

Structural and Immunological Relationships of Isoferritins in Normal and Malignant Cells¹

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SUMMARY

Ferritins from normal adult human liver and heart were compared with ferritins from a lung carcinoma metastatic to liver and from HeLa cells on the basis of their isoferritin profiles, subunit composition, and immunological relationships. Each ferritin preparation gave different isoferritin profiles, but several contained common isoferritins. All of the tumor isoferritins had counterparts in the normal tissues. All ferritins contained similar subunits but in different proportions. Qualitative differences were demonstrable in some ferritins with antibodies to different tissue ferritins. These differences correlated with the subunit composition of the ferritins. By appropriate absorption, an antibody population was obtained that was apparently specific for one subunit type. Heart ferritin gave lines of apparent identity with the tumor ferritins with these antibodies. It is concluded that tumor ferritins are not tumor-specific antigens but correspond to isoferritins in normal adult heart.

INTRODUCTION

Ferritin is an iron storage protein that occurs in probably all mammalian cells. Much of the body's ferritin is normally found in the liver, spleen, and hemopoietic tissues, but small amounts are also found in many other organs that have no major iron storage function (12). Ferritin exists in different molecular forms (isoferritins) in many tissues. At least 12 isoferritins have been found in human organs, each organ having its own characteristic isoferritin profile (8, 22). These isoferritins appear to represent populations of hybrid molecules consisting of different combinations of dissimilar subunits, so that the tissue-specific patterns seem to reflect tissue differences in the relative proportions of the different subunit types (1, 8).

Recent studies from this and other laboratories have demonstrated that the characteristic isoferritin profiles in human and animal tissues alter markedly in diseased states such as iron overload and cancer (8, 21, 24). For example, human hepatocellular (3), pancreatic, and mammary carcinomas (18) and HeLa cells (10, 24) have been found to contain isoferritins of a more acidic nature than are present in normal adult liver. Because similar isoferritins were also found in early fetal tissue, they were originally called carci-

nofetal isoferritins (3). This paper demonstrates that these isoferritins are not entirely specific to tumor or fetal cells but also occur in normal adult heart. These more acidic isoferritins common to heart, fetal, and tumor cells can be distinguished immunologically from the liver types, a finding that may prove useful in serodiagnostic tests involving these proteins.

MATERIALS AND METHODS

Animals. Male New Zealand rabbits and Hartley guinea pigs were obtained from Charles River Laboratories, Boston, Mass.

Ferritins. Ferritin was isolated from liver and heart of a normal adult male, from a liver infiltrated with metastases from a lung carcinoma, and from HeLa cells. The organs were obtained postmortem from the New England Medical Center Hospitals. Ferritin was also obtained from HeLa cells grown for 5 days in Eagle's minimum essential medium containing 20 μ g iron per ml as ferrous ascorbate (10).

Ferritin was isolated from these tissues as previously described (10, 22). The purity of all ferritins was confirmed by immunological and electrophoretic procedures (22). Some ferritins were further fractionated by chromatography on DEAE-Sephadex (18).

Antisera. Antibodies were raised in rabbits and guinea pigs against ferritins from normal adult human liver and heart and from HeLa cells. The liver ferritin was crystallized with CdSO_4 (12). All ferritins were injected intradermally at multiple sites, initially with Freund's complete adjuvant, then subsequently with Freund's incomplete adjuvant. A total of 1 and 2 mg ferritin from liver and heart, respectively, were injected into rabbits, and approximately 100 μ g were injected into guinea pigs. The antisera were shown to be specific for ferritin in crude tissue extracts (9).

Electrophoresis and Isoelectric Focusing. The ferritins were resolved into their constituent isoferritins by isoelectric focusing in slabs of high-porosity polyacrylamide gels cooled at 4° (7). Gels were stained for nonheme iron with potassium ferrocyanide and for protein by Coomassie brilliant blue. Estimates of pH gradients were made at 4°.

