Biol Metals (1990) 3:227-231

BIOLOGY OF METALS

β-Thalassaemia/haemoglobin E tissue ferritins

II: A comparison of heart and pancreas ferritins with those of liver and spleen

Kim Chi Tran[1], John Webb[1], David J. Macey[2], and Pensri Pootrakul[3]

[1] School of Mathematical and Physical Sciences, and
[2] School of Biological and Environmental Sciences, Murdoch University, Perth, WA 6150, Australia
[3] Thalassaemia Centre, Faculty of Graduate Studies and Division of Haemotology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Received August 16, 1990

Summary. Tissue ferritins from β-thalassaemia/haemoglobin E heart and pancreas were characterized by native PAGE, SDS/PAGE and isoelectric focussing, and compared with those isolated from corresponding liver and spleen tissue. On PAGE, all ferritins consisted of a single band assigned to the protein monomer. Small differences in electrophoretic mobility were found between the bands. The ferritins were resolved by SDS/PAGE into two major subunits, H and L, corresponding to molecular masses of 22.5 kDa and 19 kDa, respectively. The L subunit was predominant in all cases. The isoferritin profiles of all tissue ferritins were remarkably similar, consisting of a complex pattern of bands which were appreciably more basic than those obtained for horse spleen ferritin. The subunit composition and isoferritin profiles of the four tissue ferritins almost certainly reflect the defense mechanism of the body in synthesizing in all four tissue types a more stable long-term iron-storage isoferritin in order to detoxify and store the excess iron present due to the pathological condition of β-thalassaemia/HbE.

Key words: Ferritin - Thalassaemia - Iron overload - Protein structure - Isoferritins

Introduction

β-Thalassaemia/haemoglobin E is a genetic disorder of haemoglobin synthesis resulting in chronic haemolytic anaemia. While it is found throughout the world, it occurs at very high frequencies in certain populations in south-east Asia (Wasi 1981). Due to economic difficulties, those affected in this part of the world receive very few or no blood transfusions. However, despite this lack of treatment, they are heavily iron-overloaded due to high iron absorption from the gut, thought to be related to the hyperactive erythropoiesis (Pootrakul et al. 1988) found in these cases. Much of this excess iron is stored in the form of ferritin and haemosiderin within the tissues (Bhamarapravati et al. 1967).

In humans, the roles of ferritin are iron storage and detoxification. It is found predominantly in the spleen, liver and bone marrow, although most tissues contain some ferritin (Aisen and Listowsky 1980). Ferritin comprises a protein shell, composed of 24 subunits surrounding a hydrous iron(III) oxide core which can be crystalline in nature (Harrison et al. 1980; St. Pierre et al. 1989).

The ferritin subunits are believed to be of at least two distinctive types: H (heavy) and L (light) (Arosio et al. 1978). Ferritins composed of mainly H subunits are associated with iron uptake, oxidation and detoxification, while L subunit ferritins play a role in long-term iron storage (Kohgo et al. 1980; Bomford et al. 1981). These two subunits are encoded by a multigene family (Costanzo et al. 1984). On native PAGE, most tissue ferritins migrate as a single band (monomeric) although some, such as heart, resolve into two components (Powell et al. 1975). Liver ferritin from siderotic mice contains an extra faster migrating band of presumably smaller ferritin in addition to the major monomer band (Massover 1985). On isoelectric focussing all ferritins show a complex pattern of bands corresponding to considerable heterogeneity. Both the isoferritin profile and subunit composition vary with tissue type, with the flux of iron into the body and with certain disease states (Drysdale et al. 1977; Bomford et al. 1981).

As part of a continuing study into the structure and reactivity of iron-storage proteins in β-thalassaemia/HbE patients, ferritins were isolated from heart and pancreas and compared with those isolated previously from liver and spleen (Tran et al. 1990). Once isolated, they were then characterized to determine their degree of heterogeneity. Information of this sort is needed so that the effect of various disease states on the structure and role of ferritin in the tissues of the body can be

Abbreviations. PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulphate

Offprint requests to: D. J. Macey

better understood and thus facilitate more effective treatment.

