Hypothesis

A ROLE FOR FERRITIN IN THE REGULATION OF IRON METABOLISM

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In normal human subjects some 25% of the total body iron is present in ferritin and the closely related compound haemosiderin. Ferritin itself consists of a protein shell of diameter 120Å surrounding a ferric hydroxyphosphate core of variable size which occupies the interior of the protein (diameter 70Å). The amount of iron present in the core varies from zero up to a maximum of about 4 300 iron atoms/molecule. The iron free protein shell (apoferritin) can be isolated by chemical reduction of the iron, or by centrifugal techniques, and consists of 24 identical polypeptide chains of molecular weight 18 500 (for a more detailed account of ferritin structure see [1, 2]. There are a number of characteristics of ferritin which suggest that it would be well suited to play a key role in the regulation of iron metabolism. In the present article I would like to draw attention to these features of ferritin and to propose a model for the function of this protein in the regulation of iron metabolism.

The results of recent studies on ferritin together with earlier data are not incompatible with the view that ferritin might be of major importance in regulating iron metabolism within the cell. These observations are briefly reviewed below:

Iron deposition in ferritin is catalysed by apoferritin itself [3–5]. The oxidation of Fe^{2+} to Fe^{3+} in the presence of suitable electron acceptors such as molecular oxygen is catalysed by apoferritin with concomitant formation of ferritin. Under the same conditions a number of other proteins do not have any effect on the rate of Fe^{2+} oxidation [5]. The mobilisation of ferritin iron does not occur at physiologically significant rates with any of a number of low molecular weight reducing or chelating agents commonly found in mammals are used, except when concentrations well in excess of those found *in vivo* are employed [6]. Likewise, the enzyme xanthine oxidase, which had been suggested as the site of ferritin iron reduction [7], does not release iron from ferritin at appreciable rates even under conditions where the oxidation of hypoxanthine by the enzyme is proceeding very rapidly [6]. Evidence has been found for an NADH-dependent flavoprotein in mammalian liver which can reductively mobilise Fe^{2+} from ferritin: the name ferriductase has been proposed for this enzyme [8]. We have also observed such an enzyme activity in rat liver [6]. Thus the existence of two separate enzymic pathways for iron deposition and mobilisation respectively seems to be well established. At least in principle therefore regulation of these two enzyme activities could operate to control the balance between iron deposition and release.

The synthesis of ferritin is also subject to control. Thus, the administration of iron, either *in vivo* or to cells in culture, leads to a specific acceleration of apoferritin synthesis (reviewed, for example in [1, 9]. The effect of iron on apoferritin synthesis seems to be at the level of translation [9–11] and can be demonstrated in liver, kidney, heart, mucosa and in reticulocytes as well as in HeLa cells. Further, it has been shown that the type of ferritin synthesised in liver cells changes with development [12], ferritin from young rats being similar electrophoretically to that of adult kidney and spleen, in marked contrast to adult liver ferritin. In a great many cases it has been found that normal ferritins are replaced by electrophoretically distinct forms in cancer cells [13–15].

That tissue-specific isoferritins occur has long been postulated: the first definitive proof of such organ specific ferritins in human and horse liver and spleen has been recently presented [16]. Although ferritin

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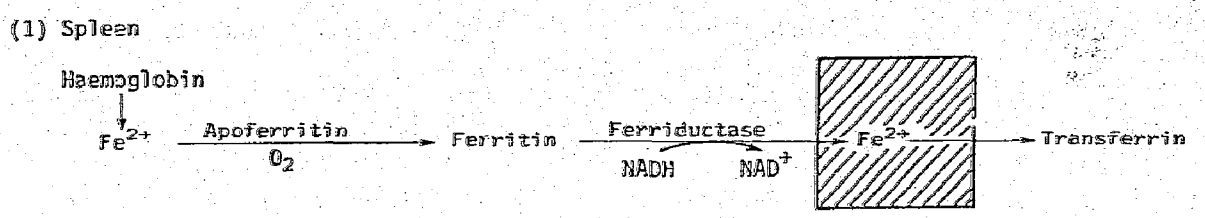

Fig. 1. The role of ferritin in regulation of iron metabolism in spleen cells. Iron released from hemoglobin breakdown is oxidised by apoferritin and stored in ferritin. Mobilisation of ferritin iron is mediated by the enzyme ferriductase, which may be membrane bound. The Fe^{2+} then passes across the cell membrane to transferrin.

occurs in the greatest amounts in liver, spleen and bone marrow, the tissue distribution of the protein seems to be ubiquitous [17]. It is also found in serum, and a significant correlation is apparent between the serum ferritin level and the state of body iron stores [18]. The distribution of ferritin in nature is extensive, ranging from mammals and invertebrates through plants and molluscs to fungi (reviewed in [2]).