Subunit analysis of the ferritins was performed by electrophoresis in acid-urea gels (20) after dissociating the multimeric shell in 67% acetic acid (1). After electrophoresis, the gels were stained with Coomassie brilliant blue R-250. The relative amounts of the separated subunits were estimated from gel scans at 550 nm and from elution of the bound dye into pyridine-water (11).

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Double diffusion studies were performed in 1% agar plates in 20 mM phosphate buffer, pH 7.6, and 0.15 M NaCl.

RESULTS

Fig. 1 shows the isoelectric focusing profiles of ferritins from a normal adult human liver, a liver with metastatic lung carcinoma, normal adult heart, and HeLa cells (a cell line derived from a cervical carcinoma). Liver ferritin was resolved into about 6 isoferritins isoelectric between pH 5.1 and 5.6. Heart ferritin contained about 8 isoferritins but with a more acidic pI range (4.8 to 5.4). Some of the least acidic heart isoferritins corresponded to the more acidic forms of liver. These results are similar to previous analyses of other liver and heart ferritins (1, 8, 22). The ferritin from the lung carcinoma had a more acidic pattern than the liver ferritin. In addition to the liver isoferritins, this tissue contained some of the isoferritins found in heart but not liver. The HeLa isoferritins had the most restricted spectrum. They focused in the pI range of 4.8 to 4.9 and corresponded to the most acidic isoferritins found in the normal adult heart.

In order to explore further the possible structural relationships of these ferritins from normal and malignant cells, we examined their subunit compositions. It has been shown that the heterogeneity in tissue ferritins is related to the presence of different subunits whose relative distribution alters through the isoferritin spectrum (1, 13). Normal human liver and heart ferritins consist of at least 2, and possibly 3, different subunits. Two subunit types, HL and L, predominate in liver and spleen, while a 3rd type, H, is characteristic of the more acidic isoferritins in heart. The H and HL types seem to represent different gene products, but the character of the L type has not yet been established (1). Fig. 2 shows that all 4 ferritin preparations examined contained both the H and HL subunit types but no detectable L type. The apparent absence of the L-type subunit is to be expected, since these analyses were performed in the presence of dithiothreitol which would cause any L subunits to comigrate with the HL type (1). No subunit other than those present in normal liver or heart was found in the tumor tissue or in the HeLa cells. Table 1 shows the relative proportions of the H and HL subunits in all 4 ferritin preparations. The HeLa ferritin, which had the most acidic spectrum, contained about 70% H and 30% HL. As the pI range of the isoferritins becomes less acidic, the proportion of H dropped in the order HeLa > heart > tumor > liver. The liver ferritin contained only about 10% of the H subunit.

The 4 ferritin preparations were further characterized by

Table 1
Subunit compositions of ferritins

Ferritins were dissociated into their constituent subunits which were then separated by gel electrophoresis (1) and quantitated by densitometric scanning of the stained gel.

	H	HL
Liver	10	90
Tumor	15	85
Heart	40	60
HeLa	70	30

examining their immunological relationships with antibodies elicited in rabbits and guinea pigs against different parts of the isoferritin spectrum. For these studies we used crystalline liver ferritin, which contains the least acidic isoferritins, and heart and HeLa ferritins, which contain the most acidic isoferritins found so far. When tested in double diffusion with rabbit antibodies to crystalline human liver ferritin, all 4 ferritin populations gave lines of apparent identity (Fig. 3, A and B). However, they appeared to differ quantitatively in their reactivities. For example, the line formed against HeLa ferritins was weaker than that given by an equivalent amount of liver ferritin. These results suggested that all 4 ferritin populations had antigenic determinants in common but differed in relative content of these determinants. A somewhat similar result was obtained with rabbit antibodies elicited against the most acidic isoferritins in heart (Fig. 3C), all ferritins giving lines of apparent identity.