Materials and methods

Samples of liver, spleen, heart and pancreas were obtained from β-thalassaemia/HbE patients at the Thalassaemia Centre, Siriraj Hospital, Bangkok, Thailand. Ferritin was isolated from these tissues as described previously (Tran et al. 1990). While ferritins could be obtained from four individual livers and three individual spleens, the limited amount of tissue available from heart and pancreas meant that samples from different individuals had to be pooled in order to obtain sufficient material. Horse spleen ferritin used as a control was isolated using a similar method to those described above. Protein and iron concentration measurements, native PAGE, SDS/PAGE and isoelectric focussing were carried out as described by Tran et al. (preceding paper), while H/L subunit ratios were measured by a laser densitometer (LKB 2202 Ultroscan).

Results

Following PAGE, a single major band was present after both protein and iron staining for ferritins isolated from the β-thalassaemia/HbE liver, spleen, heart and pancreas. Staining for protein and for iron yielded identical profiles, indicating that the only protein present was ferritin. This band was assigned to the ferritin monomer. However, the mobilities of these monomeric bands were slightly different in the various tissue types (Fig. 1). Thus, ferritin from the liver and spleen migrated at the same speed while heart ferritin was slightly faster and pancreas ferritin migrated the slowest. It is interesting to note that while the amount of ferritin applied in each track was similar (approximately 50 μg) the band densities obtained were different. Thus, the bands of heart and pancreas ferritin were more smeared down the gel when compared to those of liver and spleen ferritins. However, this smearing could not be resolved into distinct bands. No differences were found between samples of the same organ taken from different individuals.

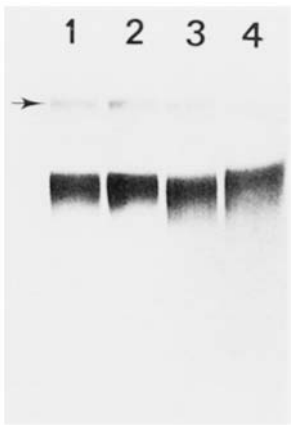

Fig. 1. Polyacrylamide gel electrophoresis of ferritins isolated from (1) β-thalassaemia/HbE spleen, (2) β-thalassaemia/HbE liver, (3) β-thalassaemia/HbE heart and (4) β-thalassaemia/HbE pancreas. In this and all subsequent figures the gels were stained for protein using Coomasie brilliant blue R-250. Arrow denotes the position of the oligomeric ferritin band found in the four tissues

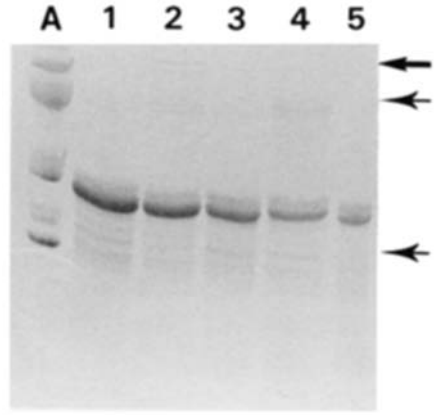

Fig. 2. SDS/PAGE, on 15% (mass/vol.) acrylamide gel of ferritins isolated from (1) β-thalassaemia/HbE liver, (2) β-thalassaemia/HbE spleen, (3) β-thalassaemia/HbE pancreas, (4) β-thalassaemia/HbE heart and (5) horse spleen. Molecular mass standards are shown in track A. Note the presence of high- and low-molecular-mass components in all samples (fine arrows) and high-molecular-mass components in β-thalassaemia/HbE spleen ferritin (broad arrow)

On SDS/PAGE, liver, spleen, heart and pancreas ferritins from β-thalassaemia/HbE patients and horse spleen ferritin gave two prominant bands in all cases indicating the presence of two different subunits (Fig. 2). The lighter or faster migrating band (L subunit) corresponded to a subunit molecular mass of 19 ± 0.5 kDa. The heavier or slower migrating band (H subunit) from human tissue ferritins corresponded to 22.5 ± 1.0 kDa while the heavy subunit from horse spleen ferritin corresponded to 21 ± 0.5 kDa.

