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Two-dimensional isoelectric focussing/sodium dodecyl sulphate polyacrylamide gel electrophoretic mapping and some molecular characteristics of the proteins of the adult guinea-pig small intestinal microvillus membrane

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(1) The adult guinea-pig small intestinal microvillus membrane was purified approximately 25-fold by both cation-precipitation and differential centrifugation methods. Comparison by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed no substantial differences in polypeptide composition between the two preparations. (2) One-dimensional SDS-PAGE and two-dimensional isoelectric focussing (IEF)/SDS-PAGE, together with Coomassie-blue, silver and lectin-staining, showed three major high molecular weight polypeptides, M_r 108000, 116000 and 127000, as well as a 47 kDa protein (actin), as major constituents of the membrane. The proteins of M_r 108000 and 116000 were strongly concanavalin A reactive. A detailed two-dimensional IEF/SDS-PAGE map of the membrane was constructed. (3) Sodium carbonate treatment showed the two concanavalin A-reactive glycoproteins, M_r 108000 and 116000, comprising the sucrase-isomaltase complex, to be loosely-associated 'extrinsic' microvillus membrane proteins. Two proteins, M_r 127000 and 135000, were tightly-associated 'intrinsic' microvillus proteins. (4) Despite regional differences in specific activity of some small intestinal microvillar enzymes, most noticeably enterokinase (EC 3.4.21.9) and dipeptidyl peptidase IV (EC 3.4.14.x), no substantial regional differences were seen in microvillus membrane polypeptide composition. In contrast, a substantial increase in the major high molecular weight proteins of M_r 108000 and 116000 accompanied a 10-fold rise in sucrase-isomaltase activity, and loss of a major protein of M_r 131000 accompanied the complete loss of lactase activity from the membrane during postnatal development.

Introduction

The mammalian small intestinal epithelium is comprised of several distinct cell populations. Within the crypts of Lieberkuhn, basally-positioned stem cells divide and produce a migratory population of immature enterocytes which subsequently move out of the crypts onto the villus surface [1,2]. During this crypt-villus transition, the immature absorptive enterocyte gradually differentiates, acquiring an elaborate apical cell surface membrane (the brush-border or microvillus membrane). More than 90% of the cells within the small intestinal epithelium at any one time belong to the differentiated absorptive cell population (discussed in Ref. 3). The relative uniformity of the differentiated cell population, together with reliable methods of microvillus membrane preparation, make the absorptive enterocyte an excellent cell type in which to study both the differentiation of, and vectorial distribution of polypeptides to, the apical cell surface.

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Much is known about the enzymology of the mammalian small intestinal brush-border and many specific hydrolases have been isolated and characterised [4]. In addition, interest in the microvillus as an example of cell surface specialisation has led to the characterisation of some of its cytoskeletal components [5–10]. However, no attempt has been made to systematically map the microvillus membrane polypeptides, as has been done with the differentiating lymphocyte cell surface [11]. Such a detailed analysis of the components of the differentiated microvillus membrane would enable the often complex changes accompanying development and differentiation of the intestinal cell surface to be more fully appreciated and reliably compared at a molecular level.

The present study was undertaken with the aim of identifying polypeptide changes in the microvillus membrane which might be amenable to further investigation using molecular cloning techniques [12]. In a previous study, we examined the changing polypeptides of the guinea-pig microvillus membrane during foetal development using one-dimensional SDS-PAGE and two-dimensional IEF/SDS-PAGE [13]. Here, we report the molecular characterisation of the polypeptides present in the adult guinea-pig microvillus membrane, together with an analysis of the major changes which occur in this membrane during the postnatal transition to the adult phenotype. Some of the biochemical characteristics of the major adult microvillus membrane proteins are also reported.

Materials and Methods

Microvillus membrane preparation by differential centrifugation and cation-precipitation methods

Microvillus membranes were purified from the adult guinea-pig small intestinal mucosa by differential centrifugation essentially as described in Ref. 14, using the homogenisation conditions described in Ref. 15. All procedures were performed at 4°C.

