Biochimica et Biophysica Acta, 601 (1980) 572–583

BBA 78945

THE UPTAKE OF IRON BY RETICULOCYTES

THE INFLUENCE OF PURIFICATION OF THE GHOSTS ON IRON-CONTAINING COMPONENTS IN THE GHOST SUSPENSION

C. VAN DER HEUL [a], M.J. KROOS [b], C.M.H. DE JEU-JASPARS [b] and H.G. VAN EIJK [a]

Institutes of [a] Haematology and [b] Chemical Pathology, Medical Faculty, Erasmus University, Rotterdam (The Netherlands)

(Received April 10th, 1980)

Key words: Iron; Transferrin receptor; Ghost purification; (Rat reticulocyte)

Summary

1. Rat reticulocytes previously incubated with (^{59}Fe, ^{125}I)-labelled transferrin were hemolysed to yield labelled ghosts.

2. The solubilized ghosts can be fractionated, by gel filtration on Sepharose 2B and 6B, into several ^{59}Fe- and ^{125}I-containing compounds, classified as A, B_1 and B_2.

3. These fractions were prepared from ghosts which were obtained at different centrifugation rates and further purified by sucrose density gradient centrifugation in order to obtain membrane compounds purified from mitochondrial and lysosomal impurities. The influence of these purification steps on the appearance and the ^{59}Fe/^{125}I activity of the three components was investigated.

4. The first Sepharose 2B fraction with high molecular mass, greater than 10^6 daltons, is an intracellular product of mitochondrial and lysosomal origin which precipitates with the membrane fractions during the preparation of the ghost. The first Sepharose 6B fraction, B_1, with $M_r \sim 10^6$ seems to be a real membrane component. The second Sepharose 6B fraction, B_2, with $M_r \sim$ 230 000 represents a real membrane receptor for transferrin.

Introduction

It is known that immature red cells are able to take up iron from the plasma iron-binding protein, transferrin, in order to utilize it for heme synthesis [1,2]. The initial step in this process is the binding of transferrin to specific receptors on the cell membrane, but the successive steps of the mechanism by which the

iron is delivered to the mitochondria have not yet been elucidated [3—5]. The idea that the transferrin molecule, after binding to the membrane receptors, enters the cell by a temperature and energy-dependent process, probably pinocytosis, has received considerable support [6—10]. However, the original idea that iron is removed from transferrin at the membrane receptor and donated to membrane and cytoplasmic carriers has not been completely rejected [11—14]. The last theory is based partly on the fact that the $^{59}Fe/^{125}I$ ratio of isolated ghosts and membrane receptors, both obtained from reticulocytes previously incubated with (^{59}Fe, ^{125}I)-labelled transferrin, is increased when compared to the $^{59}Fe/^{125}I$ ratio of the transferrin in the incubation solution [11,12,16,18].

Gel filtration of solubilized ghosts on Sepharose 2B resulted in two iron-containing fractions with $M_r > 10^6$ and $M_r \sim 10^6$, classified in the literature as A and B, respectively [12,13,17,18].

Refiltration of component B on Sepharose 6B resulted in one peak containing only ^{59}Fe activity, classified as B_1 ($M_r \sim 10^6$) and in another peak, heterogeneous in composition, containing a large proportion of the ^{59}Fe activity and nearly all ^{125}I activity. This component was classified as B_2 ($M_r \sim 230\,000$) and contains the receptor for transferrin on the cell membrane [12,17,18]. Components A and B_1 have also been considered as membrane components, which might function as intermediates for iron transport between transferrin bound to its receptor and cytoplasmic carriers of iron [12,13].

However, as isolated ghosts may contain mitochondria, it has been argued that the results mentioned above could have been disturbed by contamination of the membrane fractions with iron-containing mitochondrial components [19]. Recently, it has been shown that other factors may also influence the $^{59}Fe/^{125}I$ ratio of the membrane [20]. In the present study the effect of purification of the membrane fractions on the iron-containing components is described.

Materials and Methods

All chemicals used were of analytical reagent grade.

Reticulocytes were obtained from male Wistar rats as has been described [16]. Rat transferrin was isolated by affinity chromatography on CNBr-activated Sepharose 4B [21].

Transferrin, isotopically labelled with ^{59}Fe and ^{125}I, was produced as described previously [18].