Finally it should be pointed out that ferritin is implicated in the regulation of iron absorption from the gut, and may also serve as a precursor of haem iron in the reticulocyte. Although some controversy surrounds the role of ferritin in iron absorption, recent studies have shown that a considerable amount of iron passes through the ferritin of the mucosal cell within a few hours of administration of tracer doses of iron in normal rats [19, 20].

One final point should be made before proceeding to the model. While it is clearly possible that a small pool of iron may occur within cells as low molecular weight complexes, such iron must be in a transient state from one protein-bound form to another, otherwise hydrolysis and polymerisation of such iron leading to micellar ferric chelates would occur [21].

The model postulates that iron entering cells from transferrin is reduced by an enzyme system in the cell membrane and passes into the cells, either associated with a carrier molecule, or else by interaction with membrane bound ferritin. The Fe^{2+} is oxidised to Fe^{3+} by apoferritin with concomitant formation of ferritin. Iron may be mobilised from ferritin within the cell as required by ferriductase, as Fe^{2+}. Such intracellular mobilisation would be used to supply iron for haem synthesis, and for non-haem iron-containing enzymes. The mobilisation of iron for "export" (i.e. for transfer out of the cell to transferrin) may be carried out by the association of ferritin with a membrane-bound form of ferriductase, which mediates the transfer of Fe^{2+} to transferrin either itself, or via some carrier mechanism. Whether the Fe^{2+} must first be oxidised to Fe^{3+} prior to its binding to transferrin, or whether it may bind directly with subsequent oxidation on the transferrin molecule is not clear [22, 23]. The application of the model to account for regulation of iron metabolism in erythroid cells, spleen cells, liver cells and in cells of the intestinal mucosa is illustrated in figs. 1–4. Thus, in erythroid cells incoming iron would pass through ferritin on its way to haem. However, if the requirement for iron were considerable it is conceivable that incoming Fe^{2+} could be used directly for haem synthesis, thus bypassing the ferritin iron pool. There is a clear analogy between

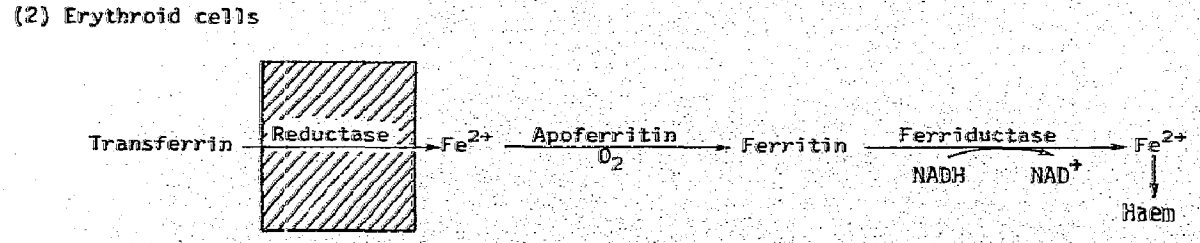

Fig. 2. In erythroid cells iron is removed from transferrin by a putative reductase in the cell membrane, and the Fe^{2+} is oxidised by apoferritin (which may be membrane bound) with formation of ferritin. The release of ferritin iron brought about by ferriductase supplies the requirements for haem synthesis.

