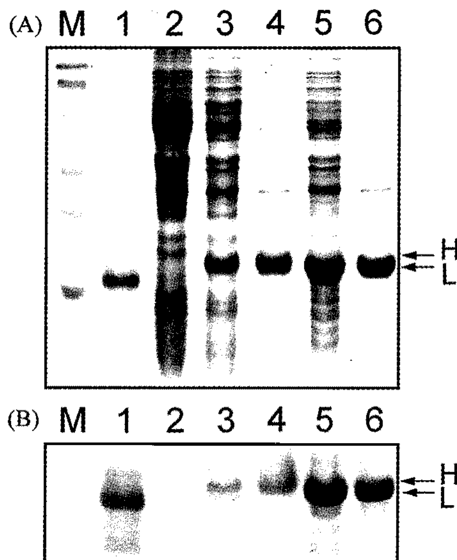

Fig. 2. 15% SDS-polyacrylamide gel electrophoresis (A) and Western blotting (B) of human H- and L-ferritin expressed in *E. coli*. The lanes are: M, size marker; (1) horse spleen ferritin; (2) JM109; (3) HFH; (4) heat-treated HFH; (5) HFL; (6) heat-treated HFL.

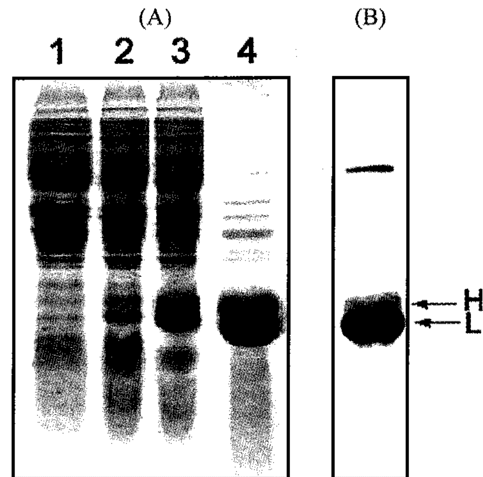

Fig. 3. 15% SDS-polyacrylamide gel electrophoresis (A) and Western blot analysis (B) of ferritin heteropolymer expressed in the H-L (*E. coli* cells transformed with the plasmid pVHFH-L). The lanes are: (1) JM109; (2) no induction; (3) induction with 0.1 mM IPTG; (4) heat-treated sample of the transformed cells being induced.

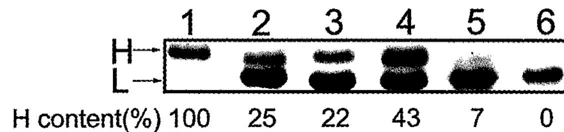

Fig. 4. 12% SDS-PAGE of ferritin heteropolymer expressed using both a bicistronic and a coplasmid expression systems. Subunit contents were estimated by densitometric analysis. Strain names are given in Materials and Methods. The lanes are: (1) HFH; (2) H-L; (3) L-H; (4) H/L; (5) L/H; (6) HFL.

SDS-PAGE and Western blotting (Fig. 2). On the SDS-PAGE gel, the extracts from the induced cells of HFH and HFL gave the major band of H-subunit (21 kDa), which covers about 25% of the soluble proteins as analyzed by densitometry. In the case of HFL, the extracts of HFL gave the L-subunit (19 kDa) band, which covers about 55% of the soluble proteins. These bands that are observed in the induced cells, when heat denatured, exhibited the same intensity, thereby proving that the expressed ferritin H- and L-subunits were as thermostable as native polypeptides (Fig. 2A; lane 4 and 6). As such, the partially purified samples, upon heat denaturation, were used to compare the expression level of ferritin subunits throughout this work. Previously, the recombinant tadpole ferritins, produced in *E. coli*, were reported to be thermostable like the native ferritin (Chang *et al.*, 1995). The overproduction of heterologous proteins was achieved in *E. coli* using an expression vector with a *tac* promoter (Min *et al.*, 1988). The major bands of the expressed proteins were further identified by Western blotting, indicating that the expressed ferritin H- and L-subunits share antigenic determinants with the native human liver ferritin (Fig. 2B).