Incubation experiments with rat reticulocytes and the labelled transferrin were also performed as previously described [18]. The incubation time was, in general, 30 min. Varying the time of incubation confirmed results described by Fielding and Speyer [12,13]. The amounts of A, B_1 and B_2 increased with time but at different rates. The ^{59}Fe activity of fraction A increased linearly with time, resembling in behavior the hemoglobin component of the cytosol. However, the amounts of ^{59}Fe in the fractions B_1 and B_2 tended to plateau with time.

Purification and solubilization of the ghost. The incubated cell suspensions were washed several times and hemolysed according to the method of Dodge et al. [22]. The membrane preparation was suspended in 2—4 ml of 5 mosM

sodium phosphate buffer, pH 7.4, and solubilized by addition of 0.25 vols. of 5% (v/v), Triton X-100 and incubated at 37°C for 15 min. The ghosts obtained were purified by sucrose gradient centrifugation as described by Lodish and Small [23].

Briefly, a discontinuous sucrose gradient was prepared by overlaying 6 ml of 60% sucrose (w/v) in centrifugation tubes which fit in the Beckman SW 27 rotor, with the following solutions: 6 ml 50%, 6 ml 45%, 6 ml 40% and 6 ml 30% sucrose. All sucrose solutions were made in 5 mM phosphate-buffered saline, pH 8.0. From the isolated ghost suspension, 0.3—0.4 ml was added to 7 ml 15% sucrose and thoroughly resuspended. The suspension was layered atop the discontinuous gradient. Centrifugation was carried out at 25 000 rev./min for 18 h at 4°C in a Beckman SW 27 rotor.

The light-scattering bands obtained were collected, diluted 10-fold with 5 mM phosphate-buffered saline, pH 8.0, and recovered by centrifugation for 1 h at 27 000 rev./min in a Beckman rotor 35. The purified bands obtained were assayed for acetylcholinesterase activity [24] (plasma membrane-bound enzyme), for succinate-cytochrome *c* reductase [25], succinate dehydrogenase [26] and α-glycerophosphate dehydrogenase [27] activity (mitochondrial enzymes) as well as for acid phosphatase activity [28] (lysosomal enzyme). Protein was determined by a modified procedure of Lowry et al. [29]. Control experiments were performed with rat erythrocytes which had not been incubated with transferrin. The control bands obtained were solubilized and purified by gel filtration on Sepharose 2B and 6B columns as has been described [18].

The influence of centrifugation rates on the compositions of ghost suspensions. A suspension of incubated reticulocytes was divided into three portions and the cells lysed with 20 mM phosphate-buffered saline, pH 7.4, as described et al. [22]. For a normal ghost preparation the suspension was centrifuged at 20 000 × *g*; in addition two other aliquots of the suspensions were centrifuged at 500 and 10 000 × *g*.

Special care was taken with the 10 000 and 20 000 × *g* ghost suspensions in order to eliminate the small greenish/brown material which appeared as a pellet at the bottom of the tubes at these centrifugation speeds [30]. The overlying pellet was used for gel filtration experiments. The 500, 10 000 and 20 000 × *g* preparations obtained were analyzed for acetylcholesterase, succinate-cytochrome *c* reductase and acid phosphatase activity. Protein was determined by using the method of Lowry et al. [33]. Enzyme activities are expressed as percent activities, as is usual for this type of study [23]. The increase in extinction in the enzyme assays over a 10 min period varied between 0.12 and 0.30. The measurements were performed in eight independent experiments in order to achieve a very small variation.

Heme iron was extracted from ghosts according to a slight modification of the procedure described by Thunell [31].

After chromatography, the fractions were assayed for ^{59}Fe and ^{125}I activity in a Packard 5120 autogamma scintillation spectrometer.

Results

Different centrifugation rates: Gel filtration

Ghost suspensions obtained at centrifugation rates of 500, 10 000 and 20 000 × *g*, were subjected to gel filtration on Sepharose 2B and 6B. The results are presented in Figs. 1 and 2.

