EFFECT OF IRON ABSORPTION ON PLASMA MEMBRANE PROTEINS OF SMALL INTESTINAL MUCOSAL CELLS FROM IRON-DEFICIENT RATS

N. Kaufman, M. Newkirk and J.C. Wyllie

Department of Pathology, Queen's University
and Kingston General Hospital

Kingston, Ontario, Canada K7L 3N6

Received October 4, 1976

Summary

Plasma membrane protein was isolated from small intestinal mucosal cells of normal and iron-deficient rats during iron absorption and examined by acrylamide gel electrophoresis. Two proteins were present in increased amounts in the iron-deficient animals 10 min after administration of iron; uptake studies with ^{3}H-leucine indicated that each had been newly-synthesized. The molecular weights of the proteins were about 60,000 and 300,000.

Introduction

Although iron absorption has been extensively studied, the precise mode of iron uptake by mucosal cells of the small intestine remains unknown (1,2). Evidence for active transport of iron across the plasma membrane, possibly by means of a carrier, has been reported (3-7). The effects of iron-deficiency and iron overload on this phase of absorption suggest that the iron requirements of the body regulate the synthesis of this carrier (8,9). We have investigated, therefore, the synthesis of plasma membrane proteins by small intestinal mucosal cells of normal and iron-deficient rats during iron absorption; the proteins were separated by acrylamide gel electrophoresis.

Materials and Methods

Iron deficiency was induced in one group of female Wistar rats by raising them from weaning on an iron-deficient diet (10 mg of iron per kg); a second group received a normal diet (210 mg of iron per kg). The weight gain in each group was similar. In animals raised on the

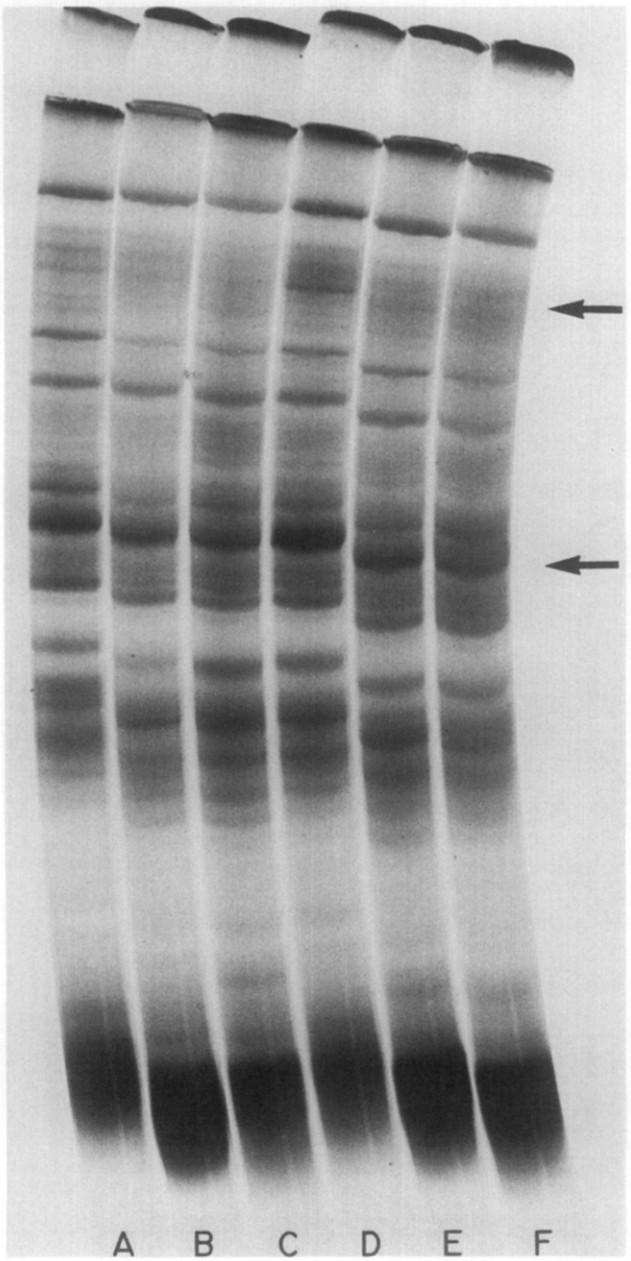

Figure 1 SDS-acrylamide gel electrophoretograms of plasma membrane protein from small intestinal mucosal cells prepared from iron-deficient or normal rats. The time intervals between administration of iron or normal saline and removal of the intestine were: 10 min, normal, iron-dosed (A); 30 min, normal, iron-dosed (B); 30 min, normal, saline-dosed (C); 10 min, iron-deficient, iron-dosed (D); 30 min, iron-deficient, iron-dosed (E) and 30 min, iron-deficient, saline-dosed (F). The locations of two proteins present in increased amounts of iron-deficient rats, 10 min after iron administration are indicated by arrows.