Intestinal segments were washed briefly with physiological saline and the mucosa scraped with a scalpel blade into homogenisation buffer (8.5% (w/w) sucrose, 1 mM $NaHCO_3$, approx. 10 ml per g tissue). An initial homogenisation with 5 strokes of a loose-fitting Dounce homogeniser was followed by 20 strokes of a Potter-Elvehjem homogeniser with a teflon pestle, rotating at 800 rpm. Filtration through nylon mesh (40 μm diameter) was followed by low-speed centrifugation (1000 × g, 10 min, MSE Mistral centrifuge). Resuspension of the resulting pellet in a similar volume of homogenisation buffer using a further 5 strokes of the loose-fitting Dounce homogeniser was followed by recentrifugation (1000 × g, 10 min). This step was repeated until the supernatant was substantially clear. The resulting pellet was resuspended at a protein concentration not exceeding 8 mg/ml in 25% (w/w) sucrose containing 1 mM $NaHCO_3$, layered on a 30/40/60% (w/w) sucrose step gradient and centrifuged for 3 h (Beckman SW28 rotor, 100 000 × g_{av}). Material collecting at the 40/60% interface was vigorously homogenised with 20 strokes of the Potter-Elvehjem homogeniser rotating at 2000 rpm, layered on a continuous 30–60% (w/w) sucrose gradient and centrifuged overnight (Beckman SW28 rotor, 100 000 × g). The gradient was fractionated and the density of each fraction determined using an Abbe refractometer. Aminopeptidase activity was used routinely as a marker enzyme activity for the microvillus membrane [16]. Purified microvillus membrane vesicles banded at an isopycnic sucrose density of approx. 1.20 g/ml.

Preparation of adult guinea-pig microvillus membrane vesicles by Mg^{2+} precipitation was exactly as described in Ref. 13, based upon the procedures of Kessler et al. [17] as modified in Ref. 18. Usually, no inhibitors of proteolytic enzymes were included during either method of preparation, since many of the enzymes whose activities were later to be determined were themselves proteases. In preliminary experiments to test for proteolysis none was observed while the vesicles were intact (i.e. in the absence of detergents) and kept rigorously at 4°C, an observation in accordance with that of Gains and Hauser [18].

SDS-polyacrylamide gel electrophoresis

One-dimensional SDS-polyacrylamide gel electrophoresis was performed using slabs of 7.5% polyacrylamide as described in Ref. 19 under reducing conditions. Protein molecular weights were estimated by reference to the migration of standard proteins (carbonic anhydrase, 29 000;

ovalbumin, 45 000; bovine serum albumin, 68 000; rabbit muscle phosphorylase *b*, 97 400 daltons; *Escherichia coli* β-galactosidase, 116 000 and rabbit muscle myosin, 205 000, all obtained from Sigma Chemicals, Poole, Dorset). Two-dimensional isoelectric focussing (IEF)/sodium dodecyl sulphate-polyacrylamide gel electrophoretic (SDS-PAGE) separations were performed essentially as described in Ref. 20, using mixtures of pH 3.5–10 and pH 6–8 ampholines (3 : 1, v/v, LKB products) for the IEF dimension and 10% acrylamide slab gels for the second, SDS-PAGE dimension.

Glycoprotein detection using concanavalin A

Transfer of electrophoretically separated proteins from SDS-polyacrylamide gels to nitrocellulose, and the subsequent identification of concanavalin A-binding glycoproteins was performed using horseradish peroxidase as described in Ref. 21.

Enzymatic determinations

Aminopeptidase (EC 3.4.11.2) and dipeptidyl peptidase IV (EC 3.4.14.x) assays were performed as described in Refs. 22 and 23, respectively. Alkaline phosphatase (EC 3.1.3.1) was assayed according to the method of Engstrom [24] as modified in Ref. 25. Lactase (EC 3.2.1.23) and sucrase (EC 3.2.1.48) were assayed by the methods of Ho et al. [26] and of Dahlqvist [27], respectively. Enterokinase (EC 3.4.21.9) was assayed as in Ref. 28, and protein was measured by the method of Lowry et al. [29].