It can be seen that the percentual ^{59}Fe activity of the first peak in Fig. 1 (peak A), calculated from eight independent experiments, increased in the 10 000 and 20 000 × *g* suspensions, while the ratio of $^{59}Fe/^{125}I$ activity did not change in the second (B) fraction. Fig. 2 shows the results of the fractionation of the B component into three fractions, referred to as B_1, B_2 and B_3, following the literature [12,13]. Together with the data given in Table I, we conclude that the percentual ^{59}Fe activity in the B fractions and the $^{59}Fe/^{125}I$ ratio in the B_2 fraction are independent of the centrifugation rate at which the ghost suspensions were prepared. Only the ^{59}Fe activity of the first fraction, component A, is influenced by the centrifugation rate at which the ghost is prepared. As mentioned above, the different ghost suspensions (500—20 000 × *g*) were fractionated. The fractions were not only analyzed for radioactivity, but

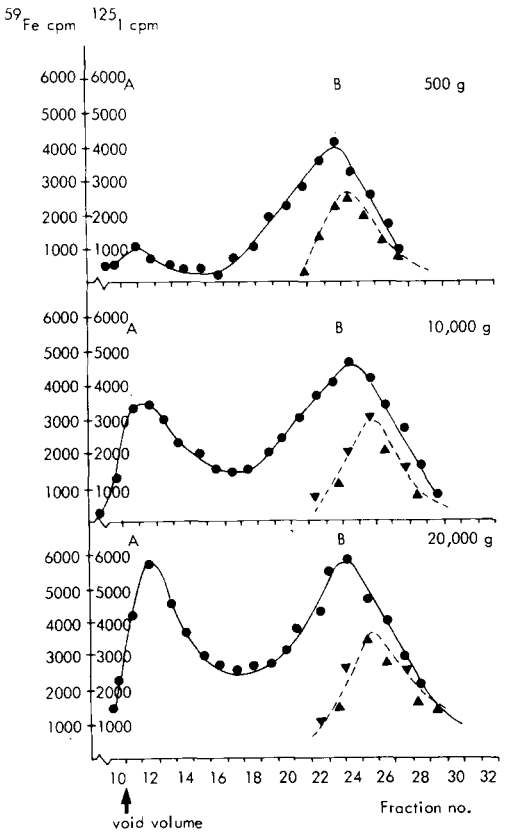

Fig. 1. Gel filtration of the solubilized reticulocyte ghosts on Sepharose 2B. The reticulocyte ghosts were obatined at centrifugation rates of 500, 10 000 and 20 000 × *g*. ●———●, ^{59}Fe; ▲- - - - -▲, ^{125}I.

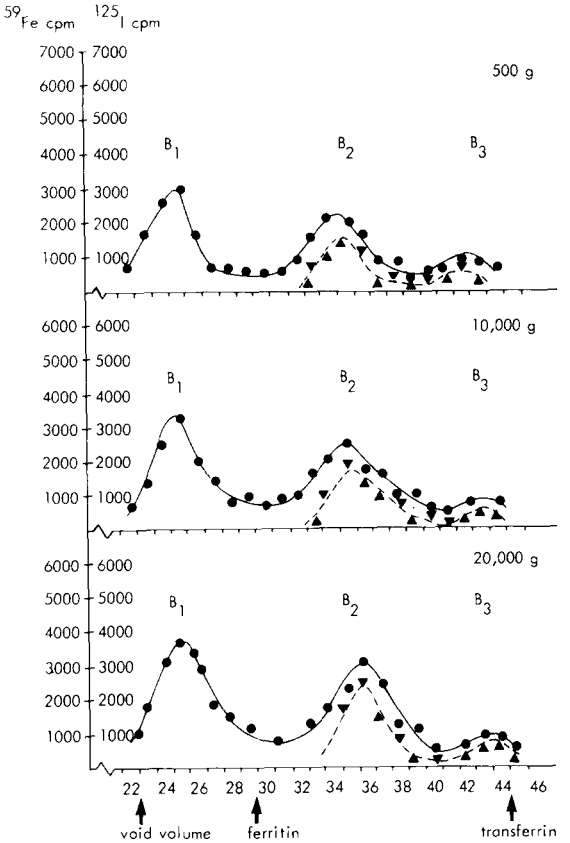

Fig. 2. Rechromatography on Sepharose 6B of the B components obtained from a Sepharose 2B column. ●——●, ^{59}Fe; ▲- - - - -▲, ^{125}I.