The H/L subunit ratios were determined by laser densitometry to be 1:6.7, 1:6.1, 1:4, 1:3.5 in the β-thalassaemia/HbE liver, spleen, pancreas and heart ferritins, respectively. While the majority of ferritin was present in the two major bands (80% for liver and spleen and 76% for heart and pancreas) several minor bands were observed at relatively low staining intensities in all tissues. Thus, for example, all organs showed slower migrating bands at 50, 48 and 44 kDa, each less than 2% of total density and faster migrating bands at 16.5 and 15 kDa at intensities corresponding to 5% and 10%, respectively. In addition spleen ferritin showed a unique set of bands at 67, 62 and 52 kDa (density equivalent to 4% of total).

The iron/protein ratios measured on tissue ferritins from different individuals gave mean results of 0.22 ± 0.02 for liver (four samples) and 0.23 ± 0.02 for spleen (three samples) ferritins, and of 0.28 and 0.26 for single pooled samples of pancreas and heart ferritin, respectively. These results indicate that these latter two ferritins, as isolated, contain somewhat higher amounts of iron than the liver and spleen ferritins. The relative amount of apoferritin in these preparations was not determined.

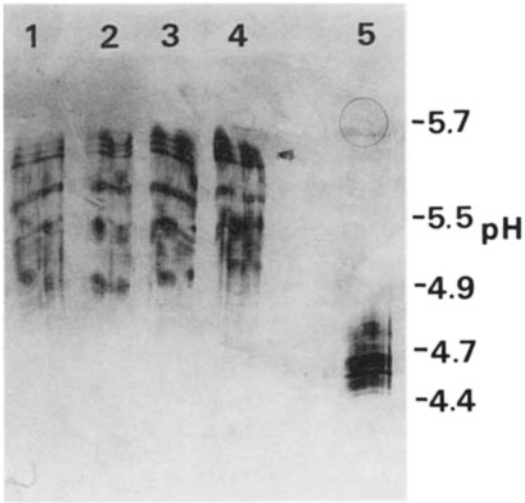

Fig. 3. Isoelectric focussing profile over the pH range 4.0–6.5 of ferritins isolated from (1) β-thalassaemia/HbE heart, (2) β-thalassaemia/HbE pancreas, (3) β-thalassaemia/HbE liver, (4) β-thalassaemia/HbE spleen and (5) horse spleen

On isoelectric focussing, ferritins from β-thalassaemia/HbE liver, spleen, heart and pancreas showed remarkably similar isoferritin profiles consisting of a complex pattern of bands (Fig. 3). These bands were appreciately more basic than those obtained for horse spleen ferritin (Fig. 3). Thus, β-thalassaemia/HbE tissue ferritins gave a range of pI values of 4.8–5.7 while the pI range for horse spleen ferritin was 4.4–4.8. In all β-thalassaemia/HbE samples the most prominant bands occurred at a pH of approximately 5.5 and 5.6. The pI gel gave an almost identical pattern on staining for iron to that found with protein staining.

