ISOLATION AND CHARACTERISATION OF RAT MUCOSAL FERRITIN

Helmut HUEBERS, Eiko HUEBERS and Robert R. CRICHTON

Institut für Pharmakologie und Toxikologie der Universität des Saarlandes 655 Homburg (Saar), Germany and Unité de Biochimie, Université de Louvain, Place Louis Pasteur 1, 1348 Louvain-la-Neuve, Belgium

Received 13 May 1974

1. Introduction

That ferritin, the major iron storage protein of mammals, occurs in mucosal cells has been known since the classic experiments of Granick [1] who showed that after feeding of a large dose of iron to guinea pigs, ferritin could be crystallised from mucosal cell extracts with cadmium sulphate. Although experiments by Brown and Rother [2] failed to demonstrate ferritin in particle-free supernatant of rat intestinal mucosal cells after administration of a physiological dose of iron, recent publications [3–9] confirm Granick's findings, even after the administration of tracer doses of iron. No agreement has been reached concerning the role of ferritin in iron absorption. Experiments of Huebers [3] and Pollack [6] indicate that, in normal and in iron deficient animals, in the first few minutes of iron absorption mucosal ferritin takes up iron without delivering it to the blood. In contrast, a second non-ferritin iron-binding protein, seems to be important in the rapid transfer of iron to the blood, particularly in iron deficient animals.

In the present communication we report the isolation and characterisation of rat mucosal ferritin.

2. Materials and methods

The starting material for the isolation of mucosal ferritin was obtained by scraping off the mucosal tissue of 50 rat duodenums and jejunums (length: 20 cm, starting from the pylorus). From animals which had been treated with ^{59}Fe [1] the wet weight obtained was 32.3 g containing 5.52 g protein as measured by the Kjedahl method, with crystalline serum albumin as standard. The tissue was suspended in 10 vol of ice-cold 1.12% aqueous KCl-solution and homogenized in a mechanically driven glass/Teflon homogenizer at 0°C at 1000 rpm and 3 strokes per minute for 3 min. The homogenate was centrifuged at 3000 *g* for 20 min and the pellet, consisting mainly of brush-borders and nuclei, was discarded. The supernatant containing up to 25% of the initial protein present in the homogenate was subjected to a heat denaturation step at 73°C for 10 min, cooled thereafter immediately in an ice bath and allowed to remain at this temperature for 12 hr. A centrifugation step at 3000 *g* for 10 min allowed a good separation between the heat denaturated proteins and the clear supernatant. The supernatant (final volume 300 ml) was applied to a Sephadex G 25 column (5.0 × 120 cm) and eluted with 0.05 M triethanolamine-buffer (pH 7.1). The ^{59}Fe-containing fraction which eluted in the void volume of the Sephadex column was applied to a column (2.5 × 40 cm) of DEAE-cellulose (Whatman DE 23) equilibrated with the same buffer. Elution was performed with a salt gradient of 0.05 M triethanolamine-buffer pH 7.1 and the same buffer containing 1 M NaCl. The ^{59}Fe-containing fractions were collected and concentrated to a final volume of 2 ml using a Diaflo XM-100 filter (Amicon, Lexington) in a micro-ultrafiltration cell (Amicon, type 8 MC). The concentrate was applied to a column of Sephadex G-200 (2.5 × 90 cm), equilibrated with 0.05 M Tris–HCl-buffer containing 1 M NaCl. The final step of purification of the ^{59}Fe-labelled fraction was achieved by gel chromatography on Sepharose 6B (1.5 × 180 cm) equilibrated with 0.05 M Tris–HCl-buffer containing 1 M NaCl. The yield was 2–4 mg of the pure protein.

Polyacrylamide gel electrophoresis was carried out as described in [3]. Sedimentation velocity analyses

North-Holland Publishing Company – Amsterdam

and polyacrylamide gel electrophoresis in sodium dodecyl sulphate were as in [10]. Amino acid analyses were performed on a Locarte acid analyser (Locarte Co, London, U.K.) after hydrolysis for 16 hr at 110°C in 6 N HCl.

3. Results and discussion

On gel chromatography of the heat denaturated particle free supernatant of rat mucosal cells on Sephadex G25 only one radioiron peak coming immediately at the void volume is observed. This confirms the results of previous papers that ^{59}Fe in the particle free supernatant of rat intestinal mucosal cells is bound by protein [3–5]. On ion exchange chromatography on DEAE-cellulose (Whatman DE 23) one iron-binding fraction is eluted at pH 8.0 and a salt concentration of 0.14 M NaCl (fig. 1). Further purification of this ^{59}Fe-fraction on Sephadex G200 (fig. 2) reveals that there is not yet coincidence of the radioiron and the protein peak indicating that the iron-binding protein is not pure. This fraction is contaminated by a protein with greater Stokes radius than the ^{59}Fe-protein as estimated by gel chromatography on Sepharose 6B (fig. 3). It should be mentioned that this protein was not found in liver and spleen preparation of the rat. As demonstrated by disc-electrophoresis on SDS-gel and by the sedimentation pattern in the analytical ultracentrifuge the preparation of ^{59}Fe-protein (mucosal ferritin) was pure.

