MOBILISATION OF IRON FROM FERRITIN FRACTIONS OF DEFINED IRON CONTENT BY BIOLOGICAL REDUCTANTS

Janine DOGNIN and Robert R. CRICHTON

Max Plank-Institut für Molekuläre Genetik, Berlin-Dahlem, BRD and Unité de Biochimie, Université Catholique de Louvain, Louvain-la Neuve, Belgium

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1. Introduction

Ferritin contains from 0–4300 atoms of ferric iron which are present as a ferric oxyhydroxyphosphate core located in the interior of the apoferritin protein shell. The iron can be released by reduction, for example with sodium dithionite [1] or with thioglycolate [2]. In the cell ferritin iron seems likely to be mobilised by a reductive system, which may involve $FMNH_2$ [3]. It is of interest to establish the nature of the biological reductant which is involved in iron release from ferritin and to analyse the effect of the iron content of the molecule on the rate of iron release. Recent reports have indicated that O-phenanthroline mobilises ferritin iron at different rates from ferritins of different iron contents [4]. We report here a survey of the rates of ferritin iron reduction by a number of biological reducing agents and confirm that there are important differences in the rates of iron reduction from ferritins of different iron content.

2. Materials and methods

Horse spleen ferritin was obtained from Serva (Heidelberg, BRD) and was fractionated by isopycnic density gradient centrifugation [5] in gradients of CsCl of initial density 1.545 using an SW65 rotor at an average centrifugal force of 182 000 for 24hr. Fractions of 200 μl were removed successively from the top of the gradients with a Carlsberg pipette and were subsequently pooled to give a series of ferritin fractions of different iron content. After dialysis against H_2O and subsequently against Tris–HCl buffer, 100 mM, pH 7.4 they were analysed for their protein and iron content. Protein concentrations were determined by a modified Lowry method [6] and by amino acid analysis with norleucine as internal standard. Iron concentration was determined after reduction with thioglycolate (1% w/v) by reaction with α,α'-bipyridyl. The iron contents of the ferritin fractions used varied from 49 atoms/molecule to 3390 atoms/molecule.

The effectiveness of a series of biological reductants and chelators in the mobilisation of iron from unfractionated ferritin was compared by dialysis of a solution of ferritin containing 1500 μg of iron against 10 vol of the appropriate concentration of chelator and/or reductant in the same buffer at room temperature. After suitable time intervals aliquots of the dialysate were removed and the concentration of Fe^{2+} determined with α,α'-bipyridyl.

Mobilisation experiments were carried out aerobically at room temperature. The different ferritin fractions in Tris–HCl buffer 0.1 M pH 7.4 were diluted with the same buffer to obtain the desired concentration in iron or in protein and 0.5 ml of this solution was added to 0.5 ml of a 0.5% w/v solution of α,α'-bipyridyl in 0.1 M Tris–HCl buffer pH 7.4. The mobilisation was then initiated by addition of 10–50 μl of a 0.1 M cysteine solution to obtain a final concentration of 0.001M-0.005M. The optical density of the solution at 520 nm was then determined at suitable intervals of time. In some experiments the cysteine was replaced by glutathione or ascorbate and in one series of measurements no reductant was added and the mobilisation due to α,α'-bipyridyl itself was measured.

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3. Results and discussion

In a survey of potential biological reducing and chelating agents which might be involved in mobilisation of ferritin iron we tried the following: ascorbic acid, cysteine, glutathione, NADH, NADPH, glucose, fructose, glycine, succinic acid, citric acid, AMP, ADP, ATP. The results for the most promising are given in table 1. From these results several conclusions may be drawn. The most effective mobilising agent is cysteine, followed by ascorbic acid; glutathione is a very poor reductant of ferritin iron. This is in agreement with earlier results of Mazur et al. [7] and Sirivech et al. [3]. Yet at concentrations of 5 mM mobilisation of only 12–15% of the iron content is obtained in 24 hr at room temperature. It does not seem likely that such reducing agents or complexants play a major role in ferritin mobilisation in vivo. By comparison, with $FMNH_2$ reduction of ferritin iron is extremely rapid (R. R. Crichton, M. Wauters and F. Roman unpublished work and [3]) 100% of the iron being reduced by 0.2 mM $FMNH_2$ in 10 min [3]. The effect of addition of chelating agents together with reducing agents did not increase the rate of iron release appreciably. We also observed

Table 1
Mobilisation of ferritin iron by biological reductants and chelators

Reductant/chelator and concn.	% Iron mobilised in function of time (hr)				
	5	24	48	72	168
Ascorbate 5 mM	3.3	11.9	18.7	23.9	34.8
25 mM	6.7	30.6	51.4	63.0	85.2
100 mM	14.0	45.4	72.4	89.5	102.1
500 mM	24.6	60.4	84.9	97.8	101.0
Cysteine 1 mM	1.6	8.5	17.3	22.2	35.1
5 mM	4.7	15.3	28.4	37.1	56.0
Glutathione 5 mM	2.1	7.0	13.5	18.3	28.0
Bipyridyl	0.7	2.4	3.5	–	–
Fructose	–	3.5	5.3	8.0	14.0

Samples of ferritin (unfractionated) containing 1500 μg of iron were dialysed as described in the text with reductants and chelators. The percentage of iron released into the dialysate as a function of time is given in the table.