TABLE I

THE RESULTS OF GEL FILTRATION OF THE 500, 10 000 AND 20 000 × *g* B COMPONENTS FROM THE SEPHAROSE 2B COLUMN ON SEPHAROSE 6B

	Centrifugation rates		
	500 × *g*	10 000 × *g*	20 000 × *g*
Mean percentual ^{59}Fe activity of the B_1 component with regard to the total activity recovered from the Sepharose 6B column	30%	33%	32%
Mean percentual ^{59}Fe and ^{125}I activity of the B_2 component with regard to the total activity recovered from the Sepharose 6B column	Fe: 29% I: 90%	30% 89%	30% 91%
$^{59}Fe/^{125}I$ ratio of the B_2 component of Sepharose 6B column	1.1	1.1	1.1
^{59}Fe $B_1/^{59}Fe$ B_2 ratio of the Sepharose 6B column	1.0	1.1	1.1

also characterized according to their protein content and enzyme activity. The protein content of a ghost suspension obtained at 20 000 × *g* was taken as 100% and the other ghost suspensions were compared with this preparation. The enzyme activities of the different fractions were determined in terms of their protein content. Enzyme activities characteristic for membranes (acetylcholesterase), mitochondria (cytochrome *c* reductase and succinate dehydrogenase) and lysosomes (acid phosphatase) are given as percent activities of that in the whole ghost suspension [23]. Since the ghost suspension spun down at 10 000 and 20 000 × *g* can be separated into a pellet, a hard brown/greenish pellet and a supernatant, we refer to this as the partially purified membrane fractions being presented in Table II.

Hardly any difference can be observed between the 10 000 and 20 000 × *g* preparations, but a real difference exists between the 500 and 20 000 × *g* preparations. The hard brown pellet contains the most mitochondrial activity, half of the lysosomal activity and a low percentage of protein and membrane activity.

Purification of the ghost suspensions by sucrose gradient centrifugation and classification of the fractions obtained

It is well known that centrifugation of ghost suspensions on a discontinuous sucrose gradient yields fractions which represent mainly plasma membranes and mitochondria. For comparison, we fractionated erythrocyte ghosts and reticulocyte ghosts (Tables III and IV).

TABLE II

THE MEAN PERCENTUAL ENZYME AND PROTEIN CONTENT OF THE GHOST SUSPENSIONS OBTAINED AT DIFFERENT CENTRIFUGATION RATES WITH REGARD TO THE ENZYME ACTIVITY AND PROTEIN CONTENT OF THE 20 000 × *g* GHOST SUSPENSION WHICH HAS BEEN CONSIDERED AS 100%

p.p.m.f., partly purified membrane fraction (see text).

	Centrifugation rates				
	500 × *g*	10 000 × *g*		20 000 × *g*	
		p.p.m.f.	hard brown pellet	p.p.m.f.	hard brown pellet
Mean percentual succinate-cytochrome *c* reductase activity	31%	96%		100%	
		24%	76% *	28%	72% *
Mean percentual acetylcholinesterase activity	59%	100%		100%	
		90%	10%	88%	12%
Mean percentual acid phosphatase activity	18%	46%		100%	
		53%	47%	59%	41%
Mean percentual protein content	61%	96%		100%	
		88%	12%	86%	14%

* Percentual distribution of the enzyme activity and protein content between the p.p.m.f. and the hard brown pellet with regard to the total activity.

TABLE III

THE DISTRIBUTION OF THE MEAN PERCENTUAL ENZYME ACTIVITY AND PROTEIN CONTENT IN THE SEVERAL SUCROSE LAYERS AFTER CENTRIFUGATION OF AN ERYTHROCYTE GHOST SUSPENSION ON A DISCONTINUOUS SUCROSE GRADIENT

	Centrifugation rate	Sucrose layer		
		40% (1.14 g/ml)	45% (1.16 g/ml)	50—60% (1.18/1.20 g/ml)
Protein	20 000 × *g*	74	24	2
Acetylcholinesterase	20 000 × *g*	76	12	12
Succinate-cytochrome *c* reductase	20 000 × *g*	no activity	no activity	no activity
Acid phosphatase	20 000 × *g*	no activity	no activity	no activity

Erythrocyte ghosts obtained at 20 000 × *g* yielded one light-scattering band, the plasma membrane fraction, at the 1.14 g/ml sucrose layer. A small amount of acetylcholineterase activity is present in the 1.18—1.20 g/ml layer, possibly due to the fact that some activity splits off from the plasma membranes during centrifugation.

The reticulocyte suspension yielded three light-scattering bands, a white opalescent band at 1.14 g/ml and two yellow-brown bands at 1.18 and 1.20 g/ml. Sometimes the last two bands coalesced. If they were separated, they coalesced after collection. The white band at 1.14 g/ml seems to be identical with the band of the erythrocyte ghosts and represents the plasma membranes of the reticulocytes because most of the acetylcholesterase activity is present in this band. The succinate-cytochrome *c* reductase activity in this band is low, which means that the contamination with mitochondria is small.

