MULTIPLE SUBUNITS IN HUMAN FERRITINS: EVIDENCE FOR HYBRID MOLECULES

T.G. Adelman, P. Arosio, and J.W. Drysdale

Department of Biochemistry and Pharmacology
Tufts University School of Medicine
Boston, Ma. 02111 U.S.A.

Received February 28,1975

SUMMARY: The subunit composition of human heart and liver ferritins was examined by both sodium dodecyl sulfate gel electrophoresis and acetic acid-urea gel electrophoresis. These analyses indicated that both tissues contained two subunit types of similar size but different surface charge. One subunit was common to both tissues. The implications of these findings in relation to the known heterogeneity of isoferritins are discussed, and a new model of ferritin structure is proposed.

INTRODUCTION

Ferritin is an iron-containing protein of wide distribution in the plant and animal kingdoms. Its chief function appears to be in iron detoxification and storage, but it may also be involved in the regulation of iron absorption and transport (1,2). The molecule consists of a multimeric shell of apoferritin, approximately 440,000 in molecular weight, which surrounds a core containing variable amounts of iron (3). Ferritins of different structure and metabolism have been found in several tissues in mammalian species (4-7). Although most tissue ferritins are thought to be discrete homogenous populations, many of these ferritins have recently been resolved into multiple isoferritins by isoelectric focusing (8-10). Thus, heterogeneity exists not only among different tissues but also within individual tissues. These isoferritins do not represent differences in aggregation or iron content but intrinsically different apoferritin shells (8). In humans, at least 12 isoferritins may be found in the tissues

Abbreviations: A, acrylamide concentration (w/v); B, N,N'-methylene-bis-acrylamide concentration (w/v); DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

of a single individual, although many are common to several tissues (7). Different tissues usually have a characteristic isoferritin profile (7), although this profile may be altered markedly in dieased states such as idiopathic hemochromatosis (11) and cancer (12,13). As yet, neither the structural basis for the multiple forms nor their structural and metabolic relationships have been established.

This paper offers a basis for the extensive heterogeneity and characteristic tissue distribution in human isoferritins. Our results indicate that liver and heart ferritins, which together contain most of the isoferritins found so far in human tissues (7), are composed of at most three discrete subunits, one of which is common to both tissues. We propose that the various tissue ferritin populations represent hybrid molecules composed of different proportions of these different subunits. This model contrasts with the presently accepted ferritin structure of a homogeneous multimeric shell consisting of only one tissue-specific subunit type.

METHODS

Ferritin from human liver and heart were purified as described previously (7). The liver ferritin was crystallized twice with $CdSO_4$ (1). Both ferritins were stored at 4° in 20 mM phosphate buffer, pH 7.6, and their purity was confirmed by immunological and electrophoretic techniques (7). Isoferritin profiles were obtained by isoelectric focusing in thin slabs of polyacrylamide gel with the composition A = 4.0%, B = 0.16% (10). Analyses of subunit composition were determined by SDS dissociation and SDS gel electrophoresis as described by Fairbanks *et al.* (14) using gels of composition A = 11.2%; B = 0.42%. Ferritin was also dissociated in 67% acetic acid (15) and the resulting subunits dialyzed overnight against 0.9 N acetic acid in 8 M urea before electrophoresis in acetic acid-urea gels (16) (A = 15.0%; B = 0.10%).

RESULTS

Figure 1 shows the isoferritin profiles of crystalline human liver ferritin and of human heart ferritin. These ferritins were chosen for analysis since they contain isoferritins from the extreme ends of the isoelectric focusing spectrum of all normal tissue ferritins (7,11). The heart contains the most acidic isoferritins (approximate pI 4.8-5.3) and the liver the least acidic isoferritins (approximate pI 5.1-5.6). Both, however, contain common isoferritins of intermediate isoelectric points. This is most clearly