Cell lysates from the bicistronic expression system (H-L) were also analyzed on SDS-PAGE (Fig. 3). The extracts from the induced cells gave the major bands of the two subunits. The major bands of the expressed protein were further identified using Western blotting (Fig. 3B).

The expression level of the human ferritin H- and L-subunits was compared in the transformants (Fig. 4). In the case of the bicistronic expression system, the expression level of the L-subunit was about three times higher than that of the H- subunit (Fig. 4; lanes 2 and 3). Changes in the order of the coding region of the two subunits hardly affected their expression. The reason is unclear, but the intrinsic translational efficiency of the *hfL* gene might be related. For example, the codon bias index is 0.28 for *hfL* and 0.17 for *hfH*. In addition, the subunit composition was modulated using a coplasmid expression system with a variation in the plasmid copy number (Fig. 4; lane 4 and 5). The expression

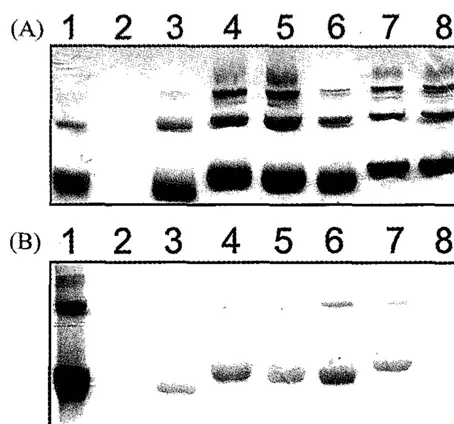

Fig. 5. Native PAGE analysis of ferritin heteropolymers. Cells were grown in a LB medium without additional iron. The crude cell extracts were heated at 75℃ for 10 min. A, stained for protein with Coomassie blue; B, stained for iron with Prussian blue. The lanes are: (1) horse spleen ferritin; (2) JM109; (3) HFH; (4) H-L; (5) L-H; (6) H/L; (7) L/H; (8) HFL.

level of the two subunits was much more differentiated by up to 13 fold. Overall, the coplasmid expression system was clearly more effective in generating dramatic alterations in the subunit composition than in the bicistronic expression system. The expression of the two subunits was confirmed in the transformed cells at the transcriptional level by Northern blotting (data not shown). Our result is consistent with an earlier report of Rucker and co-workers (1997).

Partially purified ferritins were analyzed by using PAGE (Fig. 5). The expressed H- and L-subunits were assembled into holoproteins, whose bands appeared on a non-denaturing gel with a migration somewhat comparable to horse spleen ferritin (Fig. 5). There are also a few minor bands that were assigned to ferritin oligomers. In the control cells, no band was shown at a similar position to ferritin in both the protein and iron staining gels. A weak band of bacterioferritin disappeared after heat denaturation. Recombinant H-ferritin migrated slightly faster than recombinant L-ferritin (Levi *et al.*, 1994). The band of recombinant human H-ferritin positively stained for iron, but no iron-stained band was obtained from recombinant L-ferritin. This suggests that the expressed H-ferritin forms a stable iron core *in vivo*, while the L-ferritin does not (Fig. 5; lane 8). Our results agree with an earlier report in which the recombinant human L-ferritin that is purified from the *E. coli* cells is colorless since the protein contains few iron atoms that show a distinctive brownish color (Levi *et al.*, 1989). Recombinant ferritin, produced in either the bicistronic expression (Fig. 5; lanes 4 and 5) or the coplasmid expression (Fig. 5; lane 6 and 7) system, showed a slight difference in the mobility of the protein, which might be related to the H-subunit content in the heteropolymers. As such, the ferritin heteropolymer, comprised of the 7% H-subunit content, migrated as slow as L-ferritin. Previously, Rucker and co-workers (1997) reported that ferritin heteropolymers, synthesized in bacteria, assemble into relatively homogeneous proteins, whose final subunit composition is largely dictated by the intracellular subunit population. When the cells that produce recombinant ferritin homo- and heteropolymers were grown in a medium with no additional iron added, the recombinant ferritin heteropolymers, as well as H-ferritin, incorporate iron well in their core. This suggests that H-ferritin and heteropolymer were active as natural tissue ferritins. In particular, the ferritin heteropolymer, which is composed of 7% H-subunit and 93% L-subunit (equivalent to about 2 H-subunits and 22 L-subunits), became capable of forming an iron core (Fig. 5; lane 7). The results confirm that L-ferritin, inactive alone *in vivo*, does incorporate iron in the presence of H-subunits in the cell. This indicates that iron, oxidized by the ferroxidase activity of the H-subunit, may be accumulated in the ferritin heteropolymer, as observed in the *in vitro* experiments (Levi *et al.*, 1992; Santambrogio *et al.*, 1996). The biological function of L-ferritin in the cell is yet unclear, but the overexpression of the H-ferritin reduces cell growth in transfectant HeLa cells (Cozzi *et al.*, 2000).