Sodium carbonate disruption of membrane integrity

Microvillus membrane vesicles were treated with sodium carbonate as described in Ref. 30 to separate content proteins and peripheral membrane proteins from integral membrane components. A similar approach to investigate the biosynthesis of cytoskeletal components of the pig microvillus membrane has been reported in Ref. 31.

Results and Discussion

Preparation of the microvillus membrane

Two methods of microvillus membrane pre-

TABLE I

SPECIFIC ACTIVITIES, PURIFICATIONS AND RECOVERIES OF MICROVILLAR HYDROLASE ACTIVITIES IN MICROVILLUS MEMBRANE VESICLES

The vesicles were prepared from adult guinea-pig small intestine by differential centrifugation followed by isopycnic sucrose density gradient centrifugation, as described in the text. Values are the means of three guinea-pigs. (1 unit = 1 μmol substrate hydrolysed per min)

Enzyme	Fraction	Mean specific activity (units/mg)	Enrichment (-fold)	Recovery (%)
Aminopeptidase	Mucosal homogenate	0.06	1.0	100
	Purified vesicles	1.12	18.7	14
γ-Glutamyl transpeptidase	Mucosal homogenate	$2.8 \cdot 10^{-3}$	1.0	100
	Purified vesicles	$77 \cdot 10^{-3}$	27.2	22
Alkaline phosphatase	Mucosal homogenate	179	1.0	100
	Purified vesicles	3286	18.4	14
Sucrase	Mucosal homogenate	0.42	1.0	100
	Purified vesicles	6.95	16.5	12
Isomaltase	Mucosal homogenate	0.33	1.0	100
	Purified vesicles	4.36	13.1	9
Dipeptidyl peptidase IV	Mucosal homogenate	0.015	1.0	100
	Purified vesicles	0.385	25.2	17
Enterokinase	Mucosal homogenate	$0.11 \cdot 10^{-3}$	1.0	100
	Purified vesicles	$0.72 \cdot 10^{-3}$	6.6	5

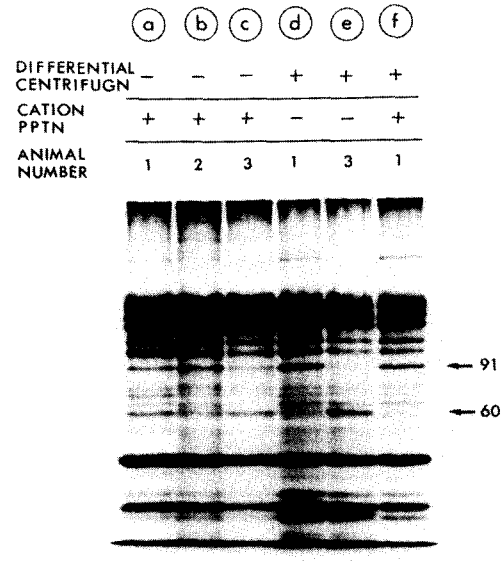

Fig. 1. Comparison of Mg^{2+} precipitation and differential centrifugation techniques for microvillus membrane preparation. Mucosal scrapings from different segments of small intestine of three guinea-pigs were halved and microvillus membrane vesicles prepared by either Mg^{2+} precipitation (tracks a–c), or differential centrifugation (tracks d, e). One sample (track f) was subjected to both differential centrifugation and Mg^{2+} precipitation prior to final purification. Purified vesicles (300 μg protein) were derivatised with SDS in the presence of β-mercaptoethanol and the component polypeptides separated by SDS-PAGE using 7.5% acrylamide slabs and stained with Coomassie blue. The observed polypeptide compositions were virtually identical. Only two proteins (arrowed) showed variation between animals. This variation was reported by both techniques and was due to the use of mucosal scrapings from different regions of the three intestines (see Fig. 5).