We next compared the 4 ferritins with antibodies elicited against the HeLa ferritins in guinea pigs. Figs. 4A and 5A show the double-diffusion patterns obtained with ferritins from HeLa, heart, tumor, liver, and a subfraction of liver which consisted largely of the most basic isoferritins. All ferritins, with the exception of the most basic isoferritins, gave 2 precipitin lines. The outer line was strongest with HeLa and heart ferritins. As the pI range of the ferritins becomes more basic, the relative intensity of the outer to the inner line decreased in the order HeLa > heart > tumor > unfractionated liver > basic liver. The lines that appear to be spurs extending from the heart and basic liver precipitin lines across the major HeLa band are an artifact from the HeLa ferritin diffusion. This is shown in Fig. 5A by the fact that they apparently stained much more heavily for iron than the precipitin line given by the most basic liver isoferritins. The major inner line was given by all ferritins, and the ratio of this line to the outer line decreased in the reverse order. A minor line was also evident from the heart ferritin with the unabsorbed antiserum. This line fused with the major inner line given by all other ferritins. The major inner line and the outer line appeared to be distinct from one another but indistinguishable from the corresponding lines in other ferritins. This result suggested that one population of antibodies recognized common antigenic determinants on all ferritins, while another population recognized determinants in HeLa and heart ferritins that were either absent or present only in trace amounts in the basic liver isoferritins. These immunological differences were made more apparent by absorbing the guinea pig anti-HeLa ferritin antiserum with the most basic liver isoferritins. Figs. 4B and 5B show that the remaining antibodies no longer precipitated the most basic liver isoferritins but did recognize and precipitate substantial amounts of heart and HeLa ferritins as well as small amounts in the tumor and liver. The precipitin line corresponded to the outer line given with the unabsorbed serum.

DISCUSSION

It is now well established that many human and animal tumors contain ferritin populations that differ from those in

normal adult liver cells and that elevated levels of serum ferritin are often associated with cancers. These observations are of interest in studies of the regulation of cell phenotype, possible tumor markers, and in possible radioimmunotherapy (2-6, 10, 14-16, 18, 19, 27). Consequently, it is important to define the structural and immunological relationships of ferritins in normal and malignant cells. The present comparisons of ferritin populations from normal and malignant human tissues indicate that the more acidic, nonhepatic isoferritins found in several cancers and early fetal tissues are not specific to cancer or fetal tissues but also occur in some normal adult tissues. This conclusion derives from our findings that the more acidic tumor isoferritins focus in the same position as isoferritins in normal adult heart, contain similar subunit types, and have immunological properties that are similar to the more acidic isoferritins in heart. Small amounts of similar isoferritins also occur in normal adult pancreas and kidney (22). An analogous situation occurs with other "carcino-fetal" isozymes. For example, the liver isozymic patterns of glycogen phosphorylase and aldolase change in hepatoma to forms found in fetal liver. However, these nonhepatic forms also occur in other normal adult tissues (25, 26). From these and other studies, it appears that the more acidic isoferritin profiles in cancer and fetal tissues (3, 10) represent a greater proportion of the H-type subunit than is present in normal adult liver. Since this subunit is present in only small amounts in normal liver, it may correspond to the apparently new form found by Alpert (2) in ferritins from hepatoma but not normal liver.

Because the isoferritin profile is largely determined by the relative amounts of the heart and liver subunit types, there may be a considerable range in isoferritin populations in cancer tissue. In some, such as the tumor studied here, the pattern may be only slightly more acidic than that of normal liver, whereas in others the pattern may be more like that of heart and HeLa cells. Furthermore, since the HeLa ferritins studied here contained about 30% of the HL subunit, it seems likely that even more acidic isoferritins may occur if ferritin molecules can be made with lower levels of the HL subunit. Conceivably, the degree of substitution with H subunit may correlate with the degree of malignancy of the tumor. Thus, the progressive increase in the electrophoretic mobilities of ferritins from a graded series of Morris hepatomas (16) may be explained by a corresponding increase in the amount of an H-type subunit.

