The effect of iron on ferritin turnover in rat liver

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It has previously been reported that in rat liver the rates of synthesis and degradation of ferritin labelled with [^{14}C]leucine or arginine are dependent on the iron status of the rats. It has also been concluded that isoferritins differ markedly in their rates of turnover. Here we provide experimental evidence which shows that the rate of degradation of rat liver ferritin (labelled by injection of [^{14}C]bicarbonate to minimise problems of reutilisation) is unaltered by repeated iron injection, although ferritin synthesis is stimulated. Our data also strongly suggest that the proposed differential degradation of isoferritins is improbable.

Ferritin *Isoferritin* *Iron* *Turnover*

1. INTRODUCTION

Although iron is required for a variety of metabolic processes, it is also a potentially toxic element. The widely distributed protein, ferritin, in which up to 4500 Fe(III) atoms can be stored inside a protein shell, provides both a reserve of this element and a means of avoiding the toxic effects of unutilized iron [1]. Ferritin and iron are mutually interactive: iron administration leads to an increase in tissue ferritin levels and hence more molecules are made available to remove the excess iron [1,2]. It is well established that a response to iron injection in rats is an immediate de novo stimulation of ferritin biosynthesis in the liver [2]. The observation that the ferritin pool continues to build up for several hours after a single iron dose could result from the combined effects of increased synthesis and a decrease in the rate of ferritin degradation. Such a decrease has been observed when the rate of decay of ferritin-associated radioactivity in rats receiving multiple iron injections was compared with that in control rats [2,3] or in rats which hd been made anaemic by bleeding [3]. However in the earlier experiments the protein was labelled with [^{14}C]leucine or [*guanidino*-^{14}C]arginine. It has been established [4,5] that use of such labelled amino acid precursors may yield seriously underestimated rates of degradation of liver proteins especially under conditions of increased protein synthesis (as in the case of ferritin when iron is administered). In the experiments reported here we have labelled rat liver ferritin by injection of $NAH^{14}CO_3$, since fixation of its radioactive carbon into amino acids (principally argininine, glutamic and aspartic acids) occurs primarily in the liver and reutilisation of labelled amino acids derived from extra hepatic tissue is minimised [4,5]. We have followed the same regime of iron injections as in [2,3], but have used immunoprecipitation for the isolation of ferritin from homogenates [6], and radial immunodiffusion [7] for its measurement, thus avoiding the more conventional purification procedures used previously [2,3] in which losses may occur. We find no effect of iron on the rate of degradation.

On isoelectric focussing ferritin has been shown to separate into several bands or 'isoferritins' [8]. Iron administration leads to a change in the focussing profile of rat liver ferritin with an increase in the more basic bands and a decrease in the more acidic bands as well as an overall increase in ferritin concentration [9,10]. Authors in [9] using a double isotope method concluded that the more

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acidic isoferritins turn over faster than the more basic isoferritins and that this difference was accentuated by iron treatment. We have shown [11] that double isotope methods are inappropriate to measurement of isoferritin turnover because of transfer of label between them. Here we confirm the shift to a more basic electrofocussing profile on iron loading, but suggest that this is unlikely to result from differential rates of degradation.

2. MATERIAL AND METHODS

2.1. *Animals and injections*

Locally bred female laboratory rats in the weight range 193 ± 10 g were used throughout and were given access to food and water. Each group consisted of 4 animals. For iron administration ferric ammonium citrate (green form, BDH, Atherstone) adjusted to pH 7.2 in 0.9% (w/v) NaCl was used, each rat receiving 400 μg Fe/100 g body wt at each injection. When isotope was administered each rat received 800 μCi of $NaH^{14}CO_3$ (spec. act. 58 mCi/mmol, Radiochemical Centre, Amersham). Iron and isotope were given by intraperitoneal injection.

2.2. *Ferritin purification*

Ferritin was prepared according to [12] except that the heat supernatant was concentrated in an 8MC ultrafiltration cell of 400 ml capacity with a PM-10 membrane (Amicon, High Wycombe) and applied directly to a Sepharose 6B column (Pharmacia, Uppsala).

Preparative isoelectric focusing was carried out as in [11].

2.3. *Ferritin analysis*

Ferritin samples were analysed by radial immunodiffusion against rabbit anti-horse spleen ferritin antiserum [7]. The standard was a purified rat liver ferritin the concentration of which had been determined by amino acid analysis [13].