paration from the adult guinea-pig small intestine were compared, one based upon low-speed differential centrifugation to isolate purified brush-border membranes which were then vesicularised and subjected to isopycnic sucrose density gradient centrifugation ([14], based upon the classical studies of Neville [32] and Coleman and Finean [33]), the other based upon magnesium precipitation to remove contaminating intracellular membranes again followed by isopycnic sucrose density gradient centrifugation ([13], based upon Refs. 34, 17 and 18). Both techniques yielded a prominent, well-separated, monodisperse membrane band in the final sucrose gradient. This band was highly enriched in the microvillus membrane marker enzymes aminopeptidase, sucrase, alkaline phosphatase, dipeptidyl peptidase IV and, in the case of the neonatal membrane, lactase. The purity of these microvillus membrane fractions was comparable to those previously reported by other investigators (Table I). Although differential centrifugation or cation precipitation alone have proved sufficient for good purification of the chick and rabbit microvillus membrane (Refs. 5 and 17, respectively), in our experience using the guinea-pig, a final sucrose gradient centrifugation step was essential to remove other contaminating membrane bands of lighter buoyant density than the microvillus membrane from the preparation.

A direct comparison by one-dimensional SDS-PAGE of the polypeptides present in microvillus membrane vesicles isolated by these two methods used in parallel (Fig. 1) showed very few differences in polypeptide composition. Two polypeptides, M_r 91 000 and 60 000, did vary between preparations but were found in subsequent experiments to be differentially-enriched in the distal and proximal regions of the small intestine, respectively. Their abundance reflected the region of the intestine used for microvillus membrane preparation. Vesicularised brush-border membranes prepared by differential centrifugation were also subjected to a Mg^{2+} precipitation step prior to isopycnic sucrose gradient centrifugation, but no additional purification of the final microvillus membrane fraction was observed by SDS-PAGE (Fig. 1f).

Polypeptide components of the microvillus membrane

Separations of adult guinea-pig microvillus membrane polypeptides by one-dimensional SDS-PAGE followed by Coomassie-blue staining (Fig. 2a) and silver-staining (Fig. 2b) showed three major high molecular weight polypeptides, M_r 108 000, 116 000 and 127 000, in addition to a very prominent polypeptide, M_r 47 000, presumed to be actin. Several polypeptides showed differential staining with the two procedures and many assumed characteristic hues when subjected to silver staining.

In parallel experiments, glycoprotein components were identified by concanavalin A overlay techniques [21]. The two major high molecular weight proteins of M_r 108 000 and 116 000 were

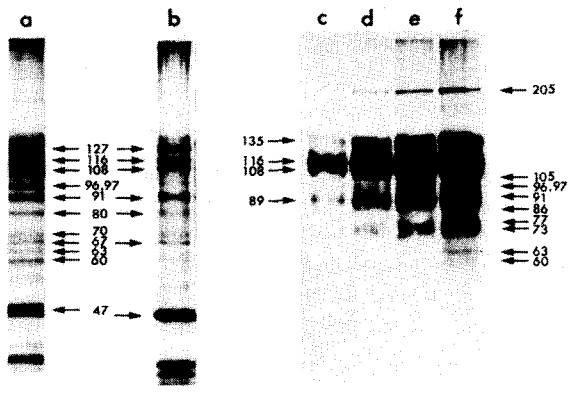

Fig. 2. Polypeptide composition of adult guinea-pig microvillus membrane vesicles. Microvillus membrane vesicle polypeptides, separated by SDS-PAGE in 7.5% acrylamide gels, were stained for proteins with either Coomassie blue (track a), or a modified silver reagent (track b) and for glycoprotein components by concanavalin A overlaying of nitrocellulose transfers (tracks c–f). Protein loading in tracks a–f was 300, 50, 50, 100, 150 and 200 μg, respectively. Molecular weights ($\times 10^{-3}$) of both major and minor constituents are indicated.

strongly concanavalin A reactive (Fig. 2c–f). Several weakly reactive or minor glycoproteins could also be distinguished (indicated in Fig. 2).

Two-dimensional IEF/SDS-PAGE mapping of the microvillus membrane polypeptides

Further characterisation of microvillus membrane polypeptides was achieved by two-dimensional IEF/SDS-PAGE performed essentially as described in Ref. 20. Most of the major high molecular weight polypeptides possessed complex charge distributions, especially those of M_r 89000, 116000, 127000 and 135000. Separation of the 108 kDa polypeptide in the IEF dimension proved difficult. This protein either remained at the top of the first dimension gel, or appeared as a diffuse streak in the second dimension (Fig. 3a). In contrast, many of the smaller polypeptides were present as discrete spots. Concanavalin A overlays detected several major glycosylated components, most notably those of 89, 116 and 135 kDa (Fig. 3b).

