The Identification of Rat Intestinal Membrane Enzymes after Electrophoresis on Polyacrylamide Gels containing Sodium Dodecyl Sulphate

By HANS-PETER HAURI and JONATHAN R. GREEN

Department of Gastroenterology, University Children's Hospital, 3010 Bern, Switzerland

(Received 20 October 1977)

Brush-border membranes were isolated from the rat small intestine and then treated with sodium dodecyl sulphate under non-reducing conditions at room temperature. Analysis of the solubilized components by polyacrylamide-gel electrophoresis identified three major glycoproteins that co-migrate with glucoamylase–maltase–sucrase, lactase and isomaltase–maltase–sucrase activities. High activities of alkaline phosphatase and trehalase were detectable, but they could not be attributed to distinct co-migrating protein bands. Analysis of mucosa from the distal small intestine by the same methods showed a pattern of bands different from that obtained with the proximal intestine, which appeared to correlate with the relative deficiency of some of the enzymes in the distal region.

The rat small intestine has been widely adopted as a system for the investigation of the processes involved in the differentiation of the enterocyte (Weiser, 1973; Galand & Forstner, 1974; Kim *et al.*, 1975; Raul *et al.*, 1977). The method of Forstner *et al.* (1968) has become the established technique for the preparation of a purified brush-border fraction from the rat small intestine. A modification of this procedure by Hopfer *et al.* (1973) gives brush-border membrane vesicles of higher purity, with the virtual absence of fibrous core material. However, the low yield of the Hopfer *et al.* (1973) method often dictates the use of the Forstner *et al.* (1968) method for the isolation of brush-border membranes in higher yield, but of lower purity, from small amounts of tissue. It was therefore considered necessary to establish the identification of the enzymes of the rat intestinal brush-border membrane with a pure preparation by the method of Hopfer *et al.* (1973), and then to compare the findings with those from a preparation by the method of Forstner *et al.* (1968). In a previous investigation using SDS/polyacrylamide-gel electrophoresis under reducing conditions, Alpers (1972) identified the protein components of brush-border membranes prepared by the method of Forstner *et al.* (1968), but the major active enzymes of the adult rat small-intestinal membrane have not been identified after electrophoresis on SDS/polyacrylamide gels. Such an identification is a prerequisite for following the molecular events occurring during differentiation of the enterocyte and the turnover of its membrane.

Abbreviations used: SDS, sodium dodecyl sulphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Methods

Preparation of brush-border membranes

The entire small intestine was removed from adult rats that had been anaesthetized with ether. The intestines were divided into three equal lengths, and the proximal and distal thirds were retained and processed identically in separate flasks. After the intraluminal contents had been rinsed out with cold 0.9% NaCl, the pieces of intestine were opened and the mucosa was scraped off lightly with a glass slide. Brush borders were then isolated by the method of Forstner *et al.* (1968) with the following modifications. All solutions were buffered with 1 mM-Tris, adjusted to pH 7.4 with Hepes. The scraped mucosa was homogenized with an Ultra-Turrax homogenizer in short bursts of 3–5 s. After each stage the homogenate was examined under the phase-contrast microscope. Homogenization was repeated until whole nuclei were no longer visible and the brush borders appeared to be free of cytoplasmic material. This usually required a total homogenization time of 10–20 s, varying with different animals and the volume of homogenate. It was essential to monitor the process in the microscope, otherwise prolonged homogenization decreased the yield of intact brush borders. Vesicles were prepared from the brush borders by following the exact procedure of Hopfer *et al.* (1973), and the disruption process in the glass/Teflon homogenizer was checked under the microscope and continued until brush borders were no longer visible. Samples of the homogenate, brush-border and vesicle preparations were retained for determination of total protein (Lowry *et al.*, 1951), alkaline phosphatase activity (Garen & Levinthal, 1960) and

glycosidase activities (Dahlqvist, 1968; Asp & Dahlqvist, 1972). Results are expressed as units of enzymic activity; 1 unit hydrolyses $1\ \mu\text{mol}$ of substrate/min under the conditions specified for the assay.