As almost all mitochondria activity is present in the coalesced 1.18 and 1.20 g/ml bands, these bands contain the mitochondria which contaminate the original reticulocyte ghost suspension.

TABLE IV

THE DISTRIBUTION OF THE MEAN PERCENTUAL ENZYME ACTIVITY AND PROTEIN CONTENT IN THE SEVERAL SUCROSE LAYERS AFTER CENTRIFUGATION OF RETICULOCYTE GHOST SUSPENSION, OBTAINED AT 500 AND 20 000 × *g*, ON A DISCONTINUOUS SUCROSE GRADIENT

	Centrifugation rate	Sucrose layer		
		40% (1.14 g/ml)	45% (1.16 g/ml)	50—60% (1.18/1.20 g/ml)
Protein	500 × *g*	52	14	34
	20 000 × *g*	48	12	40
Acetylcholinesterase	500 × *g*	69	15	16
	20 000 × *g*	68	18	14
Succinate-cytochrome *c* reductase	500 × *g*	15	21	64
	20 000 × *g*	12	22	66
α-Glycerophosphate dehydrogenase	500 × *g*	14	12	76
	20 000 × *g*	11	14	75
Succinate dehydrogenase	500 × *g*	14	16	70
	20 000 × *g*	12	20	68

Although the unpurified reticulocyte ghost suspension contained lysosomal activity, as reflected by the presence of acid phosphatase, it was not possible to recover more than 50% of the lysosomal enzyme activity in the various sucrose layers taken together. This is probably a result of the fact that the total lysosomal contamination in the ghost suspension is very low.

Gel filtration of the purified membrane fractions

The 20 000 × *g* ghost suspension of incubated reticulocytes was divided into two portions. One portion was solubilized and directly purified by gel filtration on Sepharose 2B and 6B as described. The other portion was centrifuged on a discontinuous sucrose gradient; the plasma membrane band and the mitochondrial bands were collected, solubilized in Triton X-100 and finally purified by gel filtration on Sepharose 2B and 6B. The same experiment was performed with a 500 × *g* ghost suspension. The results of ten experiments (mean values are given) are summarized in Figs. 3—5. The ^{59}Fe-containing A component disappeared almost totally from the elution pattern when the purified plasma membranes (1.14 g/ml bands) were separated by gel filtration on Sepharose 2B (Figs. 3 and 4).

It is likely that the ^{59}Fe-containing component of the mitochondrial layer

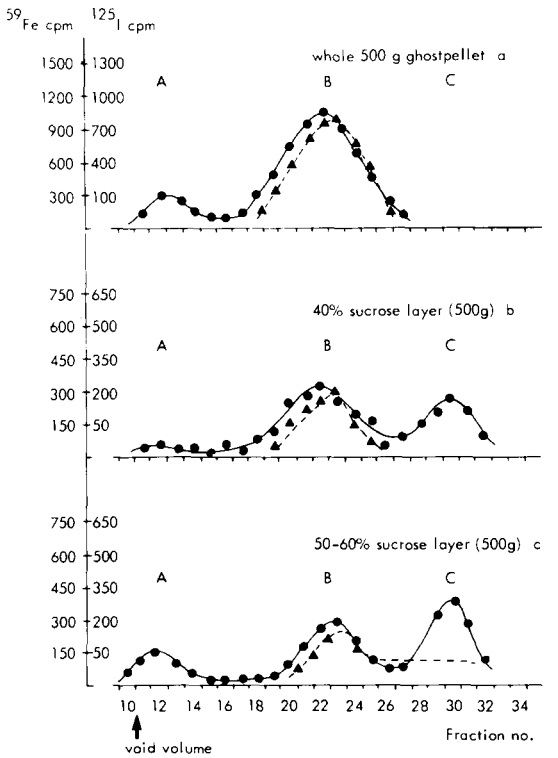

Fig. 3. Gel filtration on Sepharose 2B of 0.3 ml of solubilized reticulocyte membranes obtained at a centrifugation rate of 500 × *g* (a) and of the solubilized suspensions obtained from the 40% (b) and 50/60% (c) sucrose layers after centrifugation of 0.3 ml of the 500 × *g* ghost suspension on discontinuous sucrose gradient. ●——●, ^{59}Fe; ▲- - - - -▲, ^{125}I.