Integration of the major polypeptides within the microvillus membrane

Microvillus membrane vesicles were treated with sodium carbonate as described for microsomal membranes in Ref. 30. Such treatment removes loosely-associated (extrinsic) polypeptides from both faces of the opened membrane lamellae. Several microvillus polypeptides were removed almost quantitatively by Na_2CO_3 treatment, most noticeably the two major high molecular weight polypeptides, M_r 108000 and 116000 (Fig. 4, b and c). The two microvillus proteins of M_r 127000 and 135000 remained tightly associated with the membrane, as did all the other minor concanavalin A-reactive glycoproteins. Several non-glycosylated components, most notably the majority (but not all) of the M_r 47000 protein (actin), were also removed from the membrane. These may be cytoskeletal components [31].

It was surprising to find that the polypeptides of M_r 108000 and 116000 were released from the microvillus membrane by Na_2CO_3 treatment since these polypeptides comprise the two subunits of the sucrase-isomaltase complex (Fig. 4e). In the rabbit, one of these polypeptides (isomaltase, M_r 140000) is thought to be anchored into the microvillus membrane via an amino-terminal hydrophobic sequence [35,36]. The release of sucrase-isomaltase from the guinea-pig microvillus membrane may reflect a species difference, since the guinea-pig enzyme is slightly smaller (108 and 116 kDa) than the rabbit enzyme (120 and 140 kDa)

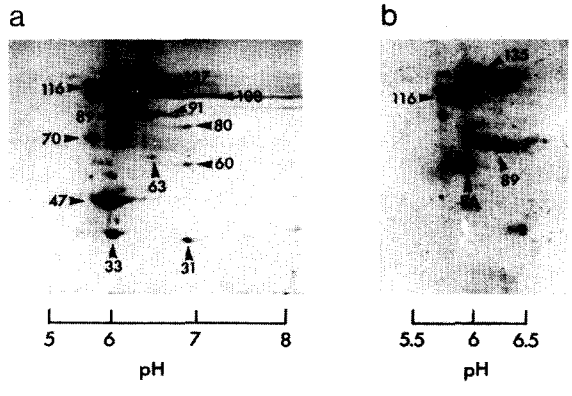

Fig. 3. 2D-IEF/SDS-PAGE mapping of the adult guinea-pig microvillus membrane polypeptides. Microvillus membrane vesicle polypeptides (300 and 100 μg, respectively) were separated by 2D-IEF/SDS-PAGE, and stained using (a), Coomassie blue, and (b), concanavalin A overlaying after transfer to nitrocellulose.

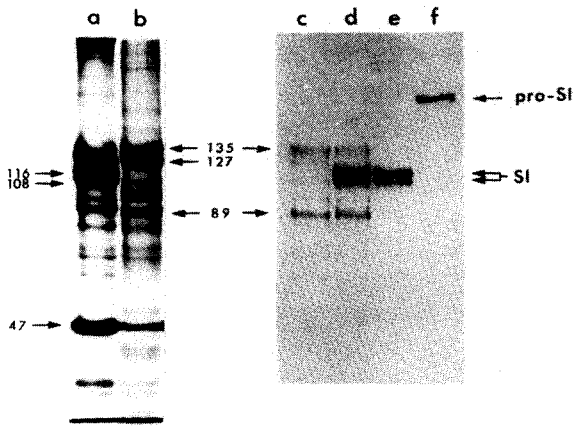

Fig. 4. Na_2CO_3 treatment of microvillus membrane vesicles. Microvillus membrane vesicles were treated with 100 mM Na_2CO_3, as described in Methods. Track (a), polypeptide components of microvillus membranes before treatment; track (b), polypeptide components of Na_2CO_3-treated membranes. Concanavalin A-reactive glycoproteins of treated (track c) and untreated (track d) membranes are shown together with purified sucrase-isomaltase (dimeric detergent-form, track e; precursor form, pro-sucrase-isomaltase, track f). The molecular weights ($\times 10^{-3}$) of the major Na_2CO_3-extracted and Na_2CO_3-resistant proteins are indicated.

and this size difference may reflect the cleavage of its membrane anchor. Experiments are underway to investigate more closely the extent of membrane-association of the sucrase-isomaltase complex using guinea-pig microvillus membrane vesicles isolated in the presence of proteolytic enzyme inhibitors.