Identification of iron-binding protein in the transformed cells To determine whether or not human ferritins that are expressed in *E. coli* are catalytically active *in vivo*, we analyzed iron-binding proteins in the cells that were cultured in a medium containing 2 mM Fe-NTA. The elution pattern on the Sephacryl S-300 gel filtration (2.6×100 cm) of the sonic extract of the *E. coli* cells of HFH, HFL, H-L, H/L and L/H is shown in Fig. 6. In all of the cases, the main iron peak occurred in the high molecular weight fractions that were subsequently identified as containing ferritin. In the control JM109 cells, the main iron peak had a similar elution volume as ferritin. This could be due to bacterioferritin or ferritin-like protein (Abdul-Tehrani 1999). This protein was undetected on native PAGE under our experimental conditions. In the HFH cells, the major iron peak reached over 90% of the total iron content detected, suggesting that the human H-ferritin is functionally active in *E. coli*, and its activity is distinctive (Fig. 6B). The elution profile of HFL was significantly different from that of HFH, which was rather comparable to that of the control JM109 (Fig. 6C). These results support the native PAGE analysis, where L-ferritin did not form a stable iron core *in vivo* (Fig. 5B).

In the bicistronic system of H-L, the main iron peak (except the peak at the column void volume) was found with a similar elution volume to ferritin (Fig. 6D). The activity of the accumulating iron in the core in L-H was comparable to H-L (data not shown). In the coplasmid system of H/L, *i.e.*, 43% H-subunit and 57% L-subunit, the ferritin peak was the major iron binding protein (Fig. 6E). The ferritin peak was also found in L/H, *i.e.*, 7% H-subunit and 93% L-subunit (Fig. 6F). This indicates that ferritin heteropolymers that are produced in *E. coli* were functionally active and able to accumulate iron in their core, as proven by the iron staining of PAGE (Fig. 5). To