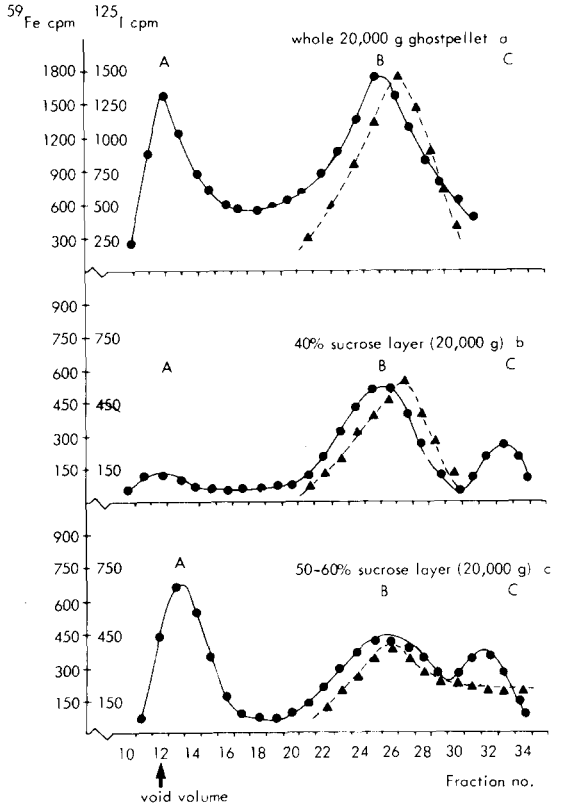

Fig. 4. Gel filtration on Sepharose 2B of 0.3 ml of solubilized reticulocyte membranes obtained at a centrifugation rate of 20 000 × *g* (a) and of the solubilized suspensions obtained from the 40% (b) and 50/60% (c) sucrose layers after centrifugation of 0.3 ml of the 20 000 *g* ghost suspension on discontinuous sucrose gradient. ●———●, ^{59}Fe; ▲- - - - -▲, ^{125}I.

(1.18 and 1.20 g/ml bands), which elutes in the void volume of Sepharose 2B, corresponds to the A component of the original unpurified ghost suspension, since its behavior on Sepharose 2B is the same. Moreover, its ^{59}Fe activity depends on the activity of the A component of the original ghost suspension as shown in the experiments with the 500 and 20 000 × *g* ghost suspensions (Figs. 3 and 4). This, together with the results of the different centrifugation rates, shows that the A component of the ghost suspension is not a membrane component and may be a part of the contaminating mitochondria or lysosomes.

In addition to the radioactive void volume peak, two other radioactive components where obtained when the sucrose layers with the contaminating mitochondria (1.18 and 1.20 g/ml bands) were purified by gel filtration on Sepharose 2B (Figs. 2 and 4, bottom).

60% of the ^{59}Fe activity of the last radioactive component C is present as heme, which is probably derived from the mitochondria. The second radioactive component contains transferrin (measured by immunoprecipitation) which may be specifically bound to an intracellular component. On the other hand, this transferrin may only be an aspecific contamination, since during the centrifugation on the sucrose gradient some transferrin is lost from its receptor

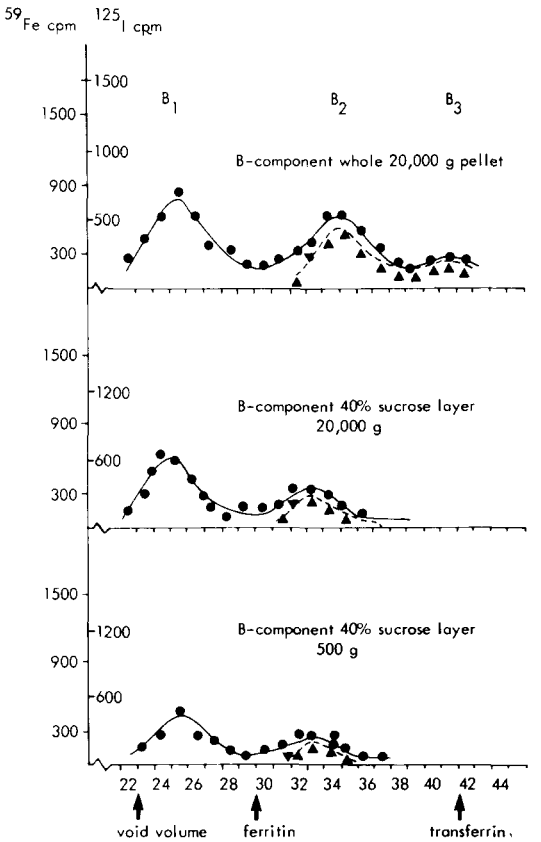

Fig. 5. Gel filtration of the B component obtained from a Sepharose 2B column on Sepharose 6B. B components were obtained from an unpurified 20 000 X *g* ghost suspension, and from the 40% sucrose layers of a 20 000 and 500 X *g* ghost suspension (containing the purified membranes).

