Biochimica et Biophysica Acta 814 (1985) 8–12
Elsevier

BBA 72497

Fe^{3+} transport by brush-border membrane vesicles isolated from normal and hypoxic mouse duodenum and ileum

Robert J. Simpson, Kishor B. Raja and Timothy J. Peters

Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ (U.K)

(Received August 5th, 1984)

Key words: Fe^{3+} transport; Brush-border membrane; Hypoxia; Membrane vesicle; (Mouse intestine)

Studies of $^{59}Fe^{3+}$ uptake by brush-border membrane vesicles prepared from mouse duodenum have indicated that uptake represents transport across the brush-border membrane which is rate-limited by the membrane-transfer step (Simpson, R.J. and Peters, T.J. (1984) Biochim. Biophys. Acta 772, 220–226). Further studies presented here reveal that the uptake rate represents the net influx rate for Fe^{3+} and is independent of Na^+ in the medium and of the method of vesicle preparation. Uptake by brush-border membrane vesicles prepared from mouse distal ileum also represents predominantly transport and is higher than that observed with duodenal brush-border membrane vesicles. Studies of the initial uptake rate by vesicles prepared from normal and hypoxic mouse intestine demonstrated an increase in Fe^{3+} transport in duodenal vesicles only.

Introduction

Recent studies on the role of the brush-border membrane in the intestinal absorption of iron have reached contradictory conclusions. Studies by Cox and O'Donnell [1–3] of Fe^{2+} binding by rabbit brush-border membrane preparations from normal and iron-deficient animals implicated a specific glycoprotein receptor which was involved in adaptive regulation of iron absorption. A similar study by Marx and Aisen [4] revealed not binding but transport of Fe^{2+} across the membranes of rabbit brush-border vesicles. They observed no effect of boiling the vesicles on this transport and concluded [5] that the brush-border membrane played an essentially passive role in Fe^{2+} absorption. Eastham et al. [6] have also demonstrated transport of Fe^{2+} by isolated rat brush-border membrane vesicles.

Studies of Fe^{3+} transport by isolated brush-border membrane vesicles have been more limited and, while Marx and Aisen [4] could not demonstrate transport of Fe^{3+} by rabbit vesicles, we have found that Fe^{3+} transport could be observed with mouse duodenal brush-border membrane vesicles, provided a sufficient free Fe^{3+} concentration was used [7]. The low uptake of Fe^{3+} observed with rabbit vesicles [1,4] is probably explained by the very low medium free Fe^{3+} used by these workers [7].

Fe^{3+} uptake by mouse duodenal brush-border membrane vesicles was further shown to be rate-limited by the brush-border membrane transport step [7]. In this paper, further studies of the nature of Fe^{3+} transport by duodenal and ileal brush-border membrane vesicles are reported. In addition, studies were performed on vesicles from animals in which the rates of iron absorption had been experimentally increased by hypoxia.

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

Materials and Methods

Brush-border membrane vesicles were prepared from mouse duodenum (proximal 4 cm) and ileum (distal 10 cm) by a modification [7] of the method of Kessler et al. [8]. Experiments were also performed with vesicles that were prepared in resuspension buffer (0.1 M mannitol/0.1 M NaCl/0.02 M Hepes (pH 7.4)/0.1 mM $MgSO_4$) following disruption of isolated brush borders with Tris [9] or NaSCN [10]. $^{59}Fe^{3+}$ uptake from an incubation medium (0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.4)) containing $^{59}Fe^{3+}$ and excess nitrilotriacetate was studied by the millipore filtration method in Ref. 7. Figures present representative experiments demonstrating qualitative results which could be reproduced at least three times.

Iron was measured by atomic absorption spectrophotometry as described in Ref. 11 with a Perkin-Elmer model 703 dual-beam atomic absorption spectrophotometer with an HGA 76 graphite furnace. Protein and enzyme determinations were performed as in Refs. 7 and 12. Reagents were obtained as described in Ref. 7.