Discussion

The overall electrophoretic mobilities of tissue ferritins obtained from β-thalassaemia/HbE patients are very different from the patterns obtained using normal tissue ferritins. Powell et al. (1975) reported that normal spleen ferritin migrates slightly more slowly than the normal liver ferritin while heart and pancreas ferritins migrate faster than ferritin from the liver. In the case of β-thalassaemia/HbE ferritins, however, those from liver and spleen migrate similarly (Fig. 1) while the heart and pancreas ferritins migrate faster and slower, respectively. It has now been well established that iron loading both affects the overall level of ferritin synthesis (Theil 1987) and leads to the L subunit being preferentially synthesized over the H subunit (Bomford et al. 1981). In addition, Massover (1985) found that iron loading induced mice to synthesize a new form of ferritin which was appreciably smaller than the normal type found in non-loaded animals. This difference in electrophoretic mobility between tissue ferritins from normal and pathological sources is almost certainly the result of increased iron loading causing an increase in the overall level of ferritin in the diseased tissues and changing the form in which it is found. In this context, it should be noted that the iron level in β-thalassaemia/HbE pancreas is 45 times that found in normal tissues (Shuler et al. 1990). Similarly, iron levels in the liver, spleen and heart are elevated by 10, 3 and 3 times, respectively (Shuler et al. 1990). Thus, it is not surprising that our results are in contrast to those obtained by Powell et al. (1975) who could not stain normal pancreas ferritin for iron using Perls reagent, suggesting that the iron content of this ferritin is appreciably less than that in the β-thalassaemia/HbE patients.

In addition, it is interesting to note that while Powell et al. (1975) identified the presence of two major bands in normal heart ferritin, in the β-thalassaemia/HbE patients, heart ferritin gave a band that smeared across a relatively broad range (Fig. 1). In the former case, the two bands were ascribed to the presence of two separate homogeneous populations of ferritin (Drysdale et al. 1977) while in the latter case the β-thalassaemia/HbE heart ferritin appears to be much more heterogeneous. The smearing and presumed heterogeneity also occurred in β-thalassaemia/HbE pancreas ferritin while normal pancreas ferritin gave only a single band on electrophoresis. No evidence was found in any tissue of the 'light' ferritin bands described by Massover (1985) as being present in siderotic mice.

The detailed 'micro structure' of ferritin seems to depend, to a large degree, on the nature and amount of iron loading. Thus, ferritins isolated from five different organs from patients with untreated idiopathic haemochromatosis and thus iron loaded, were reported to be remarkably uniform in their isoferritin profiles (Powell et al. 1974). However, heart, kidney and pancreas isoferritins isolated from normal tissues were characterized by more acidic isoferritin profiles than those of normal liver and spleen (Drysdale et al. 1977). In addition, in patients with haemochromatosis who had been treated by phlebotomy, the isoferritin pattern reverted to normal. However, the isoferritin profiles of ferritins prepared from spleen, liver and kidney of patients with transfusional iron overload showed little difference from those of ferritins isolated from the corresponding normal tissues. Ferritin prepared from the hearts of transfusional-iron overload patients, however, showed an increase in the more basic isoferritins compared with normal heart ferritin (Wagstaff et al. 1978).

In many ways, iron loading in β-thalassaemia/HbE patients is similar to that which occurs in untreated idiopathic haemochromatosis patients in that iron is absorbed from the gut throughout life. This is in contrast to the more rapid iron overload due to repeated blood transfusions. This difference in the form of iron loading is reflected in our results, namely, the similarity between the individual isoferritin profiles obtained from liver, spleen, heart and pancreas ferritins from β-thalassaemia/HbE patients.

In human and rat, the more basic isoferritins contain more L subunits while the more acidic ones contain more H subunits (Arosio et al. 1978; Bomford et al. 1981). As described, the nature of iron loading is observed to affect the structure of the protein shell in