TABLE V

THE MEAN PERCENTUAL IRON AND IODINE DISTRIBUTION AFTER GEL FILTRATION OF THE B COMPONENTS FROM A PURIFIED AND UNPURIFIED GHOST SUSPENSION ON SEPHAROSE B

B component unpurified ghost suspension = B component Fig. 4 top (a). B component purified ghost purified ghost suspension = B component Fig. 4 middle (b).

	B component unpurified ghost suspension	B component purified ghost suspension by means of centrifugation on discontinuous sucrose gradient
Mean percentual ^{59}Fe activity of the B_1 component with regard to the total activity recovered from the Sepharose 6B column	32%	42%
Mean percentual ^{59}Fe and ^{125}I activity of the B_2 component with regard to the total activity recovered from the Sepharose 6B column	^{59}Fe: 31% ^{125}I: 92%	24% 93%
^{59}Fe B_1/^{59}Fe B_2 ratio of Sepharose 6B column	1.0	1.7

on the plasma membrane (1.14 g/ml layer) and equilibrates in the 1.18—1.20 g/ml sucrose layers (see Fig. 5).

From Fig. 5 can be concluded (and it was calculated) that the ratio between B_1 and B_2 in a 20 000 × *g* pellet or in a 40% sucrose layer preparation is of the same order of magnitude, however, decreased activity in the sucrose layer preparation, as can be seen clearly for the B_1 fraction, is due to incomplete recovery (technical conditions).

Discussion

The nature of iron transport from transferrin binding sites through the membrane and across the cytosol to mitochondria is still poorly understood. Earlier work has shown that by submitting a reticulocyte ghost suspension, which has been obtained as described by Dodge et al. [22] to gel filtration using Sepharose 2B and 6B, three iron-containing components could be obtained. The components have been previously classified in the literature as A, B_1 and B_2 and have been considered as membrane components [12,13,18].

The components described have been used to support the argument that transferrin donates its iron to membrane proteins which might function as intermediates in the transport of iron from the transferrin receptor through the membrane to the cytosol. However, as reticulocyte ghosts may contain mitochondria it has been argued that some of the ^{59}Fe-containing components described might be mitochondrial components. contaminating the membranes in the ghost suspension.

The present study demonstrates that rat reticulocyte ghosts, obtained as described by Dodge et al., are contaminated with mitochondria and lysosomes, as reflected by the presence of mitochondrial and lysosomal enzyme activities in the ghost suspensions. The amount of mitochondrial and lysosomal contamination is dependent on the centrifugation rate at which the ghost suspension is obtained, as shown in Table II. We have also shown that it is possible to purify partially the ghost suspensions obtained at 10 000 and 20 000 × *g* by eliminating the hard, greenish/brown pellet from the overlying ghost suspension. This hard pellet contained most of the mitochondria, as reflected by the percentual amount and the relative specific activity of the mitochondrial enzymes (Table II). However, this means of purification did not result in a ghost suspension, which was completely free of all mitochondrial and lysosomal contamination.

Our second means of purification was a discontinuous sucrose gradient centrifugation, which yielded a purified plasma membrane fraction with very low contamination with mitochondria or lysosomes.

Since the activity in the A fraction (Fig. 1) increases with increasing centrifugation rate during preparation and the A fraction is nearly absent in a plasma membrane fraction prepared by discontinuous gradient centrifugation, but is present in the mitochondrial fraction, we conclude that the A fraction represents a non-membrane-iron-containing component. This may correspond to the iron-containing void volume component, described by Ponka et al. [19], when he submitted isolated mitochondria to gel filtration on Sepharose G-200.