6–8-week-old To-strain mice were used throughout. Males were used, except where specifically indicated. Hypoxia was induced by placing animals in a hypobaric chamber at 0.5 atmospheres for 3 days. Food and water were given freely, except on the day of killing. Experiments with pregnant mice were performed in the final week of pregnancy.

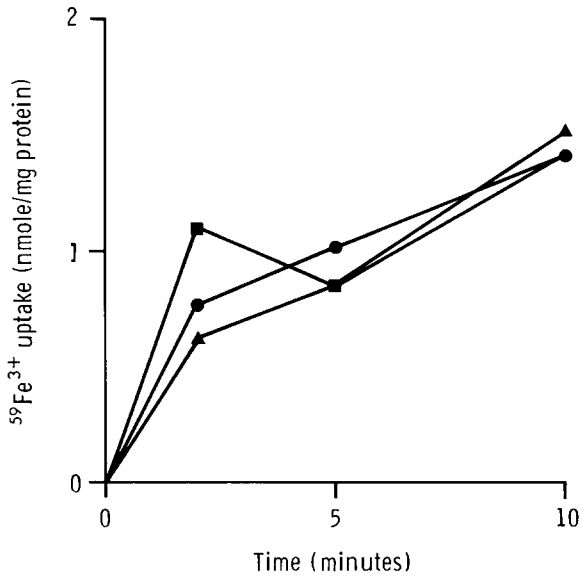

Fig. 1. Effect of preincubation of vesicles with $^{56}Fe^{3+}$ on $^{59}Fe^{3+}$ uptake. In each experiment, vesicles in 0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.4)/0.1 mM $MgSO_4$ were mixed with 10 vol. of incubation buffer (0.1 M mannitol/0.1 M NaCl/0.02 M Hepes (pH 7.4), final concentrations) containing $^{56}Fe^{3+}$-nitrilotriacetic acid giving final concentrations of 74 and 163 μM for $^{56}Fe^{3+}$ and nitrilotriacetic acid, respectively. $^{59}Fe^{3+}$-nitrilotriacetic acid was added as a small volume (less than 4% of final volume) to give concentrations of Fe^{3+} and nitrilotriacetic acid of 91 and 200 μM, respectively. The vesicles were either incubated for 2 h at 37°C before addition of $^{56}Fe^{3+}$-nitrilotriacetic acid and $^{59}Fe^{3+}$-nitrilotriacetic acid (▲) or for 2 h at 37°C with $^{56}Fe^{3+}$-nitrilotriacetic acid (●) before addition of $^{59}Fe^{3+}$-nitrilotriacetic acid or $^{56}Fe^{3+}$-nitrilotriacetic and $^{59}Fe^{3+}$-nitrilotriacetic acid were added together to vesicles without preincubation (■). $^{59}Fe^{3+}$ uptake was determined by millipore filtration [7].

Results and Discussion

Previously reported electron spin resonance studies [13] indicate that the uptake of Fe^{3+} by the vesicles represents a net movement of iron to some binding site within the vesicle with a higher affinity for Fe^{3+} than nitrilotriacetic acid. Fig. 1 shows that preloading the vesicles with $^{56}Fe^{3+}$ has little effect on the subsequent $^{59}Fe^{3+}$ uptake rate. This implies that the unidirectional influx of Fe^{3+} into the vesicles and the initial uptake rate measured with $^{59}Fe^{3+}$ are similar.

The $^{59}Fe^{3+}$ uptake rate was found to be independent of the presence of Na^+ or mannitol in the medium. In two separate experiments, vesicles prepared in 0.3 M mannitol/0.02 M Hepes buffer (pH 7.4) gave initial uptake rates of 0.15 and 0.12 nmol/mg protein per min (means of duplicate assays using 91 μM Fe^{3+} and 182 μM nitrilotriacetic acid and an incubation time of 5 min), while substituting the appropriate NaCl concentration for mannitol in the assay mixture gave uptake rates with the same preparations of 0.20 and 0.12 nmol/mg protein per min (mean of duplicate assays), respectively. All these values are within the uptake range obtained with vesicles in mannitol/NaCl/Hepes incubation buffer (see Table II).