terms of subunit synthesis. Thus, in normal tissues, the H subunit is dominant in heart ferritin while the L subunit is prominant in liver and spleen ferritins (Kohgo et al. 1980; Bomford et al. 1981). In all four β-thalassaemia/HbE tissue ferritins studied, the L subunit is predominant, occurring at a ratio of at least 3.5:1 L/H subunits. In this case, the reduction of H-chain and the increase of L-chain synthesis is almost certainly due to the different roles of the H and L subunits and their different turnover rates. The H subunit is involved in iron uptake and oxidation (Kohgo et al. 1980) since it contains the ferroxidase centre (Levi et al. 1988; Lawson et al. 1989) and H-rich ferritins are more specialised for iron detoxification (Levi et al. 1988). In contrast, ferritins rich in L subunits are less susceptible to intracellular proteolysis and survive longer than the H-rich shell and thus play the major role in long-term iron storage (Bomford et al. 1981). For example, iron administration in the rat, while stimulating overall ferritin synthesis, caused the preferential formation of L subunits and thus led to the more basic isoferritins being present (Bomford et al. 1981; Treffry et al. 1984). Under normal cellular conditions, at low Fe/apoferritin ratios, the ferroxidase activity of H chains may be needed for iron uptake, oxidation and core formation. However, L-chain homopolymers are capable of taking up iron slowly and forming the core in the absence of the ferroxidase activity associated with the H chain (Lawson et al. 1989). Thus, when relatively high iron concentrations occur, as in the case of β-thalassaemia/HbE, L-subunit synthesis is preferable in order to form stable long-term iron-storage ferritin.

While under normal conditions the heart and pancreas do not have a major iron storage role (Drysdale et al. 1977), under the dramatically increased iron loading found in β-thalassaemia/HbE patients, iron is found in these tissues (Bhamarapravati et al. 1967; Shuler et al. 1990). In these organs, a relatively high H/L subunit ratio has been maintained, presumably in order to maintain some detoxification activity.

The heterogeneity of ferritin is not well understood. While it might be derived from the difference in combination of H and L subunits (Nishi 1985), the existence of multigene families coding for ferritin has also been proposed (Costanzo et al. 1984; Theil et al. 1987) as has post-translational modification (Treffry and Harrison 1980) or a combination of such factors. Thus, the presence of minor bands on SDS/PAGE could be the results of intra-chain conformation changes, intersubunit bonding via disulfide bonds (Arosio et al. 1978) and general proteolytic processes (Lavoie et al. 1977).

In the case of β-thalassaemia/HbE, the unusual similarity between individual tissue ferritins found using a variety of techniques could result from the same processes postulated to occur in idiopathic haemochromatosis. Here, it has been suggested that there is a generalised deposition of catabolic or storage protein normally present predominantly in liver and spleen and also a decreased synthesis of the more acidic isoferritins present in the heart and pancreas (Powell et al. 1974; Wagstaff et al. 1980). This almost certainly reflects a defence mechanism of the body in synthesizing a more stable long-term iron-storage isoferritin in order to detoxify and store the iron present due to the pathological condition.

Acknowledgements. This work was supported by the award of a Commonwealth Research Scholarship to K. C. Tran, by the Australian Research Council and by the Murdoch University Special Research Grant. We thank Dr. Prapon Wilairat and the Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand for providing facilities to isolate and purify ferritin. In addition, P. P. acknowledges the support provided by the United State Public Health Research grant HL 34408 from The National Heart, Lung and Blood Institute, USA.

References

Aisen P, Listowsky I (1980) Iron transport and storage proteins. Annu Rev Biochem 49:357–393

Arosio P, Adelman TG, Drysdale JW (1978) On ferritin heterogeneity. Further evidence for heteropolymers. J Biol Chem 253:4451–4458

Bhamarapravati N, Na-Nakorn S, Wasi P, Tuchinda S (1967) Pathology of abnormal hemoglobin diseases seen in Thailand. I. Pathology of β-thalassaemia/hemoglobin E disease. Am J Clin Pathol 47:745–758

Bomford A, Conlon-Hollingshead C, Munro HN (1981) Adaptive responses of rat tissue isoferritins to iron administration. J Biol Chem 256:948–55