Regional distribution of microvillus membrane-associated hydrolytic activities and polypeptides

The specific activities of the microvillus enzymes aminopeptidase, sucrase, isomaltase, dipeptidyl peptidase IV, γ-glutamyl transpeptidase, enterokinase and alkaline phosphatase were compared in microvillus membrane vesicles prepared from six regions of the adult guinea-pig small intestine, approximately equal in length, corresponding to the duodenum (region 1), proximal and distal jejunum (regions 2 and 3), and proximal, mid- and distal ileum (regions 4–6) (Table II). Only two enzymes showed significant regional change in specific activity, enterokinase (which was restricted to the duodenum and proximal jejunum) and dipeptidyl peptidase IV (which showed a gradual increase in specific activity towards the ileum). In parallel experiments using one-dimensional SDS-PAGE, no polypeptides were seen to be exclusively localised to any one region of the small intestine (Fig. 5), although one polypeptide, M_r 91 000, was predominantly localised to the distal intestine, while another, M_r 60 000, was predominantly localised to the proximal small

TABLE II

DISTRIBUTION OF MICROVILLAR HYDROLASE ACTIVITIES ALONG THE ADULT GUINEA-PIG SMALL INTESTINE

Activities were determined in microvillus membrane vesicles prepared by differential centrifugation followed by isopycnic sucrose density gradient centrifugation, as described in the text. Values are the means (±S.E.) of five guinea-pigs.

Enzyme	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6
Aminopeptidase (nmol pNA/h per μg)	35.3± 5.3	39.0± 4.5	46.2± 5.7	45.7± 7.5	41.6± 6.9	48.4± 5.3
γ-Glutamyl transpeptidase (nmol pNA/h per μg)	7.6± 1.6	10.5± 2.0	12.9± 1.8	11.6± 2.1	10.1± 2.0	7.8± 2.0
Alkaline phosphatase (μmol pNP/h per μg)	160 ±34	155 ±22	183 ±22	185 ±27	194 ±22	219 ±15
Sucrase (nmol glucose/h per μg)	43.3± 4.8	37.3± 5.9	36.2± 6.6	35.1± 5.6	37.2± 4.5	37.8± 6.0
Isomaltase (nmol glucose/h per μg)	44.2± 4.3	38.8± 4.5	32.3± 4.2	32.9± 4.3	35.2± 2.9	35.1± 3.6
Dipeptidyl peptidase IV (nmol pNA/h per μg)	12.4± 2.5	13.7± 2.1	18.9± 3.2	21.3± 3.4	25.2± 3.3	30.1± 4.1
Enterokinase (pmol trypsin/h per μg)	70.6±20.9	10.0± 6.0	3.1± 2.0	1.1± 0.9	0.1± 0.1	0

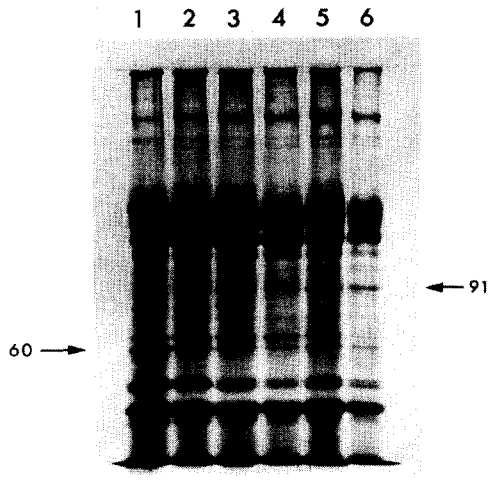

Fig. 5. Polypeptide composition of microvillar membrane vesicles prepared from different regions of the adult guinea-pig small intestine. The component polypeptides of microvillar membrane vesicles prepared from the same 6 regions of the small intestine as used for the data in Table II were separated by SDS-PAGE using 7.5% acrylamide slab gels. Regional differences in polypeptide composition are indicated.

intestine. These results are in agreement with those for the human small intestine reported by Skovbjerg [37] and Triadou et al. [38].