Studies of Fe^{3+} uptake by vesicles prepared from distal ileum demonstrated distinct qualitative and quantitative kinetics from those observed with

duodenal vesicles. Uptake experiments with vesicles in media of progressively increasing osmolarity revealed that, while uptake predominantly represented transport relating to an osmotically active space, a more significant proportion represented uptake unrelated to an osmotically active space (Fig. 2). This latter uptake may represent binding to the outside of the vesicles. The proportion of uptake which is apparent binding should therefore be taken into account in experiments with distal ileal vesicles. This proportion (30–45%) was found to be roughly constant as a function of time up to 20 min incubation and was unaffected by increasing the medium Fe^{3+} content from 100 to 400 μM at constant nitrilotriacetic acid/Fe^{3+} ratio. Increasing the medium iron concentration over this range was found to have a small effect on initial vesicle Fe^{3+} uptake (1.7 ± 0.5-fold (mean $\pm$ S.E., $n = 3$)), similar to that noted with duodenal vesicles over the same concentration range (1.5 ± 0.2-fold (mean $\pm$ S.E., $n = 3$)). The effect of adding 1 mM $CaCl_2$ to the incubation medium on Fe^{3+} uptake (91 μM Fe^{3+}, 182 μM nitrilotriacetate) was a (3.5 ± 0.1)-fold increase (mean $\pm$ S.E., $n = 3$). When the degree of binding during Ca^{2+}-stimulated uptake was measured, the proportion was found to drop from about 40% to 25%, thus the effect of Ca^{2+} on the transport rate is about a 4-fold increase. This effect of 1 mM Ca^{2+} on transport is lower than

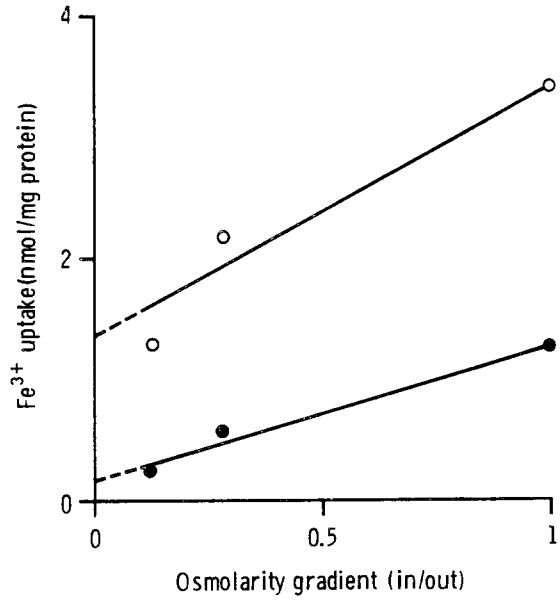

Fig. 2. Effect of medium osmolarity on $^{59}Fe^{3+}$ uptake. Brush-border membrane vesicles were prepared as in Fig. 1 from duodenum (●) and ileum (○) and mixed with media containing ^{59}Fe-nitrilotriacetic acid, 0.1 M mannitol, 20 mM Hepes (pH 7.4) and sufficient NaCl to generate the indicated osmolarity gradient to start the uptake time-course.

was noted with duodenal vesicles [7]. It should be noted that the assumption of binding represents a relatively small correction to the results presented above.

Table I presents data for Fe^{3+} uptake by mem-

TABLE I

UPTAKE OF Fe^{3+}, IRON CONTENT AND BRUSH-BORDER MARKER ENZYME ACTIVITY IN VESICLE PREPARATIONS FROM DIFFERENT SOURCES

Uptake, after 5 min incubation at 37°C, of $^{59}Fe^{3+}$ from 400 μM Fe^{3+}/790 μM nitrilotriacetic acid/0.1 M NaCl/0.1 M mannitol/20 mM Hepes (pH 7.4 ± 0.1) by various brush-border membrane vesicles preparations. All data are mean $\pm$ 95% confidence limit for (n) membrane preparations. n.d., not determined.