Gel electrophoresis and eluate analysis

Gel electrophoresis in the presence of SDS was performed on 7.5% (w/v) acrylamide gels as described by Laemmli (1970) under non-reducing conditions (Hauri *et al.*, 1977). After electrophoresis the gels were split longitudinally, one half was stained with Coomassie Blue or Schiff/periodate stain and then scanned, and the other half was sliced into 0.5 mm sections that were eluted overnight in 1 ml of water at 4°C. The following glycosidase activities were then measured in the eluates by using the method of Dahlqvist (1968): glucoamylase (exo-1,4- α -glucosidase, EC 3.2.1.3) with glycogen as substrate, isomaltase (oligo-1,6-glucosidase, EC 3.2.1.10) with palatinose as substrate, lactase (β -D-galactohydrolase, EC 3.2.1.23) in the presence of *p*-chloromercuribenzoate as recommended by Asp & Dahlqvist (1972), maltase (α -glucosidase, EC 3.2.1.20), sucrase (sucrose α -glucohydrolase, EC 3.2.1.48) and trehalase ($\alpha\alpha$ -trehalase, EC 3.2.1.28). Alkaline phosphatase (EC 3.1.3.1) was measured in the eluates by the method of Garen & Levinthal (1960). The relative mobilities of the stained protein or glycoprotein bands and those of the regions of enzymic activity from the two halves of the same gel were then calculated and compared.

Results and Discussion

The use of SDS/polyacrylamide-gel electrophoresis has been successful in the analysis of the protein and enzymic composition of human (Maestracci *et al.*, 1975), hamster (Critchley *et al.*, 1975) and guinea pig (Green & Hadorn, 1977) brush-border membranes. In an initial attempt to identify rat brush-border enzymes on SDS/polyacrylamide gels after electrophoresis, Alpers (1972) was only able to detect alkaline phosphatase and low maltase activity by histochemical techniques. We now report the identification of protein bands that co-migrate with maltase, glucoamylase, lactase, sucrase, isomaltase, alkaline phosphatase and trehalase activities after electrophoresis on SDS/polyacrylamide gels under non-reducing conditions.

The brush borders prepared from proximal small intestine by the method of Forstner *et al.* (1968) gave a similar protein pattern on gels (Fig. 1a) to that obtained with membrane vesicles prepared by the method of Hopfer *et al.* (1973) (Fig. 2a). The only major differences in the high-molecular-weight region ($R_F < 0.5$) are the absence of peaks 3 and 4 from the gel of the vesicle preparation and the decrease of peak 7 to a shoulder. However, in the

lower-molecular-weight region ($R_F > 0.5$) several protein bands are absent from gels of the vesicle preparation, and this may be attributed to the removal of the fibrillar core proteins by the homogenization procedure, as originally observed in the electron microscope by Hopfer *et al.* (1973). Direct visual examination of the gels of brush-border membranes prepared from the proximal small intestine revealed that peak 1, just discernible as a split peak on the gels stained for glycoprotein (Figs. 1a and 2a), comprised two distinct sharp bands, so the designation of the glucoamylase-maltase-sucrase enzymic activity to one or both of these bands was initially not possible. Further examination of the same samples on 6% (w/v) acrylamide gels (instead of 7.5%) clearly separated the two bands, and all the enzymic activity migrated in a position corresponding to the faster band (gel scans not shown). Lactase activity coincides with glycoprotein band 5 and isomaltase-maltase-sucrase with glycoprotein band 6 (Fig. 2). The heterogeneity of rat intestinal alkaline phosphatase has previously been established (Malik & Butterworth, 1976) and is now confirmed by our finding of a broad region of alkaline phosphatase activity comprising several adjacent peaks of R_F values from 0.20 to 0.32, of which the major one at R_F 0.32 co-migrates with glycoprotein band 10. In contrast with the other glycosidases, the relative mobility of trehalase (R_F 0.58) is high, but this is consistent with its reported mol.wt. of 90000 on SDS/polyacrylamide gels (Sasajima *et al.*, 1975). On the 7.5% acrylamide gel system used in the present studies, proteins of this size form broad bands, so it is not possible to assign the enzymic activity to a single sharp protein band (Fig. 2). Nevertheless there is protein present in this region on the gel. No enzymic activity has yet been attributed to the other three major glycoprotein bands 2, 8 and 12, so these may be membrane structural components or additional intestinal enzymes that have been inactivated during electrophoresis. In particular, leucine naphthylamide-hydrolysing activity appears to be very sensitive to SDS (Maestracci *et al.*, 1975).