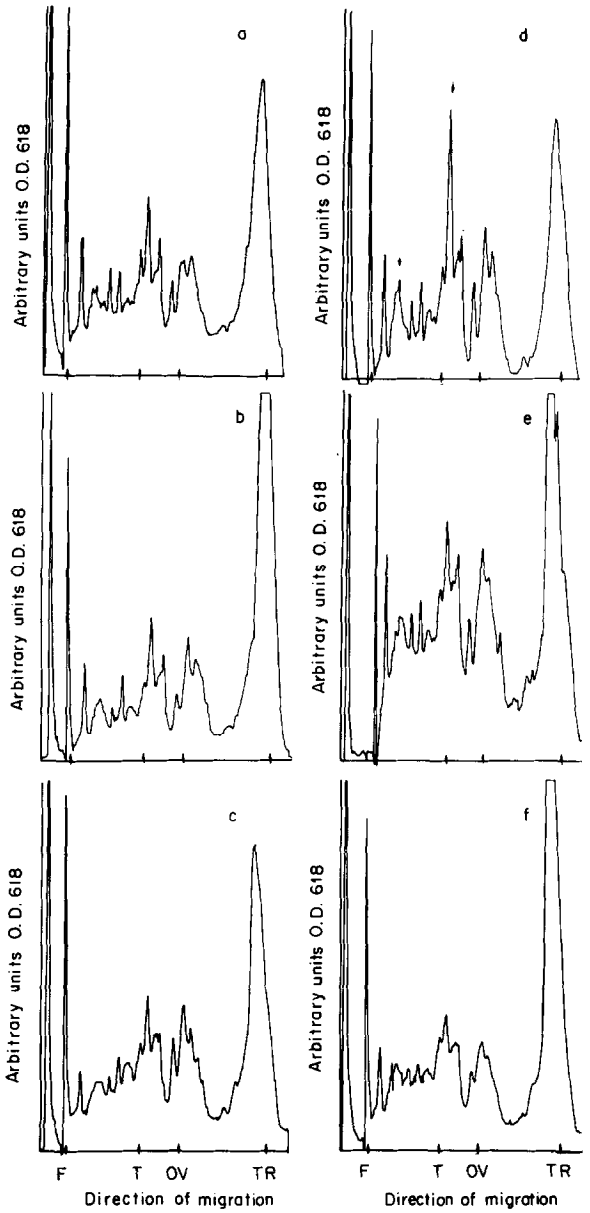

Figure 2 Densitometric tracings of SDS-acrylamide gels of plasma membrane protein from normal (A-C) or iron-deficient (D-F) rats. The time intervals of iron or saline incubation were: 10 min iron-dosed (A,D); 30 min iron-dosed (B,E); and 30 min, saline-dosed (C,F). The locations of two proteins present in increased amounts in iron-deficient rats, 10 min after iron administration, are indicated by arrows above the tracing (D). The marker proteins, ferritin (F), transferrin (T), ovalbumin (OV) and trypsin (TR) were run in a parallel gel and are indicated by arrows below the tracings.

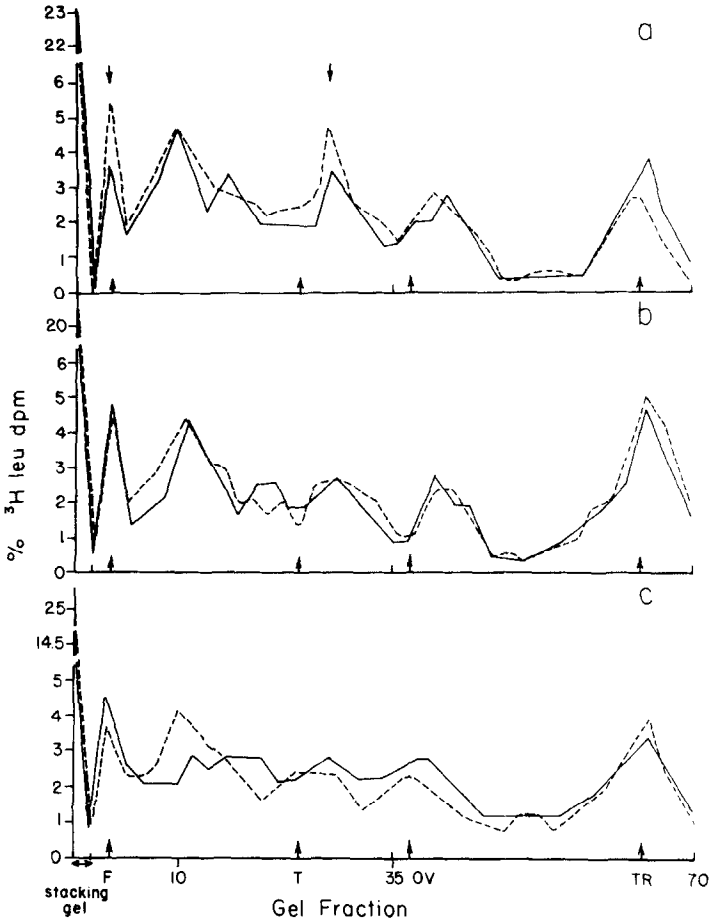

Figure 3 SDS-acrylamide gel electrophoresis of plasma membrane proteins obtained from iron-deficient (-----) or normal (———) rats and pulse-labelled with ^{3}H-leucine. The time intervals between administration of iron or normal saline were: 10 min, iron-dosed (a); 30 min, iron-dosed (b) and 30 min, saline-dosed (c). The two proteins present in increased amounts in iron-deficient rats, 10 min after iron administration, are indicated by arrows above the graph (a). The marker proteins, ferritin (F), transferrin (T), ovalbumin (OV) and trypsin (TR) were run in a parallel gel and are indicated by arrows below the graph.