The immunological relationships of the various ferritins studied here indicate that the different proportions of H and HL subunits result in different antigenic structures which give rise to distinct antibody populations. The double-diffusion patterns in Fig. 4 indicate that the inner of the 2 lines is formed to molecules consisting largely of the HL type while the outer line is formed to ferritins rich in the H type. By absorbing the guinea pig antiserum produced against HeLa ferritin with the most basic liver isoferritins, we have obtained antibodies that seem to recognize only those isoferritins with high levels of the H subunit type.

The structural and immunological relationships of tissue ferritins bear on recent interest in serum ferritin as a possible tumor marker and as an index of body iron stores.

Normally, most of the body's ferritin is found intracellularly. However, small but significant amounts are also found in serum. The levels of serum ferritin, or β -fetoprotein as it has been called (4), usually reflect the amount of iron stores, but grossly elevated levels are found in several cancers where there is no clear correlation with iron stores or tissue damage (4-6, 15, 19). Although the source of the serum ferritin in many cases is not known, Powell *et al.* (23) have recently shown that the characteristic shift in isoferritin profiles in hepatoma is also reflected in the serum ferritin profile. By contrast, in normal persons and in patients with iron overload, the serum ferritin is derived from liver-type isoferritins. Thus, although ferritin may not be a tumor-specific antigen, it may nevertheless be a useful tumor marker since most of the body's ferritin seems to be of the liver type.

The immunological differences in tissue ferritins are particularly pertinent in considering quantitation of serum ferritin levels. Our results suggest that the values found will presumably depend on both the source of the serum ferritin and the specificity of the antibodies for the various isoferritins. In most procedures, serum ferritin is quantitated by radio-immune assays utilizing antibodies raised against liver or spleen ferritins (14, 17). Such assays may, therefore, considerably underestimate the amount of heart-type isoferritins in serum. For example, we have found that in competitive systems in which the serum ferritin competes with labeled liver ferritin for a limiting amount of liver ferritin antibodies, the heart or tumor forms may be underestimated by 2 orders of magnitude. Conversely, by using a system based on the HeLa ferritins and an appropriately absorbed anti-HeLa antiserum such as that described here, it is possible to selectively quantitate the more acidic isoferritin populations in heart and tumors (M. Yokota, P. Arosio, J. T. Hazard, and J. W. Drysdale, unpublished data). Such assays might allow a clear distinction between elevated serum ferritin in iron overload or liver damage and in cancer.

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Fig. 1. Isoelectric focusing profiles of human ferritins from normal adult liver (*L*), liver with metastatic lung carcinoma (*T*), normal adult heart (*H*) and HeLa cells (*He*) in the pH range 4 to 6 in polyacrylamide gel. Prussian blue stain for nonheme iron.

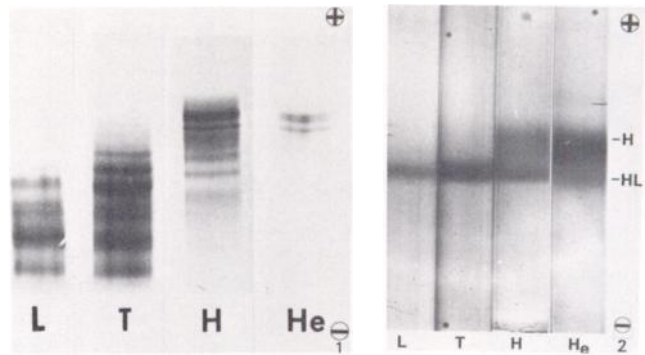


Fig. 2. Acetic acid-urea electrophoresis of ferritin subunits. Same abbreviations as in Fig. 1. Subunit nomenclature as in Ref. 1. Coomassie brilliant blue stain for protein.