Although regions of enzymic activity have been shown to coincide with stained protein bands it was necessary to demonstrate that the enzymic activity was an intrinsic property of the major coincident protein band and not due to a small amount of very active protein masked by the other bands. In work with human brush-border membranes the absolute identity of enzymic activities with protein bands has been facilitated by the study of individuals who lack specific intestinal enzymic activities. From the cases examined so far, it has been shown that the absence of lactase (Freiburghaus *et al.*, 1976), sucrase-isomaltase (Preiser *et al.*, 1974; Freiburghaus *et al.*, 1977) and glucoamylase-maltase (H.-P. Hauri, unpublished work) activities is also accompanied by the disappearance of the corresponding major coincident

Fig. 1. Electrophoretic patterns for protein and glycoprotein on polyacrylamide gels of brush borders from the rat small intestine

The Forstner *et al.* (1968) method was used for the preparation of brush borders from rat proximal (a) and distal (b) small intestine. The gels, loaded with 50 μ g of protein, were run as described by Laemmli (1970). Protein was stained with Coomassie Blue and scanned at 550 nm (—); glycoprotein was stained with Schiff/periodate reagent and scanned at 520 nm (---).

protein band from the gels. Since similar enzyme deficiencies have not been described in laboratory animals, we have used the distal region of the rat small intestine as a model of a partially enzyme-deficient mucosa from the proximal small intestine. Previous studies with homogenized mucosal scrapings have shown that the activity of intestinal enzymes is decreased in the distal region of the small intestine (Dahlqvist, 1963; Stevens & Kidder, 1972), and this is confirmed by our own results (Table 1). Alkaline phosphatase, lactase and trehalase activities in the distal region are decreased to less than 5% of their values in the proximal small intestine, whereas the other enzymes are decreased to 30–70% of the activity in the proximal small intestine. Thus the mucosa of the distal small intestine may be regarded as partially enzyme-deficient and this should be

apparent in a comparison of the gel patterns of protein and enzymes from proximal (Figs. 1a and 2) and distal (Figs. 1b and 3) small-intestinal brush-border membranes. The disappearance of lactase from the distal mucosa is accompanied by the absence of the coincident glycoprotein peak 5. Therefore it appears that the major band 5 was the protein possessing the lactase enzymic activity. Similarly, the disappearance of peaks 10 and 11 from the gel of material prepared from the distal small intestine is concomitant with the decrease of alkaline phosphatase activity in the same region of the gel (Figs. 2 and 3). However, in view of the results of Booth & Kenny (1976), showing that the high activity of alkaline phosphatase in kidney microvillus membrane arises from a very low amount of protein, it is doubtful whether the intestinal alkaline phosphatase enzyme

Fig. 2. Protein, glycoprotein and enzyme patterns on polyacrylamide gels of brush-border membranes from the proximal region of the rat small intestine

Brush-border membrane vesicles were prepared by the method of Hopper *et al.* (1973). The gels, loaded with 50 μ g of protein, were run as described by Laemmli (1970). (a) Protein stained with Coomassie Blue and scanned at 550 nm (—); glycoprotein stained with Schiff/periodate reagent and scanned at 520 nm (----). (b) Enzyme pattern in eluates of a sliced corresponding half gel in arbitrary units. Isomaltase, measured with palatinose as substrate, is not shown, but low activity was found in a position corresponding to peak 6. The peak numbering system is based on that of the gel given in Fig. 1(a). ▲, Alkaline phosphatase; △, glucoamylase; ■, lactase; ●, maltase; ○, sucrase; ×, trehalase.

contributes significantly to the protein bands visible on a stained gel. A conclusion about trehalase is not possible, because the enzymic activity could not be assigned to a sharp protein band on the gels of material from the proximal small intestine (Fig. 2). Nevertheless the corresponding region on the gel of material from the distal small intestine lacks the low broad peaks present on the gel of material from the proximal small intestine (Fig. 3). Although precise quantitative measurement of the peaks on the gel scans was not attempted, the decrease in the activities of the other enzymes appears to be consistent with a decrease in the intensity of the stained protein bands.

