SUBCELLULAR LOCALISATION OF IRON DURING ACCELERATED FERRITIN SYNTHESIS

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1. Introduction

It is now generally accepted that the locus for the induction of ferritin synthesis by iron is post-transcriptional [1–3]. The mechanism has been advanced that iron binds to a specific cyto-plasmic repressor molecule, thereby removing the inhibition and allowing the synthesis to proceed at an increased rate [2]. This hypothesis invites experimental verification by attempting to label the repressor with radioiron, if a means could be devised of preferentially directing the isotope and of distinguishing repressor labelling from that of ferritin and other proteins unrelated to ferritin biosynthesis.

This paper describes experiments in which the subcellular distribution of ^{59}Fe in rat liver has been measured. Since the maximum rate of ferritin synthesis (corresponding to maximum repressor binding to iron) occurs 5 h after administration of iron [4], radioactivity was measured after this time interval. In addition, radioactive labelling associated with ferritin synthesis was determined by comparing the results in two groups of rats, one given isotope only (Group I), and the other (Group II) given the same dose of isotope along with sufficient iron to stimulate ferritin synthesis at the optimal rate. The time course of iron labelling in one subcellular fraction was also studied.

2. Materials and methods

Protein was estimated by the method of Lowry et al [5]; RNA by the orcinol method of Schneider [6].

Iron for injection was prepared by adding 0.4 ml $FeCl_3$ solution or water to 1 μCi of $^{59}FeCl_3$ (Radiochemical Centre) contained in 0.4 ml. The final concentration of iron in the former solution was 1 mg/100 g rat body weight. This dosage produces the optimal response in the rate of amino acid incorporation into ferritin [4]. The quantity of iron in the tracer dose alone (5–20 μg) is insufficient to cause a significant rise in ferritin synthesis. Before administration into muscle, the iron was reduced to Fe^{II} by adding 0.05 M ascorbic acid dropwise until the brown colour of Fe^{III} was discharged.

The rats were killed and exsanguinated 5 h after administration of iron. The livers were removed immediately and placed in 3 vol (w/v) of 'TKM' buffer, pH 7.40, at 0°–2°C (0.005 $MgCl_2$ 0.025 M KCl, 0.05 M Tris–HCl) for homogenisation using a piston homogeniser. Since uniformity of homogenisation is essential for reproducible cell fractionations [7], the same piston and container were used for all homogenisations. All subsequent steps were at 0–2°C.

The homogenate was subjected to a simple fractionation into nuclear, mitochondrial-lysosomal, microsomal, and supernatant fractions by differential ultracentrifugation [7] as shown in fig.1. In later experiments, free and membrane-bound ribosomes were prepared as shown in fig.2 [8]. It is important to note that (a) the ribosomes were prepared using sucrose solutions containing ribonuclease inhibitor extracted from rat liver [9] thus reducing monosome formation during sedimentation; (b) the method gives quantitative preparation of ribosomes [9], but that there may be up to 20% contamination of free ribosomes by bound ribosomes which have become detached during preparation, and conversely up to 7% of free ribosomes fail to sediment and

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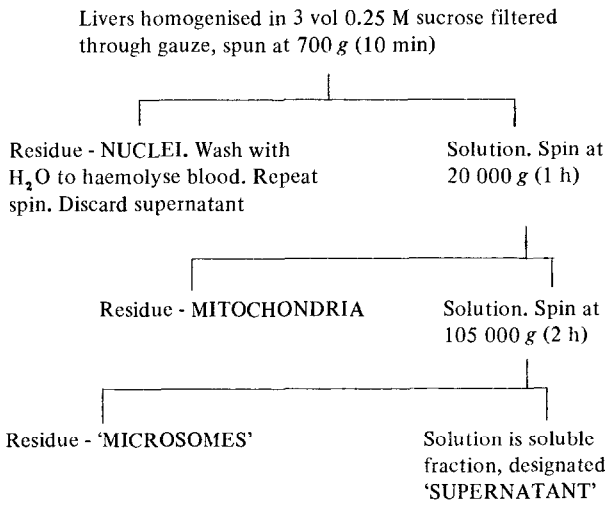

Fig.1. Scheme of liver cell fractionation into 'nuclear', 'mitochondrial', 'microsmal', and 'supernatant' fractions. All operations were carried out at 0–2°C [7].

therefore contaminate the 'bound' fraction [10]; and (c) electron microscopy shows the free ribosome preparations to be uncontaminated by ferritin (Dr R. F. Macadam, personal communication).

The time course of radioiron incorporation into free and bound ribosomes was measured by administering radioiron with added iron as above (modified by the use of iron–sorbitol–citrate 'Jectofer' rather than ferric chloride, and omitting the reduction step) and sacrificing the animals in groups of three 0.5, 1, 3, 5, 8, 12, (or 16), and 24 h later.