Vesicle source	$^{59}Fe^{3+}$ uptake (nmol/mg protein)	Iron content (nmol/mg protein)	Brush-border marker enzyme (munits/mg protein)
Normal male duodenum	0.89 ± 0.24 (16)	8.4 ± 4.4 (6)	10.6 ± 1.6 (10) [c]
Normal male ileum	3.3 ± 1.2 (9) [b]	4.8 ± 0.9 (5)	4.4 ± 0.8 (8) [a]
Hypoxic male duodenum	1.38 ± 0.26 (17) [a]	7.6 ± 2.8 (7)	10.1 ± 2.0 (10)
Hypoxic male ileum	1.83 ± 0.37 (6) [b]	n.d.	4.1 ± 0.7 (6) [a]
Normal female duodenum	1.09 ± 0.47 (4)	n.d.	11.3 ± 2.0 (4)
16-day-pregnant female duodenum	0.87 ± 0.21 (4)	n.d.	11.0 ± 1.6 (4)

[a] $P < 0.01$.

[b] $P < 0.001$.

[c] The value quoted in Ref. 7 is 10-fold high.

brane preparations from duodenum and ileum of control and hypoxic male mice and from duodenum of control and pregnant female mice. The initial rate of uptake by normal male mouse ileum brush-border membrane vesicles was higher than that found for normal duodenal vesicles. Only vesicles from duodenum showed a significant increase in uptake rate in hypoxic mice compared to normals. There was no difference in Fe^{3+} uptake by duodenal vesicles from pregnant mice compared with controls.

Table I also shows that brush-border membrane vesicles from mice contain considerable amounts of iron and that this is unchanged in duodenal vesicles from hypoxic mice. It can be seen that the iron content of vesicles from distal ileum is slightly lower than that of normal duodenal brush-border membrane vesicles, though this does not reach statistical significance. It is not known whether this vesicular iron is of significance in Fe^{3+} uptake; however, since it does not change in hypoxia, we may conclude that the change in Fe^{3+} uptake is probably not due to changes in occupancy of iron-binding sites.

The specific activity of the brush-border membrane marker enzyme Zn^{2+}-resistant α-glucosidase is unchanged in vesicle preparations from hypoxic mice (Table I), as is the activity in whole duodenal tissue (normal; 0.055 ± 0.005 munits/mg wet wt. (mean $\pm$ S.E., $n = 16$); hypoxic, 0.046 ± 0.007 munits/mg wet wt. (mean $\pm$ S.E., $n = 10$)), suggesting that the increase in $^{59}Fe^{3+}$ uptake rate elucidated by hypoxia is a specific effect on a brush-border Fe^{3+} transport system. The lower specific activity of the brush-border membrane marker in the distal ileal preparations presumably reflects lower activity of this enzyme in ileum [14].

Table II presents evidence that changing the method of vesicle preparation does not greatly affect the Fe^{3+} uptake rate. In particular, the method employed in this paper, involving treatment with Mg^{2+} [7], gives similar uptake to vesicles prepared with EDTA, followed by disruption of brush borders [9,10].

These data, coupled with that previously reported [7,13] strongly suggest that $^{59}Fe^{3+}$ uptake by mouse duodenal brush-border membrane vesicles represents the net influx rate for Fe^{3+} across isolated brush-border membrane. There is no indication of a Na^+ dependence of Fe^{3+} transport, and vesicles prepared by different procedures (Table II) show similar uptake rates.