Further evidence to support the identification of an enzymic activity with a stained protein band can be obtained from a comparison of the specific activity of an enzyme in the brush-border membrane with its specific activity in a pure form. The gel scans shown in

Fig. 2 were obtained with brush-border membranes, prepared by the method of Hopper *et al.* (1973), in which the specific activities of lactase and sucrase were 0.19 and 1.2 units/mg of protein respectively. Published data, for enzymes solubilized from the brush-border membrane with papain, give the specific activity of pure lactase as 22 units/mg of protein (Schlegel-Haueter *et al.*, 1972), and that of pure sucrase as 11.8 units/mg of protein (Kolinska & Kraml, 1972). From these data it can be calculated that the brush-border membrane comprises 0.86% lactase enzyme and 10.2% sucrase enzyme. Given the indirect method of deriving these values, they support the designation of lactase and sucrase enzymic activities to the major co-migrating bands on a gel. Unfortunately, this approach cannot be used to calculate the specific activity of enzymes in eluates from gels because certain uncharacterized contaminants, which react strongly with the protein

assay, are eluted out of the gel together with the proteins. However, the recovery of enzymic activity in the eluates could be measured and was as follows: alkaline phosphatase, 24–26%; glucoamylase, 17–24%; lactase, 8–14%; maltase, 8–16%; isomaltase, 7–17%; sucrase, 7–13%; trehalase, 17–35%.

A frequent anomaly observed throughout this work

has been that the gels of material from the proximal small intestine appeared to contain more protein than those of the distal region, despite the fact that they were always loaded with 50 μ g of protein. Given that the sample from the distal small intestine contains less of the high-molecular-weight components, the anomaly may arise from the additional lower-molecular-weight proteins being less well fixed and stained. It was also observed that the gels of material from the distal small intestine had a higher non-specific background staining than those containing material from the proximal small intestine. On the other hand it must be borne in mind that protein assays by the method of Lowry *et al.* (1951) and by dye staining are based on different properties of the proteins, so the two measurements need not necessarily agree. Moreover, it is well established that the binding of Coomassie Blue is variable from one protein to another (Fishbein, 1972; Bray & Thomas, 1975). A further basic difference between the samples prepared from proximal and distal small intestine was indicated by the considerably higher yield (5–7-fold) of proximal than of distal brush-borders obtained from equal weights of mucosa. This may be explained partly by

Table 1. Enzyme activities in mucosal homogenates from proximal and distal thirds of adult rat small intestine Results are mean values for two animals. Further details are given in the Methods section; 1 unit of enzymic activity hydrolyses 1 μ mol of substrate/min under the conditions specified for the assay.

Enzyme	Specific activity (units/g of protein)	
	Proximal	Distal
Alkaline phosphatase	2810	128
Glucoamylase	205	118
Lactase	28.1	1.2
Maltase	352	254
Isomaltase	15.9	7.1
Sucrase	71.2	21.6
Trehalase	199	8.1

Fig. 3. Protein, glycoprotein and enzyme patterns on polyacrylamide gels of brush-border membranes from the distal region of the rat small intestine

Brush-border membrane vesicles were prepared by the method of Hopfer *et al.* (1973). Other details and symbols are as in Fig. 2.

morphological differences between the proximal and distal regions of the small intestine, and also by the apparent greater fragility of distal brush borders, as discussed by Koldovsky (1969). However, the procedure of Forstner *et al.* (1968) for the isolation of brush borders from homogenates resulted in similar sucrase purification factors for proximal and distal tissue (11–16-fold), and an additional purification of 1.3–1.8-fold was achieved for both samples with the vesicle technique of Hopfer *et al.* (1973). This increase in purity obtained with the Hopfer *et al.* (1973) method was not at the expense of a specific loss of any of the active enzyme components from the brush-border membrane.