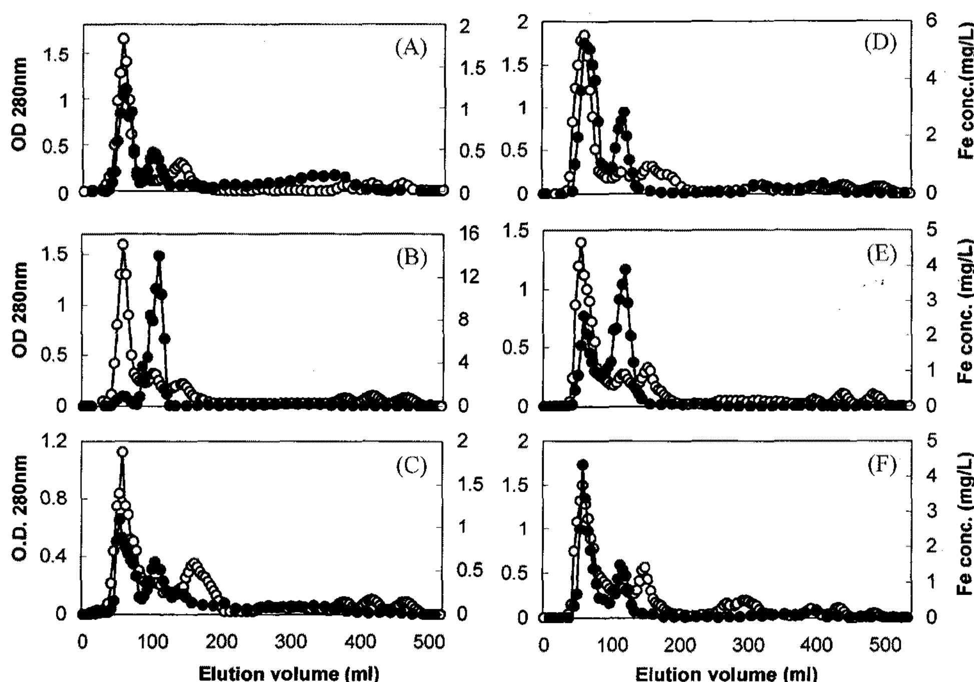

Fig. 6. Chromatography on Sephacryl S-300 of the sonic extract of the transformed cells, which were cultured in a medium that contained 2 mM Fe-NTA. (○), Optical density at 280 nm; (●), Fe concentration (mg/L). (A) JM 109; (B) HFH; (C) HFL; (D) H-L; (E) H/L; (F) L/H.

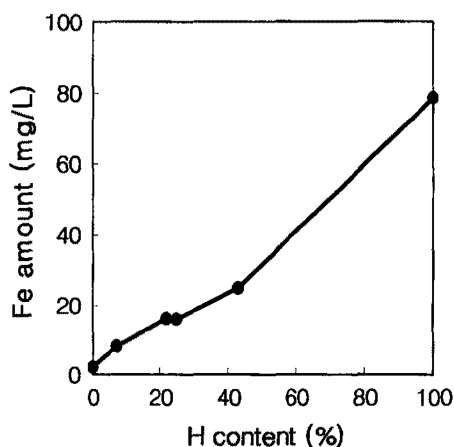

Fig. 7. Biological activity of ferritin heteropolymers. Ferritin heteropolymers were analyzed by both gel filtration chromatography and atomic absorption spectrometry. Fe amounts that bound to the ferritin molecule were obtained by adding up the iron contents of the ferritin peak area in Fig. 6.

compare the biological activity of ferritin heteropolymers, the amount of iron that bound to the protein molecule was plotted against the H-subunit content (Fig. 7). The amount of total iron was obtained by adding up the iron contents of the ferritin peak area (approximately equivalent to the molar concentration of ferritin) of the chromatography. The activity, which was in proportion to the content of the H-subunits, reflects the iron bound to the recombinant ferritins *in vivo*. The slope increased with a gentle gradient up to 43% H-subunit with a steeper gradient beyond the H-subunit contents. This phenomenon implies the synergic effect of the two subunits on iron uptake in the ferritins in the cells. From the previous experiments with ferritin heteropolymers *in vitro*, the initial rate of iron uptake increased from 0 to about 35% H chain, and reached a plateau from 35 to 100% (Santambrogio *et al.*, 1993). However, the results could not be compared to our results since the iron uptake mechanism of ferritin is far more complex. It involves iron oxidation, iron transfer, nucleation, and core formation. For comparison, measurements on the initial rate of iron uptake need to be performed with purified ferritin heteropolymers. Our heteropolymer expression system, however, did contribute to the understanding of the function of each subunit of ferritin and the iron uptake mechanism *in vivo*.

Acknowledgments This work was supported by a grant from the National R & D Program of the Korean Ministry of Science and Technology and a grant from the Chonbuk National University to K.-S. Kim.

References

Abdul-Tehrani, H., Hudson, A. J., Chang, Y. S, Timms, A. R., Hawkins, C., Williams, J. M., Harrison, P. M., Guest, J. R. and Andrews, S. C. (1999) Ferritin mutants of *Escherichia coli* are iron deficient and growth impaired, and *fur* mutants are iron deficient. *J. Bacteriol.* **181**, 1415-1428.

Beveridge, T. J. (1989) Metal ions and bacteria; in *Metal Ions and Bacteria*, Beveridge, T. J. and Doyle, R. J. (eds.), pp. 1-29, John Wiley & Sons, New York.

Burnette, W. N. (1981) Western blotting: Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **112**, 195-203.

Chang, S.-R., Kim, Y.-T. and Kim, K.-S. (1995) Purification and characterization of recombinant tadpole H-chain ferritin in *Escherichia coli. J. Biochem. Mol. Biol.* **28**, 238-242.