The uptake of $^{59}Fe^{3+}$ by vesicles prepared from distal ileum has distinctive properties when compared with duodenal vesicles. The initial rate of uptake is higher in distal ileal vesicles, a finding which appears to run counter to in vivo studies [15] which suggest that the specific transport of Fe^{3+} is localised to the proximal small intestine. It should be noted, however, that it is the capacity to transfer iron to the blood which is lacking in ileal mucosa, which can still take up Fe^{2+} and Fe^{3+} from the intestinal lumen [15].

Hypoxia induces increased rates of iron absorption, as has been demonstrated by both in vivo [16] and in vitro [17] techniques. An increase in Fe^{3+} transport by isolated duodenal but not distal ileal brush-border membrane vesicles from hypoxic mice implies that a specific, adaptable Fe^{3+} transport system may exist in the duodenal brush-border membrane. This finding is similar to observations of Fe^{2+} uptake by normal and iron-deficient rabbit duodenal brush-border membrane preparations [3].

TABLE II

UPTAKE OF Fe^{3+} BY BRUSH-BORDER MEMBRANE VESICLES PREPARED BY DIFFERENT METHODS

Uptake was determined in duplicate, after 5 min incubation at 37°C, by millipore filtration [7]. The medium Fe^{3+} concentration was 91 μM and nitrilotriacetic acid was 182 μM. Values given are mean $\pm$ S.E. for (n) preparations.

Vesicle preparation method	Zn^{2+}-resistant α-glucosidase activity (munits/mg protein)	$^{59}Fe^{3+}$ uptake rate (nmol/mg protein per min)
Mg^{2+} precipitation [7]	$11.1 + 1.2$ (14)	0.15 ± 0.02 (14)
Tris disruption [9]	20.8 ± 2.1 (3)	0.11 ± 0.01 (3)
NaSCN disruption [10]	21.4 (2)	0.16 (2)

The increase in Fe^{3+} transport was small (less than 2-fold) and no increase in Fe^{3+} transport by brush-border membrane in pregnancy was observed. These observations, when compared with larger increases noted in whole tissue [17,18], suggest that the properties of transport by isolated brush-border membrane may not account fully for in vivo Fe^{3+} absorption.

Acknowledgements

R.J.S. is an MRC Training Fellow. We thank Ms. Rosamund Greensted for typing the manuscript.

References

1 Cox, T.M. and O'Donnell, M.W. (1981) Biochem. J. 194, 753–759
2 O'Donnell, M.W. and Cox, T.M. (1982) Biochem. J. 202, 107–115
3 Cox, T. and O'Donnell, M. (1980) Biochem. Int. 1, 446–454
4 Marx, J.J.M. and Aisen, P. (1981) Biochim. Biophys. Acta 649, 297–304
5 Aisen, P. (1982) Clin. Haematol. 11, 241–257
6 Eastham, E.J., Bell, J.I. and Douglas, A.P. (1977) Biochem. J. 164, 289–294
7 Simpson, R.J. and Peters, T.J. (1984) Biochim. Biophys. Acta 772, 220–226
8 Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 394, 361–376
9 Critchley, D.R., Howell, K.E. and Eicholz, A. (1975) Biochim. Biophys. Acta 394, 361–376
10 Hopfer, U., Crowe, T.O. and Tandler, B. (1983) Anal. Biochem. 131, 447–452
11 Selden, C. and Peters, T.J. (1979) Clin. Chim. Acta 98, 47–52
12 Peters, T.J. (1976) Clin. Sci. Mol. Med. 51, 557–574
13 Simpson, R.J., Weir, M., Gibson, J.F. and Peters, T.J. (1984) Biochem. Soc. Trans. 12, 859
14 Batt, R.M. and Peters, T.J. (1976) Clin. Sci. 50, 511–523
15 Forth, W. and Rummel, W. (1973) Physiol. Rev. 53, 724–792
16 Hathorn, M.K.S. (1971) Gastroenterology 60, 76–81
17 Raja, K., Simpson, R.J. and Peters, T.J. (1983) Clin. Sci. 65, 40P
18 Batey, R.G. and Gallacher, N.D. (1977) Gastroenterology 73, 267–272