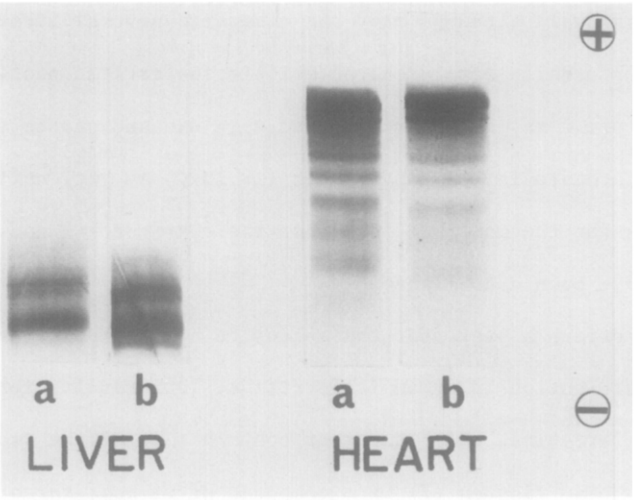

Figure 1. Isoelectric focusing of human liver and heart isoferritin. 30 µg of crystalline human liver ferritin and 100 µg of human heart ferritin were focused in duplicate on parallel tracks in a thin polyacrylamide gel slab. a, protein stained with Coomassie Brilliant Blue; b, iron stained with Prussian blue.

indicated by protein staining, since Prussian blue preferentially stains the iron-rich isoferritins and may not reveal isoferritins of low iron content (7). After dissociation into subunits by treatment with SDS and DTT, SDS electrophoresis of both heart and liver ferritins (Figure 2) indicated the presence of one major subunit species of molecular weight about 19,000 in both cases. The minor low-molecular weight species found in both liver and heart types probably represent degradation products from the treatment with SDS inasmuch as similar peptides are released by SDS degradation of the major subunit in crystalline horse spleen ferritin (17). While SDS gel electrophoresis shows only one class of subunits in liver and heart, subsequent analyses by acid urea electrophoresis revealed multiple subunit types in both cases (Figure 3). This electrophoretic technique resolved proteins by charge as well as by molecular volume. Heart ferritin gave rise to two bands, designated H and HL. HL is apparently common to both heart and liver ferritin as judged by co-electrophoresis (Figure 3, gel 3). In addition to HL, liver ferritin also contained a major band (L) of faster mobility, together with several

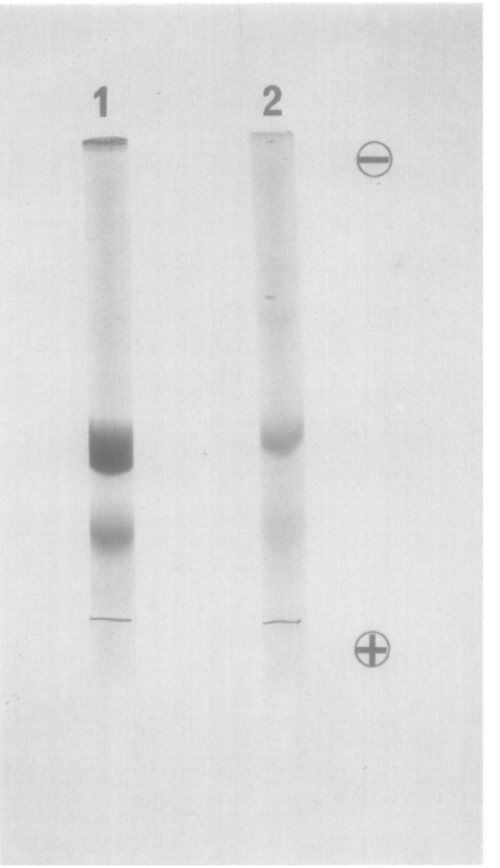

Figure 2. SDS electrophoresis of human ferritin subunits. Ferritins were dissociated with SDS and DTT and electrophoresed as described in Methods. The pin at the bottom of the gels indicates the position of the pyronine dye marker before staining with Coomassie Brilliant Blue (14). 1, 25 µg crystalline human liver ferritin; 2, 25 µg human heart ferritin.