All ferritin samples were counted after precipitation of 50 μg with rabbit anti-horse spleen ferritin antiserum. The cpm were corrected to dpm using a ferritin quench curve.

To calculate the amount of ^{14}C-activity in ferritin the homogenates were treated with 1% deoxycholate/1% Triton X-100 and ferritin measured by radial immunodiffusion to give total ferritin/liver. Ferritin (50 μg) was then precipitated from the homogenate with antiserum, washed and centrifuged through a sucrose gradient to remove any adhering non-specific precipitate [6].

2.4. *^{14}C-activity in total liver protein*

Liver homogenate (0.25 ml) was heated for 5 min at 80°C with 1 ml of 0.01 N NaOH. The protein was then precipitated with 5% trichloroacetic acid, washed twice and redissolved in 0.5 N NaOH. It was then assayed for protein and for radioactivity.

3. RESULTS AND DISCUSSION

Table 1 shows that, as in [2,9,10], ferritin/liver is increased in rats receiving single or multiple doses of iron as compared with rats on standard diets. Again, similar to [2,9] a single iron dose administered 2 h prior to $NaH^{14}CO_3$ caused increased incorporation of radioactivity into ferritin as shown by the higher levels of total and specific ac-

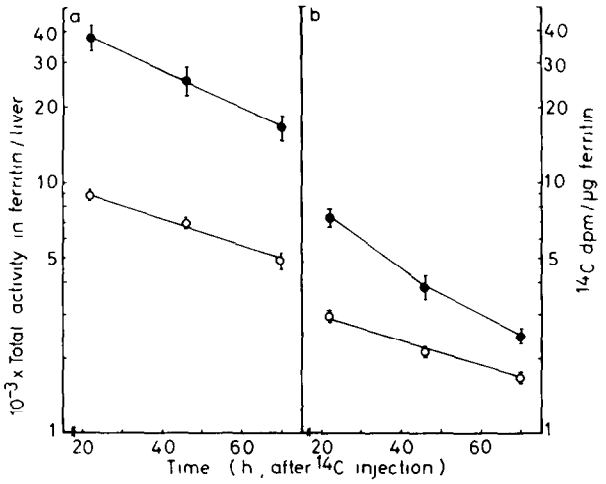

Fig. 1. Decay in ^{14}C-total activity (a) and specific activity (b) after $NaH^{14}CO_3$ injection. Rats were each injected with 800 μCi $NaH^{14}CO_3$ and killed at the specified times after labelling. (○), control rats; (●), iron-treated rats (Exp. 1, table 1). All iron-treated rats received 400 μg Fe/100 g body wt 2 h before the bicarbonate labelling. The first group was killed 22 h after $NaH^{14}CO_3$ administration. The second group received a further two iron injections at 22 and 34 h after labelling and killed at 46 h and the third group received iron injections at 22, 34 and 46 h and killed at 70 h after labelling. Each point represents a group containing 4 rats.

Table 1

Recovery of ferritin protein and ^{14}C-activity from rat livers

	Normal rats			Iron-treated rats				
				Expt. 1			Expt. 2	
Time (h) after ^{14}C	22	46	69	22[a]	46[b]	70[c]	46[b]	70[c]
mg Fe/100 g body wt	0	0	0	0.4	1.2	1.6	1.2	1.6
mg ferritin/liver	2.97	3.23	2.92	4.74	6.03	6.25	6.65	7.65
μg ferritin/g liver	407	434	387	639	874	950	1012	1108
^{14}C dpm/μg ferritin	2.98 ± 0.10	2.13 ± 0.06	1.68 ± 0.13	7.37 ± 0.58	3.84 ± 0.40	2.44 ± 0.14	3.55 ± 0.15	1.97 ± 0.02
$\frac{^{14}C \text{ dpm}/\mu\text{g ferritin}}{\text{total liver protein}}$	3.18	3.10	3.30	5.04	3.52	2.80	4.50	3.39
Ferritin dpm as % total liver protein dpm	0.64	0.72	0.65	1.50	1.45	1.44	2.50	2.13

All groups of rats were given single injection of $NaH^{14}CO_3$, and killed at times shown after administration of ^{14}C. Results represent average values for 4 rats. Those in the iron-treated groups were given Fe injections (400 μg/100 g body wt) at: [a]0 h, [b]0, 24 and 36 h, [c]0, 24, 36 and 48 h, the initial injection (0 h) being 2 h before $NaH^{14}CO_3$

tivities in these animals as compared with controls (fig. 1). However, unlike [2,3], no reduction in the rate of degradation was apparent in the iron-injected rats. Radioactivities followed an exponential decay unlike those reported for iron-treated rats in [2,3]. Hence, for the preparation as a whole; degradation appears to be a random process, unaffected by iron-loading.