Postnatal rearrangements of microvillus polypeptide expression

Microvillus membrane vesicles prepared from the small intestine of guinea-pigs of different postnatal ages were analysed by one-dimensional SDS-PAGE (Fig. 6) and the polypeptide profiles compared to the changing patterns of enzyme expression during the same period (Table III). Several changes in microvillus membrane polypeptides were seen during this time, most noticeably the gradual loss of a major polypeptide, M_r 131 000, and the concomitant increase in level of the two major polypeptides of M_r 108 000 and 116 000 (Fig. 6). Parallel changes were observed over the same period in both lactase and sucrase-isomaltase activities. Both enzymes have been purified to homogeneity in our laboratory which has confirmed the 131 kDa protein as lactase (McAllister, G. and Bailey, D.S., unpublished

TABLE III

MICROVILLAR HYDROLASE ACTIVITIES DURING POSTNATAL DEVELOPMENT OF THE GUINEA-PIG SMALL INTESTINE

Activities were determined in microvillus membrane vesicles prepared by differential centrifugation followed by isopycnic sucrose density gradient centrifugation, as described in the text. Also shown is the change in isopycnic density displayed by the purified microvillar membrane vesicles. Values are the means (±S.D.) of three guinea-pigs allowed to suckle ad libitum during the postnatal period.

Enzyme	3 day	10 day	17 day	24 day	40 day	Adult
Aminopeptidase (nmol pNA/h per μg)	29.7± 4.7	60.7± 4.5	58.7± 1.1	61.2±1.7	51.4± 8.9	42.7± 9.6
γ-Glutamyl transpeptidase (nmol pNA/h per μg)	6.0± 2.7	2.2± 0.1	4.1± 0.1	3.3±0.3	2.8± 0.6	10.1± 2.5
Alkaline phosphatase (μmol pNP/h per μg)	195 ±32	225 ±20	163 ±20	163 ±2	147 ±15	183 ±38
Sucrase (nmol glucose/h per μg)	3.5± 0.7	6.5± 0.9	6.7± 0.3	10.6±0.4	37.9± 4.8	38.0±10
Isomaltase (nmol glucose/h per μg)	3.7± 1.6	5.6± 1.5	8.3± 0.7	12.0±0.8	41.1± 4.5	35.7± 6.3
Dipeptidyl peptidase IV (nmol pNA/h per μg)	22.8± 4.9	25.0± 2.5	30.0± 3.1	26.0±2.1	19.0± 2.3	20.2± 4.9
Enterokinase (pmol trypsin/h per μg)	8.5± 6.8	32.8± 1.8	8.0± 4.3	20.6±3.9	11.5± 1.2	14.1± 8.0
Lactase (nmol galactose/h per μg)	28.3± 1.4	22.8± 4.4	7.6± 3.7	3.5±0.8	0.1± 0.05	0
Isopycnic density (w/w sucrose)	40.2± 1.1	41.9± 0.4	43.5± 0.1	43.8±0.3	44.0± 0	44.5± 0.6