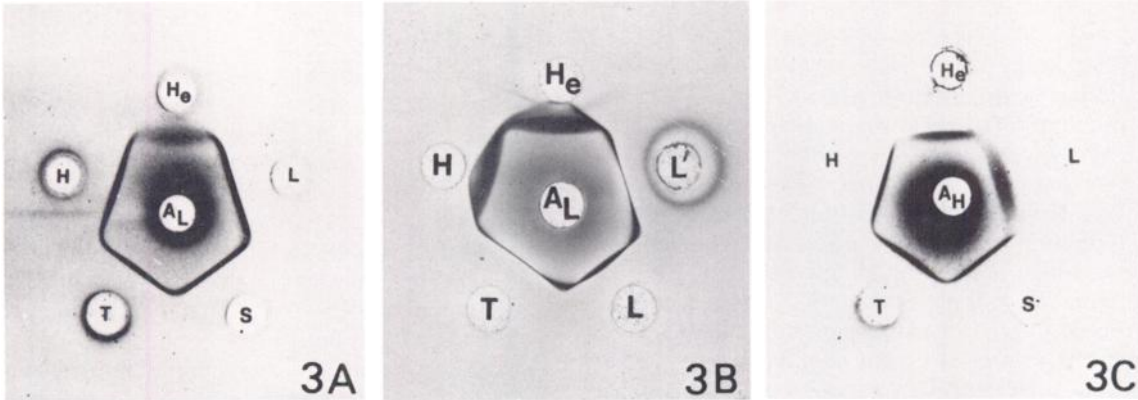


Fig. 3. A, double diffusion of human ferritins from liver (*L*), spleen (*S*), liver with metastatic lung carcinoma (*T*), heart (*H*), and HeLa cells (*He*) against rabbit antibodies to crystalline human liver ferritin. Outer wells contained approximately 5 μ g ferritin. B, as in A, except that 5 times more HeLa and heart ferritins were used to enhance the precipitin lines, and the most basic liver isoferritins (*L'*) were substituted for spleen ferritin. C, as in A, except the rabbit antibodies were raised against the most acidic heart isoferritins.

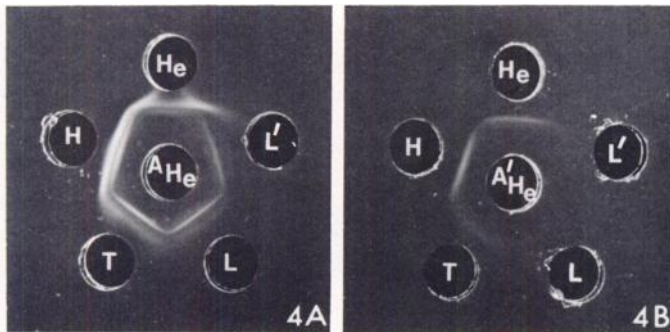


Fig. 4. Same as in Fig. 3B, except that the antiserum is to HeLa ferritin in guinea pigs (*A_{He}*); A, original antiserum; B, same antiserum absorbed with the most basic liver isoferritins *A'_{He}*. Direct photograph.

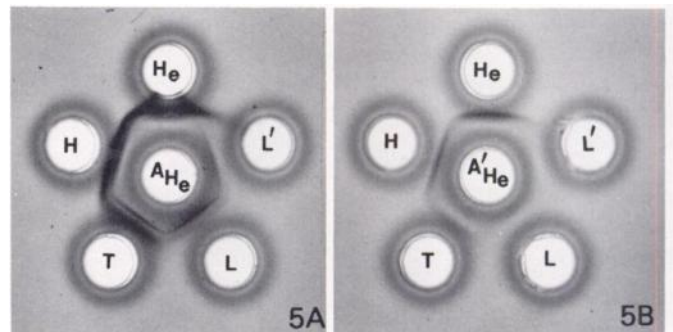


Fig. 5. Gels from Fig. 4 after Prussian blue stain for iron.