---

low-iron diet the hemoglobin was 7.0 gm/100 ml (normal 15.0 gm/100 ml), the hematocrit 22% (normal 45%), serum iron 26 µg/100 ml (normal 200 µg/100 ml) and total iron-binding capacity 810 (normal 400). Iron absorption was investigated from 70 to 90 days of age. The iron-deficient rats were divided into two groups; one group received 0.075 M $FeCl_3$ in 0.15 M NaCl, containing 0.002 M sodium ascorbate, by the *in vivo* closed duodenal loop technique (10); the other group of iron-deficient rats and the normal rats received an identical volume of 0.15 M NaCl. Each rat was given 100 µCi

of ^{3}H-leucine, I.P., 4 hr prior to sacrifice in order to label newly-synthesized mucosal cellular protein (11). Rats were killed 10 or 30 min after the administration of iron or saline. The first 45 cm of small intestine were resected, the mucosa was removed and homogenized (12). Purified plasma membranes were prepared by the method of Coleman *et al* (13) and solubilized in 6 mM Tris-phosphoric acid buffer (pH 6.7) containing 1% dodecyl sulphate (SDS), 1% β-mercaptoethanol, 10% glycerol and 0.1% bromophenol blue (14). Plasma membrane proteins were resolved on a 10% SDS-acrylamide gel for 5 hr at 2-3 ma/tube (75 V) and stained with Amido black (14). Tracings of the protein patterns were obtained at O.D. 618 with an E-C 810 densitometer. A parallel gel contained the molecular weight markers ferritin (400,000), transferrin (74,000), ovalbumin (43,500) and trypsin (23,000). The gels were sliced and the radioactivity (^{3}H) in each fraction was measured. The distribution of ^{3}H in the gel was correlated with the corresponding densitometric tracing.

Results and Discussion

Increased quantities of two proteins were apparent in plasma membranes prepared from iron-deficient rats 10 min after the onset of iron absorption; by 30 min, however, each had declined in amount (Figures 1 and 2). The proteins had molecular weights of about 60,000 and 300,000. Increased amounts of each protein were also identified in the gel pattern of newly-synthesized plasma membrane protein at 10 min (Figure 3). The quantities of these proteins present in saline-dosed iron-deficient and normal rats at 10 and 30 min were similar; protein patterns obtained at 30 min are shown in Figures 1 and 2.

Our findings suggest that two proteins (molecular weights 60,000 and 300,000) accumulate preferentially in plasma membranes isolated from small intestinal mucosal cells of iron-deficient rats during the early phase of iron absorption. The rapidity with which these proteins wax and wane in quantity is of particular interest. While it is possible that luminal iron initiates synthesis of the proteins, the interval of time for such a process is very brief. It appears more probable that each protein is continually synthesized and is available in the cell sap for insertion into the plasma membrane when required for iron uptake. Mucosal cells from iron-deficient rats contain more of each protein than do those from normal rats.

Acknowledgement

This work was supported by a grant from the Medical Research Council of Canada.

References

1. Forth, W., and Rummel, W. (1973) Physiol. Rev. 53, 724-792.
2. Turnbull, A. (1974) in Iron in Biochemistry and Medicine, eds. Jacobs, A., and Worwood, M. (Academic Press, New York), pp. 370-403.
3. Brown, E.B., and Justus, B.W. (1958) Am. J. Physiol. 194, 319-326.
4. Manis, J.G., and Schachter, D. (1962) Am. J. Physiol. 203, 73-80.
5. Greenberger, N.J., Balcerzak, S.P., and Ackerman, G.A. (1969) J. Lab. Clin. Med. 73, 711-721.
6. Thomson, A.B.R., Shaver, C., Lee, D.J., Jones, B.L., and Valberg, L.S. (1971) Am. J. Physiol. 220, 674-678.
7. Howard, J., and Jacobs, A. (1972) Br. J. Haematol. 23, 595-603.
8. Manis, J.G., and Schachter, D. (1964) Am. J. Physiol. 207, 893-900.
9. Forth, and Rummel, W. (1965) Arch. Exp. Path. Pharmak. 252, 205-223.
10. Wheby, M.S., Jones, L.G., and Crosby, W.H. (1964) J. Clin. Invest. 43, 1433-1442.
11. Alpers, D.H., (1972) J. Clin. Invest. 51, 167-173.
12. Worwood, M., and Jacobs, A. (1971) Br. J. Haemat. 20, 587-597.
13. Coleman, R., Michell, R.H., Finean, J.B., and Hawthorne, J.N. (1967) Biochim. Biophys. Acta, 135, 573-579.
14. Meldolesi, J., and Cova, D. (1972) J. Cell Biol. 55, 1-18.