The recoverable quantity of the B_1 fraction (Sepharose 6B) in the membrane

suspension remains almost unchanged after purification and the percentage of ^{59}Fe activity in B_1 is not influenced by the centrifugation rates. Consequently, it is very likely that B_1 is an iron-containing membrane component, which is extremely strongly attached to the membrane. By means of immunoprecipitation with anti-rat hemoglobin antibodies, it was shown that B_1 does not contain hemoglobin, neither did it contain heme as measured by the slightly modified procedure of Thunell [31].

References

1 Walsh, R.J., Thomas, E.D., Chow, S.K., Fluharty. R.G. and Finch, C.A. (1949) Science 10, 396—398
2 Jandl, J.H., Inman, J.K., Simmons, R.L. and Allan, D.W. (1959) J. Clin. Invest. 38, 161—185
3 Jandl, J.H. and Katz, J.H. (1963) J. Clin. Invest. 42, 314—326
4 Morgan, E.H. and Laurell, C.B. (1963) Br. J. Haematol. 9, 471—483
5 Baker, E. and Morgan, E.H. (1969) Biochemsitry 8, 1133—1141
6 Morgan, E.H. and Appleton, T.C. (1969) Nature 223, 1371—1372
7 Martinez-Medellin, J. and Schulman, H.M. (1972) Biochim. Biophys. Acta 264, 272—284
8 Borova, J., Ponka, P. and Neuwirt, J. (1973) Biochim. Biophys. Acta 320, 143—156
9 Hemmaplardh, D. and Morgan, E.H. (1974) Biochim. Biophys. Acta 373, 84—99
10 Hemmaplardh, D. and Morgan, E.H. (1977) Br. J. Haematol. 36, 85—96
11 Katz, J.H. and Jandl, J.H. (1964) Iron Metabolism (Gross, F., ed.), pp. 103—117, Springer-Verlag, Berlin
12 Speyer, B.E. and Fielding, J. (1974) Biochim. Biophys. Acta 332, 192—200
13 Fielding, J. and Speyer, B.E. (1974) Biochim. Biophys. Acta 336, 387—396
14 Workman, E.F. and Bates, G.W. (1974) Biochem. Biophys. Res. Commun. 58, 787—794
15 Glass, J., Nunez, M.T. and Robinson, S.H. (1977) Biochem. Biophys. Res. Commun. 75, 226—232
16 Verhoef, N.J. and Noordeloos, P.J. (1977) Clin. Sci. Mol. Med. 52, 87—96
17 Van der Heul, C., Kroos, M.J. and van Eijk, H.G. (1977) in Proteins of Iron Metabolism (Brown, E., Aisen, P., Fielding, J. and Crichton, R.R., eds.), pp. 299—305, Grune and Stratton, New York
18 Van der Heul, C., Kroos, M.J. and van Eijk, H.G. (1978) Biochim. Biophys. Acta 511, 430—441
19 Ponka, P., Neuwirt, J.M., Borova, J. and Fuchs, O. (1977) in Iron Metabolsm Ciba Foundation Symposium 51, Excerpta Medica, Amsterdam.
20 Martinez-Medellin, J. and Benavides, L. (1979) Biochim. Biophys. Acta 584, 84—93
21 Van Eijk, H.G. and van Noort, W.L. (1976) J. Clin. Chem. Clin. Biochem. 14, 475—478
22 Dodge, J.T., Mitchel, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
23 Lodish, H.F. and Small, B. (1975) J. Cell. Biol. 65, 51—64
24 Ellman, G.L., Courtney, K.D., Andres, V., Jr. and Featherstone, R.M. (1961) Biochem. Pharmacol. 7, 88—95
25 Sottoca, G.L., Kuylenstierna, B., Ernster, l. and Bergstvand, A. (1967) J. Cell. Biol. 32, 415—428
26 Meyer, A.E.F.H., Benson, D. and Scholte, H.R. (1977) Histochemistry 51, 297—303
27 Scholte, H.R., Jenneken, F.G.I. and Bouvy, J.J.B.J. (1979) J. Neurol. Sci. 40, 39—51
28 De Duve, C., Pressman, B.C., Gianetto, R., Watticusx, R. and Appelmans, F. (1955) Biochem. J. 59, 426—433
29 Markwell, M.A.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206—210
30 Nunez, M.I., Glass, J. and Robinson, S.H. (1978) Biochim. Biophys. Acta 509, 170—180
31 Thunell, S. (1965) Clin. Chim. Acta 11, 321—333
32 Blackburn, G.W. and Morgan, E.H. (1977) Biochim. Biophys. Acta 497, 728—744
33 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275