minor bands. Reduction with DTT altered the mobility of L and the minor bands so that they appeared to comigrate with HL. After this reduction, a tail appears above HL in gel 1 (Figure 3). This tail can not be identical with the heart subunit H, as the tail derives from reduction of L type subunits which are not found in heart. The electrophoretic mobilities of the subunits from heart ferritin were not altered by treatment with DTT. These results suggest that the fastest and slowest bands in liver represent structures with intra- and intermolecular disulfide bridges respectively. A similar phenome-

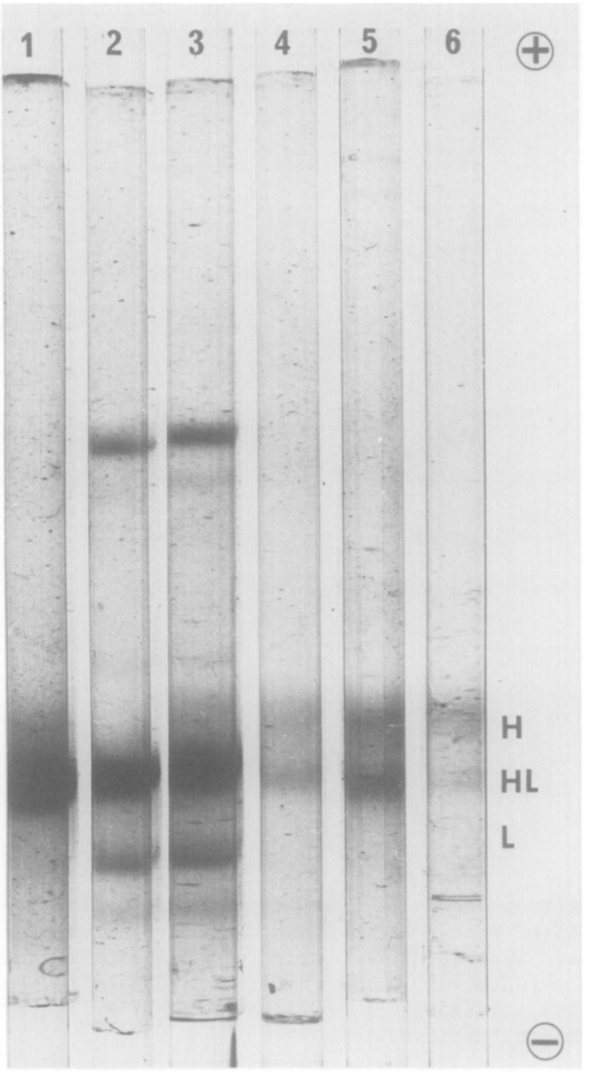

Figure 3. Acetic acid-urea electrophoreis of human ferritin subunits. Ferritins dissociated in acetic acid were electrophoresed as described in Methods for 4 hr. Protein was stained with Coomassie Brilliant Blue (14). 1, 25 µg human liver ferritin + 40 mM DTT; 2, 25 µg human liver ferritin; 3, 25 µg human liver ferritin and 10 µg human heart ferritin; 4, 10 µg human heart ferritin; 5, 25 µg human heart ferritin; 6, 10 µg human heart ferritin + 40 mM DTT.

---

non has been documented in crystalline horse spleen ferritin (17).

DISCUSSION

Although the currently accepted model of ferritin structure holds that each tissue ferritin is composed of a single tissue-specific subunit, our

experiments indicate that human heart and liver ferritins are each composed of two different subunits; one of these subunits is common to both ferritins. Acid-urea gel electrophoresis of heart ferritin demonstrated the existence of two subunits, designated H and HL, both of which are stable to DTT reduction. A similar analysis of liver ferritin also revealed the presence of the HL subunit, as well as other liver-specific subunits whose mobilities changed after DTT treatment. Although reduced L appears to comigrate with HL, we think that HL and L represent two unique primary structures. This conclusion is supported by the finding of more cyanogen bromide peptides from liver ferritin than would be expected from the assigned methionine levels of a single polypeptide chain (7), as well as from differences in amino acid composition of individual liver isoferritins (18).