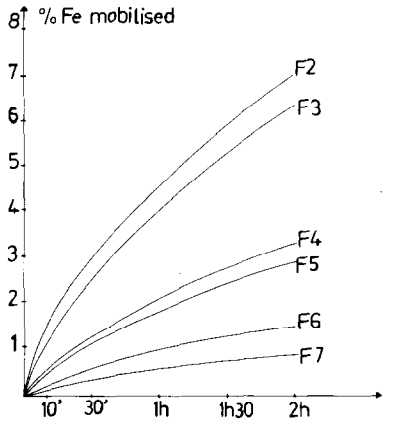

Fig.1. Mobilisation of iron from ferritins of different iron content at constant iron concentration by 5 mM cysteine. The iron content of the different fractions (atoms/molecule of protein) was 423, 762, 1170, 1440, 1989, 3390 for F2–F7 respectively.

that there was no direct relation between rate of iron reduction and concentration of reductant. Thus for example for cysteine, on going from 10^{-3} M to 5×10^{-3} M, there is an increase in the rate of mobilisation over the first 2 hr of 2–2.5-fold, which indicates that the kinetics of iron mobilisation are dependent on the square root of the concentration in reductant.

With a series of ferritins of different iron content we followed the mobilisation at constant iron and at constant protein concentration using cysteine, ascorbic acid, glutathione and also bipyridyl alone. Fig.1 shows the results for six ferritin fractions with 5 mM cysteine at a constant iron content of 10^{-3} M. Similar results were obtained for the other reductants. Fig.2 shows the same effect with bipyridyl alone at a concentration of 1.6×10^{-3} M. The mobilisation is slower, but again the most rapid release of iron is from the fraction of lowest iron content.

In marked contrast is the effect of iron content on the mobilisation at constant protein concentration. Fig.3 shows a typical result for 5 mM cysteine where the concentration in protein was constant for each fraction at 10^{-4} M. In general fractions of intermediate iron content (800–1400 iron atoms/molecule) released their iron more rapidly than molecules of lower or higher iron content. In the course of a series of

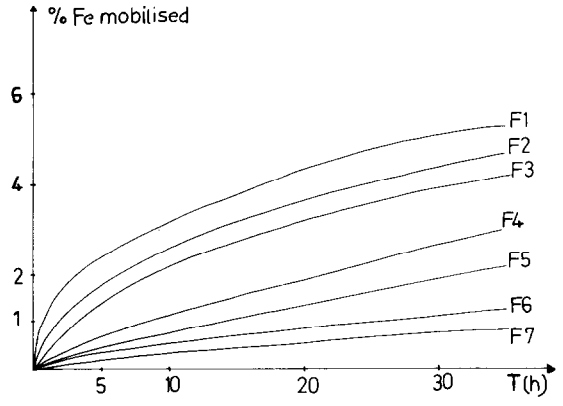

Fig.2. Mobilisation of iron from ferritins of different iron content at constant iron concentration by 16 mM α,α'-bipyridyl. The iron content was as in fig.1: F1 contained 49 atoms of iron/molecule of protein.

experiments we found that the fractions of 1200–1400 iron atoms gave most rapid reduction of iron.

These results confirm the observations of Hoy et al. [4] obtained with *o*-phenathroline as photosensitive reductant. The greater percentage of ferritin iron mobilised in samples of low iron content is in accord

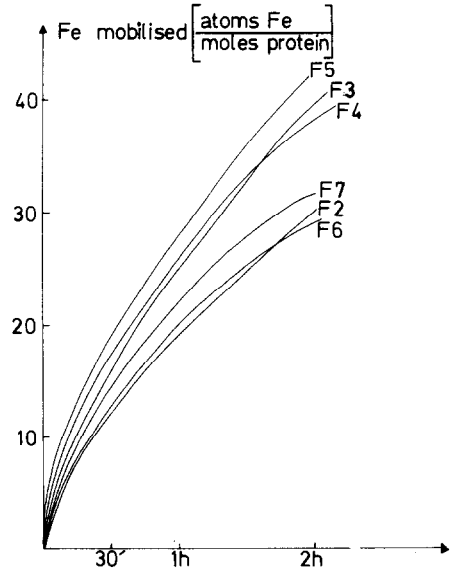

Fig.3. Mobilisation of iron from ferritins of different iron content at constant protein concentration by 5 mM cysteine. The iron content of the fractions was as in fig.1.

with suggestions that there is an equilibrium between core iron and small quantities of iron on the surface, or in inter-subunit channels, of the protein [7,8]. However, to explain the observation that the number of atoms of iron mobilised per molecule of protein is maximal for ferritins containing about one-third of their total iron capacity is less easy. Hoy et al. [4] have proposed a model for the shape of the iron core in which a reasonable correlation is obtained between rate of release of iron and the calculated micelle surface area. This model does not however seem to account for the correlation between iron content and percentage of iron mobilised (figs.1 and 2). We would suggest that a more likely explanation is that there are two mechanisms whereby iron can be mobilised using reductants namely mobilisation from redox sites in the interior of the protein shell or, perhaps more plausibly, in the channels between subunits, and mobilisation by direct reduction of iron on the surface of the micelle. Whether the latter mechanism plays a role in the mobilisation of ferritin iron by reduced flavin mononucleotide remains to be established.

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