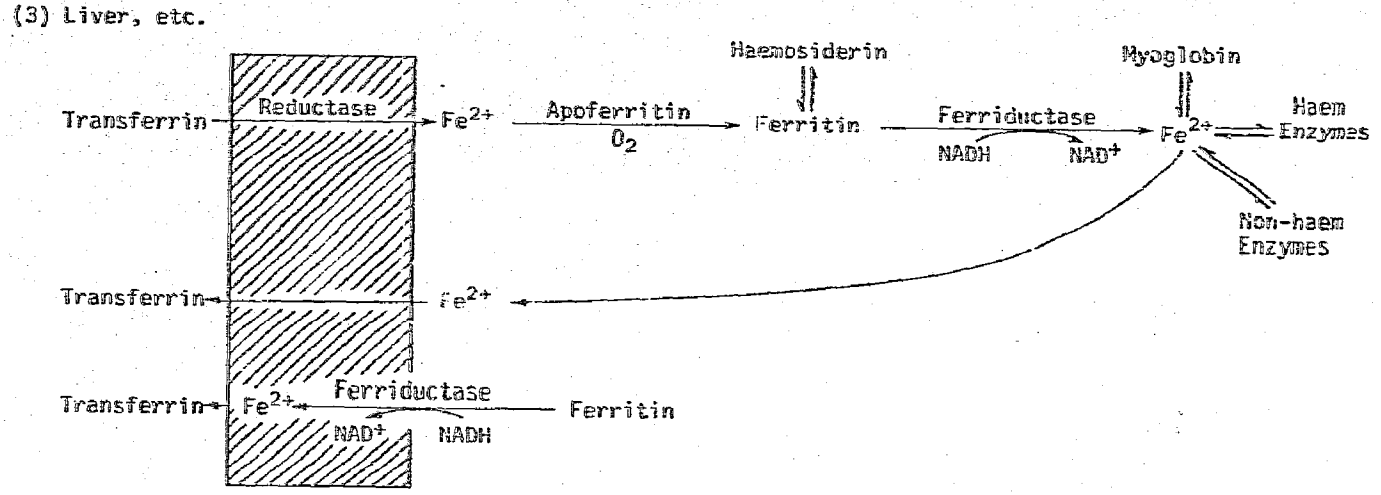

Fig. 3. In cells such as liver and spleen incoming iron from transferrin is stored in ferritin, as outlined for spleen cells (fig. 1). It can be mobilised as required for the synthesis of haem, non-haem enzymes and for haem enzyme synthesis, and iron released from the breakdown of such iron-containing proteins is subsequently incorporated into ferritin by the action of apoferritin. Iron release from such sources may also pass directly to transferrin, or else may occur via a membrane bound ferriductase. Ferritin iron may also be stored as haemosiderin.

the regulatory mechanisms operative in spleen and in mucosal cells. Both receive iron within the cell as Fe^{2+}, and both cells export a large part of their ferritin iron. An alternative pathway for iron transfer from mucosal cells is indicated: there is recent evidence for a transferrin-like iron carrier in mucosal cells that seems to be of great importance in iron absorption in iron deficient animals [19, 20]. The liver cells operate a system which is a composite of the system in erythroid and spleen cells, with both soluble ferriductase for haem and non-haem iron protein synthesis, as well as a membrane bound ferriductase for export of iron.

There is one further alternative to the present model which could be considered, namely that transferrin iron was reduced by a membrane bound redox-system that could also reduce ferritin iron on the inner side of the membrane. This would dispense with the requirement for membrane bound ferriductase, but such an enzyme system would be more difficult to regulate.

The present model makes a number of predictions, all of which are amenable to rigorous analysis. All cells should contain ferritin and ferriductase. The distribution of ferriductase within the cell should vary with cell type: thus spleen and mucosal cells may possess a membrane-bound ferriductase, whereas reticulocytes should have an exclusively cytoplasmic enzyme. In liver, muscle, etc., the ferriductase may occur in both free and membrane-bound forms. Further, the model predicts that the tissue specificity of ferritins

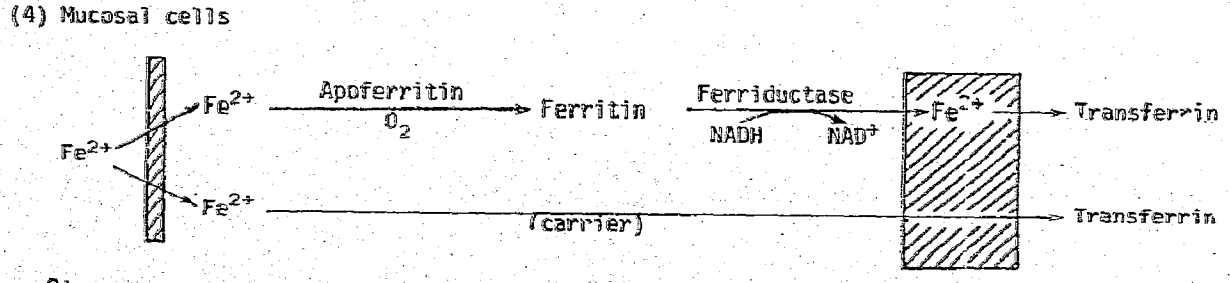

Fig. 4. Fe^{2+} entering mucosal cells from the gut may pass to transferrin through oxidation by apoferritin and reduction by ferriductase (which, as in spleen cells may be membrane bound), or may, in phases of rapid absorption, pass directly to transferrin bound to an intracellular carrier (which may be an intracellular form of transferrin).

reflects distinct functional properties: erythroid cell ferritin would be adapted to take up Fe^{2+} from the cell membrane whilst mucosal and spleen ferritins could more readily take up free Fe^{2+} in the cytoplasm. In contrast, the erythroid ferritin would be adapted to releasing iron to soluble ferriductase, whilst the mucosal and spleen ferritins would be best suited for iron mobilisation at the cell membrane. Perhaps the most striking prediction of the present model is that ferritin iron is used within the cell for the synthesis of all iron-containing proteins, such as haemoglobin, myoglobin, etc.

The model presented here would imply that intracellular iron metabolism can be regulated by the activities of apoferritin in the oxidation of Fe^{2+} and of ferriductase in the mobilisation of Fe^{2+} from ferritin. That such a mechanism could operate to regulate iron metabolism should become clear from further research.

Acknowledgements

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