The existence in heart and liver ferritins of different subunit types, with one type common to both ferritins, now provides a structural basis for the extensive heterogeneity and the characteristic isoferritin profiles found in mammalian tissue ferritins. It has recently been demonstrated that the isoferritins in horse spleen are hybrid molecules with different combinations of two dissimilar subunits (19,20). By analogy, a similar but more complex situation might account for the isoferritins from different human organs. We suggest that the most acidic isoferritins in heart may contain a high proportion of the H subunit with only a small proportion of the HL subunit. With increasing pI up to 5.3, the H type is replaced by the HL type so that heart isoferritins which are also found in liver might consist largely of the common subunit HL. In isoferritins of higher pIs, e.g. - pI 5.3-5.6, the HL subunit is gradually replaced by the liver subunit L, so the L type predominates in the least acidic liver isoferritins. This model will account for the overlap of the isoferritin profiles of different human ferritins and for differences in amino acid composition both between heart and liver ferritins and within liver isoferritins (11,18).

Our analyses to date suggest that the isoferritin spectrum in human

tissues is unlikely to contain all possible combinations of a random distribution of three dissimilar subunits. If one accepts the estimated molecular weight of the three subunits at around 19,000 and the multimeric shell as 440,000, one would expect 20-24 subunits per shell. If 2 dissimilar subunits are randomly distributed in the multimer,as is the case with lactate dehydrogenase, one would expect at least 2^{20} combinations. Clearly this heterogeneity is not present, or the various permutations are not resolved by our procedures of isoelectric focusing. If the former explanation holds, it seems likely that only a limited number of combinations exist in vivo.

The authors gratefully acknowledge the technical assistance of Mr. Dan Brooks, Ms. Heather Duram, and Ms. Joanne Hazard. This work was supported by grants AM-17775 and AM-16781 from the National Institutes of Health.

REFERENCES

1. Granick, S. (1951) Physiol. Rev. 31, 489-511.
2. Munro, H.N. and Drysdale, J.W. (1970) Proc. Fed. Amer. Soc. Exp. Biol. 29, 1469-1473.
3. Harrison, P. (1964) Iron Metabolism (F. Gross, ed.) pp. 40-56, Spinger-Verlag, Berlin.
4. Alfrey, C.P., Lynch, E.C., and Whitely, C.E. (1967). J. Lab. Clin. Med. 70, 419-428.
5. Gabuzda, T.G. and Pearson, J. (1968) Nature (London) 220, 1234-1235.
6. Linder, M.C. and Munro, H.N. (1973) Amer. J. Pathol. 72, 263-282.
7. Powell, L.W., Alpert, E., Isselbacher, K.J. and Drysdale, J.W. (1975) Brit. J. Haematol. 30, 47-55.
8. Drysdale, J.W. (1970) Biochim. Biophys. Acta 207, 256-258.
9. Urushizaki, I., Niitsu, Y., Ishitoni, K., Matsuda, M., and Fukuda, M. (1971) Biochim. Biophys. Acta 243, 187-192.
10. Drysdale, J.W. (1974) Biochem. J. 41, 627-632.
11. Powell, L.W., Alpert, E., Isselbacher, K.J. and Drysdale, J.W. (1974) Nature (London) 250, 333-335.
12. Alpert, E., Coston, R.C., and Drysdale, J.W. (1973) Nature (London) 242, 194-196.
13. Drysdale, J.W. and Singer, R.M. (1974) Cancer Res. 34, 3352-3354.
14. Fairbanks, A., Steck, T.L., and Wallach, D.F.H. (1968) Biochemistry 10, 2606-2617.
15. Harrison, P.M., and Gregory, D.W. (1968) Nature (London) 220, 578-579.
16. Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346.
17. Niitsu, Y., Ishitani, K., and Listowsky, I. (1973) Biochem. Biophys. Res. Commun. 55, 1134-1140.
18. Drysdale, J.W., Arosio, P. Adelman, T.G., Hazard, J.T., Brooks, D. and Duram, H. manuscript in preparation.
19. Drysdale, J.W., Hazard, J.T., and Righetti, P. (1975) Advances in Isoelectric Focusing and Isotachophoresis (ed. P. Righetti), in press, Elsevier Press.
20. Ishitani, K., Listowsky, I., Hazard, J. and Drysdale, J.W. manuscript submitted for publication.