Chung, Y.-J., Kim, K.-S., Jeon, E.-S., Park, K.-I. and Park, C.-U. (1998) Effect of the *Vitreoscilla* hemoglobin gene on the expression of the ferritin gene in *Escherichia coli. J. Biol. Mol. Biol.* **31**, 503-507.

Cozzi, A., Corsi, B., Levi, S., Santambrogio, P., Albertini, A. and Arosio, P. (2000) Overexpression of wild type and mutated human ferritin H-chain in HeLa cells: *in vivo* role of ferritin ferroxidase activity. *J. Biol. Chem.* **275**, 25122-25129.

Cozzi, A., Santambrogio, P., Levi, S. and Arosio P. (1990) Iron detoxifying activity of ferritin. *FEBS Lett.* **277,** 119-122.

Harrison, P. M., Andrews, S. C., Artymiuk, P. J., Ford, G. C., Lawson, D. M., Smith, J. M. A., Treffry, A. and White, J. L. (1990) Ferritin; in *Iron Transport and Storage*, Ponka, P., Schulman, H. M., Woodworth, R. C. (eds.), pp. 82-101, CRC Press, Boca Raton, Florida.

Harrison, P. M. and Arosio, P. (1996) The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim. Biophys. Acta* **1275**, 161-203.

Lawson, D. M., Treffry, A., Artymiuk, P. J., Harrison, P. M., Yewdall, S. J., Lussago, A., Cesareni, G., Levi S. and Arosio P. (1989) Identification of the ferroxidase center in ferritin. *FEBS Lett.* **254**, 207-210.

Levi, S., Corsi, B., Rovida, E., Cozzi, A. Santambrogio, P., Albertini, A. and Arosio, P. (1994) Construction of a ferroxidase center in human ferritin L-chain. *J. Biol. Chem.* **269**, 30334-30339.

Levi, S., Salfeld, J., Franceschinelli, F., Cozzi, A., Dorner, M. H. and Arosio, P. (1989) Expression and structural and functional properties of human ferritin L-chain from *Escherichia coli. Biochemistry* **28**, 5179-5184.

Levi, S., Yewdall, S. J., Harrison, P. M., Santambrogio, P., Cozzi, A., Rovida, E., Albertini, A. and Arosio, P. (1992) Evidence that H- and L-chains have cooperative roles in the iron-uptake mechanism of human ferritin. *Biochem. J.* **288**, 591-596.

Mejáre, M. and Bülow, L. (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends Biotech.* **19**, 67-73.

Min, S. Y., Park, H. Y., Kim, M. H. and Kim, J. I. (1988) High-level expression of the insecticidal crystal protein gene from *Bascillus thuringiensis* subsp. *kurstaki* HD-73 in *Escherichia coli. J. Biol. Mol. Biol.* (formerly *Korean Biochem. J.*) **21**, 372-377.

Rose, R. E. (1988) The nucleotide sequence of pACYC184. *Nucleic Acids. Res.* **11**, 335-355.

Rucker, P., Torti, F. M. and Torti, S. V. (1997) Recombinant ferritin: modulation of subunit stoichiometry in bacterial expression systems. *Protein Eng.* **271**, 33352-33357.

Santambrogio, P., Levi, S., Arosio, P., Palagi, L., Vecchio, G., Lawson, D., Yewdall, S. J., Artymiuk, P. J., Harrison, P. M., Jappelli, R. and Cesareni, G. (1992) Evidence that a salt bridge in the light chain contributes to the physical stability difference between heavy and light human ferritins. *J. Biol. Chem.* **267**, 14077-14083.

Santambrogio, P., Levi, S., Cozzi, A., Rovida, E., Albertini, A. and Arosio, P. (1993) Production and characterization of recombinant heteropolymers of human ferritin H and L chains. *J. Biol. Chem.* **268**, 12744-12748.

Santambrogio, P., Levi, S., Cozzi, A., Corsi, B. and Arosio, P. (1996) Evidence that the specificity of iron incorporation into homopolymers of human ferritin L- and H-chains is conferred by the nucleation and ferroxidase centers. *Biochem. J.* **314**, 139-144.