^{59}Fe activity was measured using a Nuclear Enterprises 8312 spectrometer (S.E. ± 2%).

Ferritin was assayed immunologically as previously described [2].

3 Results and discussion

The distribution of radioactivity in the four subcellular fractions analysed are given in table 1. There was a significant difference in the distribution of radioactivity, most marked in the microsomal fraction which was greater in Group II rats, i.e., those with increased rates of ferritin synthesis.

The possibility that this difference was merely due to increased incorporation of radioactive iron into newly synthesised ferritin sedimenting with the micro-

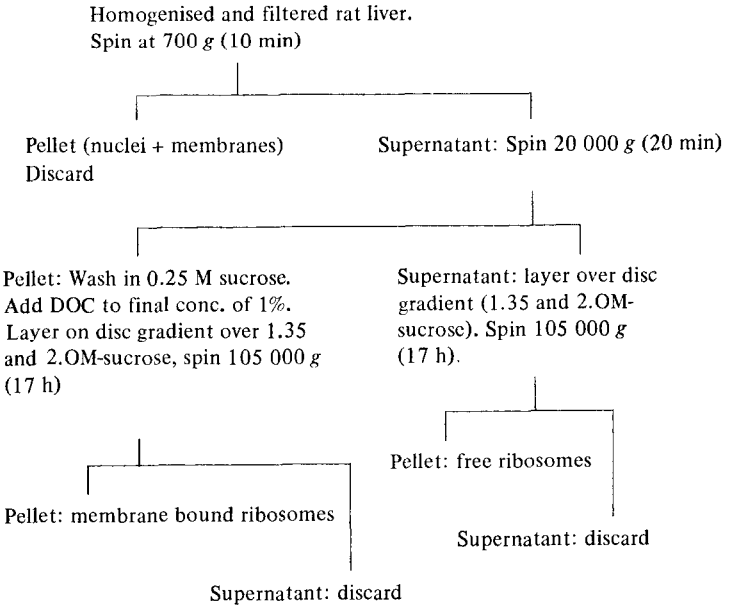

Fig.2. Preparation of free and membrane-bound ribosomes. Heavy sucrose solutions contained a ribonuclease inhibitor, as described [9]. All stages were carried out at 0°–2°C. DOC = deoxycholate.

Table 1
Subcellular distribution of ^{59}Fe in two groups of rats as described in the text

Fraction	^{59}Fe radioactivity (% total liver activity)	
	Group I: ^{59}Fe only n = 4	Group II: ^{59}Fe + 1 mg Fe/100 g b.w. n = 4
Nuclear	7 ± 5	8 ± 3
Mitochondrial/lysosomal	10 ± 5	11 ± 3
Microsomal	38 ± 11	57 ± 8
Supernatant	35 ± 8	21 ± 10
% recovery	90	97
Ferritin activity (n = 2)	33.7	30.7

Results are given as mean ± S.D. The difference in microsomal radioactivity is significant ($p < 0.001$).

somal pellet was tested by precipitating ferritin specifically using immune serum and measuring ^{59}Fe activity in the precipitate. No difference between the two groups was noted (table 1).

The distribution of ^{59}Fe between free and membrane-bound ribosomes is shown in fig.3. The total (free + membrane-bound) radioactivity was greater in Group II rats, thus confirming the previous results. 90% of the increased microsomal radioactivity in group II was found to be associated with the free ribosomes. Free ribosomal activity was greater than that of the membrane-bound ribosomes in both groups, but the difference was greater in the stimulated animals.

Time course studies confirmed that there is a maximum of ^{59}Fe radioactivity associated with the free ribosomes occurring 5 h after iron administration (fig.4). It is of interest that the pattern of ^{59}Fe binding to free ribosomes almost exactly parallels the incorporation of [^{14}C]leucine into ferritin after iron administration [4,11]. A similar result has been found in the