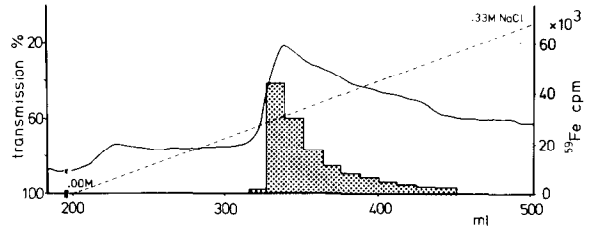

Fig. 1. Ion exchange chromatography of ^{59}Fe-labelled protein fraction obtained from the heat denaturated particle free supernatant of rat mucosal homogenate after Sephadex G25 chromatography. *Conditions:* Column 2.5 × 40 cm, DE 23 cellulose equilibrated with 0.05 m Tris–HCl buffer pH 8.0; elution by a linear salt gradient (OM–0.33 M NaCl) in the same buffer. Fraction volume 12 ml;

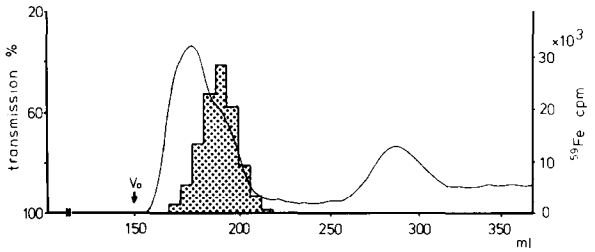

Fig. 2. Gel chromatography of the ^{59}Fe-labelled protein fraction from intestinal mucosal tissue obtained after ion-exchange chromatography (DE 23) on Sephadex G-200. *Conditions:* Column: 2.5 × 90 cm, gel equilibrated with 0.05 m Tris–HCl buffer (containing 1 M NaCl), pH 8.0; fraction volume: 6 ml; void volume: 150 ml.

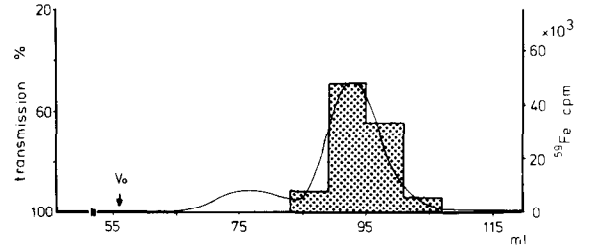

Fig. 3. Gel chromatography on Sepharose 6 B of the ^{59}Fe-protein fraction from mucosal tissue of rats after gel chromatography on Sephadex G-200. *Conditions:* Column: 1.5 × 90 cm, gel equilibrated with 0.05 m Tris–HCl buffer (containing 1 M NaCl), pH 8.0; fraction volume: 6.0 ml void; volume: 56 ml.

The mucosal ferritin gave a single band on polyacrylamide gel electrophoresis at pH 8.1 and 5.4. The isoelectric point is approximately pH 4.9 (Huebers, H., Huebers, E. & Crichton, R. R., in preparation). Sedimentation velocity analysis of the apoferritin (prepared by reduction of the iron as described in [9]) gave a sedimentation constant (uncorrected for protein concentration) of 17.5 S. Polyacrylamide gel electrophoresis in sodium dodecyl sulphate revealed a single band of mol. wt. 19 200.

The amino acid composition of the rat mucosal ferritin is given in table 1. For comparison we have included the amino acid composition of horse spleen ferritin. It is clear that the composition of the mucosal ferritin differs markedly from that of the horse spleen protein, although the percentage of non polar amino acids remains constant.

Table 1
Amino acid composition of rat mucosal ferritin and horse spleen apoferritin. The results are expressed as mole residues of each amino acid per mole of protein.

	Rat mucosal ferritin	Horse spleen apoferritin
Aspartic acid	21.2	17.3
Threonine	8.4	5.5
Serine	11.8	9.0
Glutamic acid	24.2	23.9
Proline	5.6	2.8
Glycine	11.6	9.9
Alanine	13.6	14.0
Valine	7.8	6.9
Methionine	2.0	2.8
Isoleucine	19.1	25.0
Tyrosine	5.1	5.0
Phenylalanine	5.8	7.3
Histidine	6.5	7.3
Lysine	10.4	8.7
Arginine	7.6	9.5

The content of cysteine and tryptophan has not been determined for the rat protein.

In conclusion we have demonstrated the presence in normal rat mucosa of ferritin, which has a similar quaternary structure to that of other ferritins isolated from mammals, and which can be labelled in vivo with physiological doses of radioiron. Recent comparative studies (Huebers, H., Huebers, E. and Crichton, R. R. in preparation) have established that rat mucosal ferritin differs in its primary structure from the ferritin of rat liver and spleen.

References

[1] Granick, J. (1946) J. Biol. Chem. 164, 737–748.
[2] Brown, E. B. and Rother, M. L. (1963) J. Lab. Clin. Med. 62, 357–373.
[3] Huebers, H., Huebers, E., Forth W. and Rummel, W. (1971) Life Sci. 10, 1141–1148.
[4] Huebers, H., Huebers, E., Forth, W. and Rummel, W. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 1156–1158.
[5] Worwood, M. and Jacobs, A. (1971) Life Sci. 10, 1363–
[6] Pollack, S., Campara, T. and Arcarcio, A. (1972) J. Lab. Clin. Med. 80, 322–332.
[7] Halliday, J. W. and Powell, L. W. (1973) Clin. Chim. Acta 43, 267–276.
[8] Yoshino, Y. and Manis, J. (1973) Am. J. Physiol. 225, 1276–1281.
[9] Sheehan, R. G. and Frenkel, E. P. (1972) J. Clin. Invest. 51, 224–231.
[10] Crichton, R. R., Millar, J. A., Cumming, R. L. C. and Bryce, C. F. A. (1973) Biochem. J. 131, 51–59.