Table 2

Rat liver isoferritin distribution in response to iron administration

Iso-ferritin	pH	Iron administered (mg/100 g body wt)							
		0[a]		0.4[b]		1.2[c]		1.6[d]	
		μg ferritin/liver	%	μg ferritin/liver	%	μg ferritin/liver	%	μg ferritin/liver	%
2	4.83	74 ± 28	2.5 ± 1.0	79 ± 3	1.7 ± 0.1	99 ± 50	1.7 ± 0.8	87 ± 47	1.3 ± 0.7
3	4.95	490 ± 217	17.0 ± 7.5	190 ± 28	4.2 ± 0.6	220 ± 177	3.8 ± 3.0	211 ± 114	3.1 ± 1.7
4	5.04	745 ± 128	25.8 ± 4.4	564 ± 27	12.4 ± 0.6	741 ± 294	12.8 ± 5.1	763 ± 68	11.3 ± 1.0
5	5.14	779 ± 139	27.0 ± 4.8	1216 ± 264	26.8 ± 5.8	1309 ± 310	22.6 ± 5.4	1788 ± 419	26.4 ± 6.2
6	5.25	466 ± 157	16.2 ± 5.4	1624 ± 58	35.7 ± 1.3	2452 ± 941	42.4 ± 163	3006 ± 1000	44.4 ± 14.8
7	5.32	239 ± 63	8.3 ± 2.2	631 ± 37	13.9 ± 0.8	770 ± 120	13.3 ± 2.1	757 ± 162	11.2 ± 2.4
8	5.44	88 ± 19	3.0 ± 0.6	137 ± 11	3.0 ± 0.2	185 ± 47	3.2 ± 0.8	155 ± 14	2.3 ± 0.2

The isoferritins were prepared as described in section 2. Although visible, isoferritin band 1 was present in a very small amount and is not included in the table

[a] Average of 7 experiments

[b] Rats were given a single iron injection of 400 μg Fe/100 g body wt and killed 24 h later. Average of 2 experiments

[c] Rats were given 3 iron injections of 400 μg Fe/100 g body wt at 0, 24 and 36 h and killed at 48 h. Average of 3 experiments

[d] Rats were given 4 iron injections of 400 μg Fe/100 g body wt at 0, 24, 36 and 48 h and killed at 72 h. Average of 2 experiments.

As expected for a steady-state situation the rates of synthesis (fall in specific acitivities, fig. 1b) and degradation (fall in total activities, fig. 1a) are the same in the untreated rats. However in rats receiving iron the rate of decay in specific activity is greater than that in total activity. This implies that the rate of synthesis exceeds that of degradation. Our results therefore suggest that the increase in ferritin/liver in response to iron results solely from increased synthesis and not from decreased degradation.

Table 2 shows that when ferritin is subjected to preparative isoelectric focussing the distribution of protein among the isoferritin bands is dependent on the iron status of the rats, a shift to the basic being observed on iron-loading as noted previously [9,10]. Unfortunately we cannot measure rates of degradation or synthesis of the individual isoferritins, because of the complex distribution of radioactivities observed among them, with some isoferritins gaining label, as found earlier [11] and again here. However we can conclude that differential rates of degradation are unlikely for the following reasons. Table 2 shows that in control rats bands 4 and 5 account for more than half of the total ferritin population, while band 6 is only 16%. After 3 iron injections band 6 increases to 42% and band 4 decreases to only 13% of the ferritin pool and this distribution is maintained after the fourth injection. Nevertheless the rate of degradation of the whole ferritin pool remains unaltered. Thus it seems that the change in isoferritin composition must result either from alteration in the isoferritins being synthesised or from an accentuation by iron of the movement of ferritin from acidic to basic observed in control rats [11]. Our recent unpublished results suggest that both processes contribute to the observed effect.

The double-labelling data of [9,10] indicated that within any given isoferritin subunits turn over at the same rate and therefore ferritin molecules are degraded as whole units. This conclusion is difficult to reconcile with the apparently widely divergent degradation rates [9] of isoferritins differing only to a small extent in subunit composition. If, as we suggest, all isoferritin molecules degrade at the same rate in rat liver, this problem is avoided.

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