Costanzo F, Santoro C, Colantuoni V, Bensi G, Raugei G, Romano V, Cortese R (1984) Cloning and sequencing of a full-length cDNA coding for a human apoferritin H chain: evidence for a multigene family. EMBO J 3:23–27

Drysdale JW, Adelman TG, Arosio P, Casareale D, Fitzpatrick P, Hazard JT, Yokota M (1977) Human isoferritins in normal and disease states. Semin Hematol 14:71–88

Harrison PM, Clegg GA, May K (1980) Ferritin structure and function. In: Jacobs A, Worwood M (eds) Iron in biochemistry and medicine II. Academic Press, London, pp 131–171

Kohgo Y, Yokota M, Drysdale JW (1980) Differential turnover of rat liver isoferritins. J Biol Chem 255:5195–5200

Lavoie DJ, Marcus DM, Ishikawa K, Listowsky I (1977) Ferritin and apoferritin from human liver: aspect of heterogeneity. In: Brown EB, Aisen P, Fielding J (eds) Proteins in iron metabolism. Grune and Stratton, New York, pp 71–78

Lawson DM, Treffry A, Artymiuk PJ, Harrison PM, Yewdall SJ, Luzzago A, Cesareni G, Levi S, Arosio P (1989) Identification of the ferroxidase centre in ferritin. FEBS Lett 254:207–210

Levi S, Luzzago A, Cesareni G, Cozzi A, Franceschinelli F, Albertini A, Arosio P (1988) Mechanism of ferritin iron uptake: activity of the H-chain and deletion mapping of the ferro-oxidase site. J Biol Chem 263:18086–18092

Massover WH (1985) Molecular size heterogeneity of ferritin in mouse liver. Biochim Biophys Acta 829:377–386

Nishi M (1985) Comparison of biochemical characteristics between human liver and spleen ferritin. Nippon Ika Daigaku Zasshi 57:165–177

Pootrakul P, Kriengkrai K, Yansukon P, Wasi P, Fucharoen S, Charoenlarp P, Brittenham G, Pippard M, Finch CA (1988) The effect of erythroid hyperplasia on iron balance. Blood 71:1124–1129

Powell LW, Alpert E, Isselbacher KJ, Drysdale JW (1974) Abnormality in tissue isoferritin distribution in idiopathic haemochromatosis. Nature 250:333–335

Powell LW, Alpert E, Isselbacher KJ, Drysdale JW (1975) Human isoferritins: organ-specific iron and apoferritin distribution. Br J Haematol 30:47–55

Shuler TR, Pootrakul P, Yansukon P, Nielsen FH (1990) Effect of thalassaemia/hemoglobin E disease on macro, trace and ultra-trace element concentrations in human tissue. J Trace Elements Exp Med 3:31–43

St. Pierre TG, Webb J, Mann S (1989) Ferritin and hemosiderin: structural and magnetic studies of the iron core. In: Mann S, Webb J, Williams RJP (eds) Biomineralization: chemical and biochemical perspectives. VCH Verlagsgesellschaft, Weinheim, pp 295–344

Theil EC (1987) Ferritin: structure, gene regulation, and cellular function in animals, plants and microorganisms. Annu Rev Biochem 56:289–315

Tran KC, Webb J, Macey DJ, Pootrakul P, Yansukon P (1990) β-Thalassaemia/haemoglobin E tissue ferritins. I: Purification and partial characterization of liver and spleen ferritins. Biol Metals 3:222–226

Treffry A, Harrison PM (1980) Evidence for post-translational changes in rat liver ferritin. Biochim Biophys Acta 610:421–424

Treffry A, Lee PJ, Harrison PM (1984) Iron-induced changes in rat liver isoferritins. Biochem J 220:717–722

Wagstaff M, Worwood M, Jacobs A (1978) Properties of human tissue isoferritins. Biochem J 173:969–977

Wasi P (1981) Haemoglobinopathies including thalassaemia. 1: Tropical Asia. Clin Hematol 10:707–729