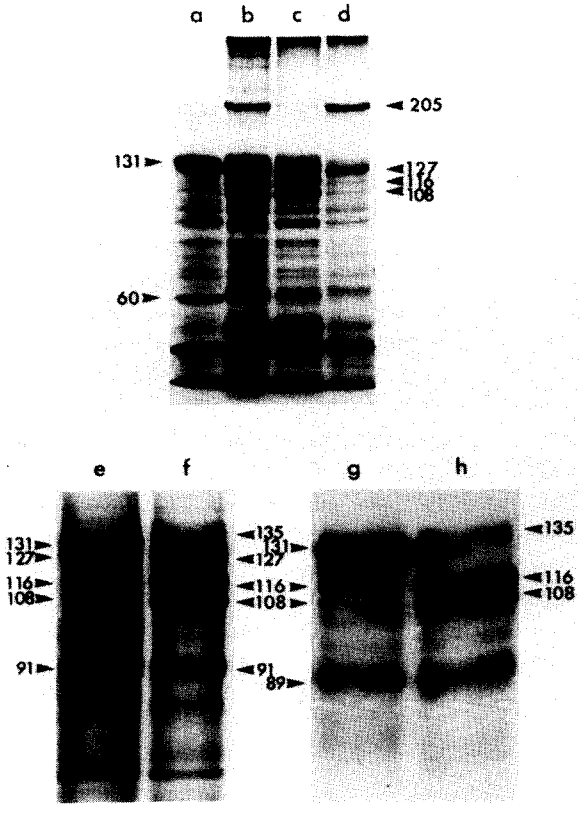

Fig. 6. Polypeptides of the microvillus membrane during postnatal development. Microvillus membrane vesicles were isolated from the small intestine of 3-day-old, 13-day-old and 40-day-old guinea-pigs and their polypeptides compared with those of microvillus membrane vesicles prepared from Thiry-Vella loops of adult (day 40) guinea-pig small intestine (tracks a–d, respectively). Detailed comparison of the day 3 and day 40 polypeptides was performed by silver-staining (tracks e, f) and concanavalin A overlaying (tracks g, h). The major developmentally-specific polypeptides are indicated.

data), and the 108/116 kDa complex as sucrase-isomaltase (Ref. 39 and Fig. 4c). Although sucrase-isomaltase showed a substantial postnatal increase in both enzymatic activity and polypeptide concentration in the microvillus membrane, both activity and polypeptides of the complex were present at birth (Fig. 6, a, e and g). This observation confirms our previous finding that sucrase activity can be detected in the early foetal small intestine [13]. Thus sucrase-isomaltase represents a microvillus membrane component whose level of expression is greatly amplified during postnatal development, but which is not induced de novo at that time. In contrast, lactase, both as an enzyme activity and as a polypeptide, is not found in the early foetal guinea-pig small intestine [13] and is completely lost during postnatal development. Guinea-pig lactase thus represents a transient microvillus membrane component.

Another interesting observation concerns expression of pro-sucrase-isomaltase. The detergent form of this enzyme can be isolated by its affinity for sepharose, as previously described for the cleaved subunits [39] and has an M_r of 205 000 (Fig. 4f). In isolated Thiry-Vella loops, the majority of the sucrase activity is present as the uncleaved precursor enzyme (Fig. 6d), and it is from such isolated intestinal loops that successful purification of the precursor in large yields has been achieved (Christianson and Bailey, unpublished work). In the adult intestine (Fig. 6c) most of the sucrase-isomaltase exists as the cleaved enzyme. However, at early times during postnatal development very substantial amounts of the precursor form may be detected (Fig. 6b). We have confirmed this result by immunoblotting using a rabbit antiserum specific for sucrase (data not shown). Such incomplete proteolytic processing suggests that the cleavage enzyme which breaks pro-sucrase-isomaltase into its two subunits (thought to be elastase [40]) is not fully active during early postnatal life. The role of a pancreatic protease in this process, as previously suggested by experiments using pancreatic duct ligation [41], is consistent with the absence of substantial cleavage in the isolated Thiry-Vella loop (Fig. 5d).

In conclusion, the present study of the guinea-pig small intestinal microvillus membrane has identified major rearrangements in the polypeptide structure of this membrane domain during postnatal development. Such changes have been precisely mapped using two-dimensional IEF/SDS-PAGE techniques in conjunction with concanavalin A-overlaying to identify the major concanavalin A-reactive glycoproteins. Comparison of these results with previous work from our laboratory using similar two-dimensional techniques [13] enables a clear appreciation of the complex changes which occur during both foetal and postnatal development of this highly specialised cell surface domain. Of equal interest, however, is the con-

servation of certain polypeptides, most notably those of molecular weights 91 000, 89 000, 80 000, 70 000, 63 000, 47 000, 33 000 and 31 000 (Fig. 3a) within this domain throughout development. Such proteins may be structural components of the microvillus, showing relatively little developmental change.

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