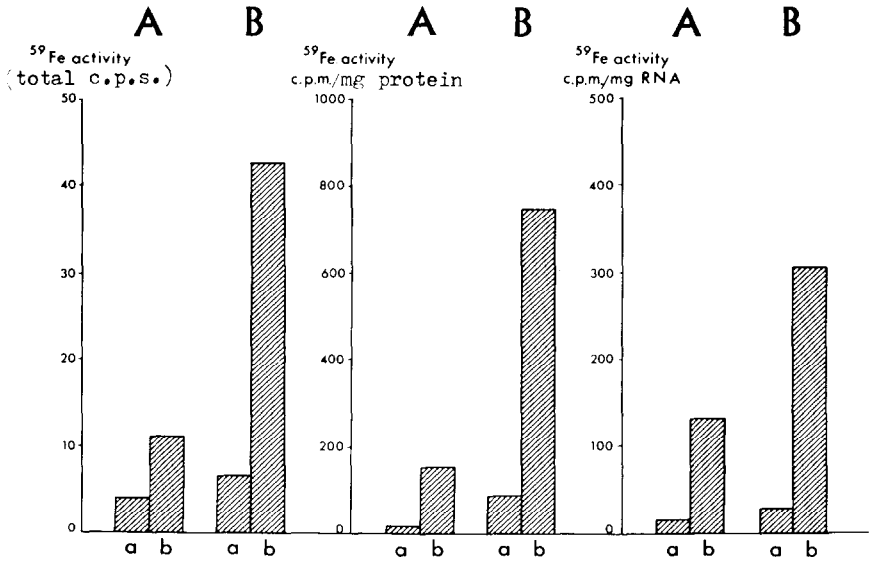

Fig.3. ^{59}Fe activity in free and membrane-bound ribosomes from rat liver. (A) group I; (B) group II; (a) bound ribosomes; (b) free ribosomes.

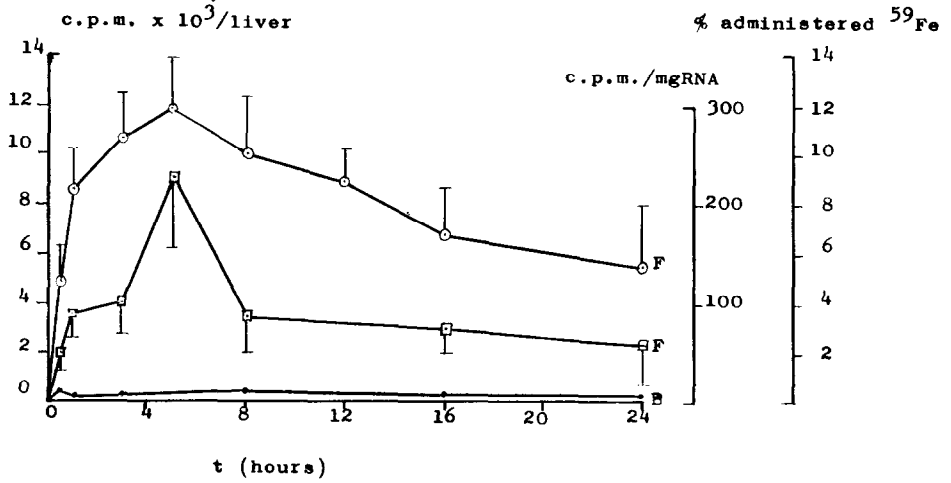

Fig.4. Time course of ^{59}Fe incorporation into free (F) and bound (B) ribosomes from rat liver. (⊙——⊙) total cpm/liver, n = 6 (mean + S.D.); (⧈——⧈) cpm/mg ribosomal RNA n = 3 (mean − S.D.).

microsomal fraction from intestinal musocal cells of rats after administration of iron orally [12]. These cells are also active in ferritin synthesis [13].

These results show that the subcellular distribution of iron alters in conditions of varying rates of ferritin synthesis. The principle used here is that differences in labelling under these conditions are likely to be related to the synthesis of apoferritin. Thus the similar levels of radioactivity found in the nuclear and mitochondrial/lysosomal compartments in groups I and II suggest that this labelling, although perhaps meaningful, is unrelated to ferritin synthesis. On the other hand, the significantly increased radioactivity found in the microsomal fraction in the iron-stimulated rats is more likely to be related to the biosynthetic process.

The possibility that it was due to increased uptake into ferritin was excluded.

That the bulk of the microsomal radioactivity is associated with the free ribosomes is in accord with in vitro experiments with cell-free protein synthesising systems which show increased [^{14}C]leucine incorporation into ferritin produced by free ribosomes. In these systems membrane-bound ribosomes have diminished capacity to incorporate [^{14}C]leucine into ferritin; in the present study they showed decreased uptake of ^{59}Fe also [8,14]. The results from cell-free systems have to be interpreted with caution [15]. There is some evidence that both free and bound ribosomes synthesise ferritin peptide chains [16]. Linder et al. showed that separate subunit peptides are synthesised by each class of ribosome [17]. However they found in rat liver that the effect of iron was to increase disproportionately the rate of subunit synthesis by the free ribosomes.

The model of ferritin synthesis under test is that iron stimulates synthesis by binding with a specific repressor molecule in the cytoplasm, thus allowing an increased rate of synthesis. Kinetic considerations would demand that such a molecule should have a high rate of turnover relative to ferritin and a low molecular weight. The findings here are consistent with this model, although they do not exclude alternative explanations such as binding or iron to nascent subunit polypeptides on the ribosome. More detailed analysis of free ribosomes using the comparative labelling technique described here should be productive in further elucidiation of the ferritin–iron system.

Acknowledgements

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