The enzyme and protein patterns given above for the intestinal brush border of the adult rat represent the final product of two distinct developmental processes. Postnatal changes in the enzyme complement of the intestinal membrane are well documented (Galand & Forstner, 1974; Kim *et al.*, 1975), as are the events occurring during the differentiation and maturation of the intestinal epithelial cells as they migrate from the crypts to villi tips (Weiser, 1973; Kim *et al.*, 1975). The molecular events accompanying these processes now require investigation. Further, the enzyme and protein scans given above closely resemble those obtained with human brush borders (Maestracci *et al.*, 1975; Freiburghaus *et al.*, 1976; Hauri *et al.*, 1977) and therefore the rat microvillus membrane may serve as a tentative model for the human one.

This work was supported by grants nos. 3.547-0.75 and 3.676-0.75 from the Swiss National Science Foundation. We are grateful to Professor B. Hadorn for his invaluable advice, to Mrs. C. Ryser for secretarial assistance, and to U. Luginbühl and A. Kúpfer for technical assistance.

References

- Alpers, D. H. (1972) *J. Clin. Invest.* **51**, 2621–2630
- Asp, N. G. & Dahlqvist, A. (1972) *Anal. Biochem.* **47**, 527–538
- Booth, A. G. & Kenny, A. J. (1976) *Biochem. J.* **159**, 395–407
- Bray, D. & Thomas, C. (1975) *Biochem. J.* **147**, 221–228
- Critchley, D. R., Howell, K. E. & Eichholz, A. (1975) *Biochim. Biophys. Acta* **394**, 361–376
- Dahlqvist, A. (1963) *Biochem. J.* **86**, 72–76
- Dahlqvist, A. (1968) *Anal. Biochem.* **22**, 99–107
- Fishbein, W. N. (1972) *Anal. Biochem.* **46**, 388–401
- Forstner, G. G., Sabesin, S. M. & Isselbacher, K. J. (1968) *Biochem. J.* **106**, 381–390
- Freiburghaus, A. U., Schmitz, J., Schindler, M., Rothauwe, H. W., Kuitunen, P., Launiala, K. & Hadorn, B. (1976) *N. Engl. J. Med.* **294**, 1030–1032
- Freiburghaus, A. U., Dubs, R., Hadorn, B., Gaze, H., Hauri, H. P. & Gitzelmann, R. (1977) *Eur. J. Clin. Invest.* **7**, 455–459
- Galand, G. & Forstner, G. G. (1974) *Biochem. J.* **144**, 281–292
- Garen, A. & Levinthal, C. (1960) *Biochim. Biophys. Acta* **38**, 470–483
- Green, J. R. & Hadorn, B. (1977) *Biochim. Biophys. Acta* **467**, 86–90
- Hauri, H. P., Kedinger, M., Haffen, K., Freiburghaus, A., Grenier, J. F. & Hadorn, B. (1977) *Biochim. Biophys. Acta* **467**, 327–339
- Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
- Kim, Y. S., Perdomo, J., Ochoa, P. & Isaacs, R. A. (1975) *Biochim. Biophys. Acta* **391**, 39–50
- Koldovsky, O. (1969) *Development of the Functions of the Small Intestine in Mammals and Man*, p. 10 and pp. 125–127, S. Karger, Basel
- Kolinska, J. & Kraml, J. (1972) *Biochim. Biophys. Acta* **284**, 235–247
- Laemmli, U. K. (1970) *Nature* **227**, 680–685
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maestracci, D., Preiser, H., Hedges, T., Schmitz, J. & Crane, R. K. (1975) *Biochim. Biophys. Acta* **382**, 147–156
- Malik, N. & Butterworth, P. J. (1976) *Biochim. Biophys. Acta* **446**, 105–114
- Preiser, H., Menard, D., Crane, R. K. & Cerda, J. J. (1974) *Biochim. Biophys. Acta* **363**, 279–282
- Raul, F., Simon, P., Kedinger, M. & Haffen, K. (1977) *Cell Tissue Res.* **176**, 167–178
- Sasajima, K., Kawachi, T., Sato, S. & Sugimura, T. (1975) *Biochim. Biophys. Acta* **403**, 139–146
- Schlegel-Haueter, S., Hore, P., Kerry, K. R. & Semenza, G. (1972) *Biochim. Biophys. Acta* **258**, 506–519
- Stevens, J. A. & Kidder, D. E. (1972) *Br. J. Nutr.* **28**, 129–137
- Weiser, M. M. (1973) *J. Biol. Chem.* **248**, 2536–2541