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# Biosynthesis of intestinal microvillar proteins

## Pulse–chase labelling studies on aminopeptidase N and sucrase–isomaltase

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The biogenesis of two microvillar enzymes, aminopeptidase N (EC 3.4.11.2) and sucrase (EC 3.2.1.48)–isomaltase (EC 3.2.1.10), was studied by pulse–chase labelling of pig small-intestinal explants kept in organ culture. Both enzymes became inserted into the membrane during or immediately after polypeptide synthesis, indicating that translation takes place on ribosomes attached to the rough endoplasmic reticulum. The earliest detectable forms of aminopeptidase and sucrase–isomaltase were polypeptides of $M_r$ 140000 and 240000 respectively. These polypeptides were susceptible to treatment with endo-β-*N*-acetylglucosaminidase H (EC 3.2.1.96), suggesting that the microvillar enzymes during or immediately after completion of protein synthesis become glycosylated with a 'high-mannose' oligosaccharide structure similarly to other plasma-membrane and secretory proteins. After 20–40 min or 60–90 min of chase, respectively, aminopeptidase N and sucrase–isomaltase were reglycosylated to give the polypeptides of $M_r$ 166000 (aminopeptidase N) and 265000 (sucrase–isomaltase). These were expressed at the microvillar membrane after 60–90 min. During the entire process of synthesis and transport to the microvillar membrane the enzymes were bound to membranes, indicating that the biogenesis of aminopeptidase N and sucrase–isomaltase occurs in accordance with the membrane flow hypothesis.

Intestinal aminopeptidase N (EC 3.4.11.2) and sucrase (EC 3.2.1.48)–isomaltase (EC 3.2.1.10) are the best characterized enzymes of the microvillar membrane. Both have been purified as their detergent-solubilized forms, and their structural properties have been studied in great detail (reviewed by Kenny & Maroux, 1982). Less, however, is known about the biogenesis of these or any other of the microvillar enzymes. By using labelling *in vivo* in the rat, Hauri *et al.* (1979) found that sucrase–isomaltase rapidly appears in the Golgi-membrane fraction as a single large polypeptide. Furthermore, it has been observed that sucrase–isomaltase, purified from enterocytes that had not been exposed to pancreatic enzymes, was exclusively in the form of the single large polypeptide (Sjöström *et al.*, 1980; Hauri *et al.*, 1980).

We previously reported the finding of new forms of aminopeptidase N and sucrase–isomaltase with different $M_r$, obtained from a $Ca^{2+}$-precipitated fraction of enterocytes (Danielsen *et al.*, 1981*a*). From their location and the relatively small amounts present, these enzyme forms were suggested to represent precursor forms of the final microvillar enzymes. The observation that some of the $Ca^{2+}$-pellet polypeptides of aminopeptidase N, sucrase–isomaltase and two other microvillar enzymes, maltase–glucoamylase (EC 3.2.1.20) and lactase (EC 3.2.1.23)–phlorizin hydrolase (EC 3.2.1.62), are susceptible to treatment with endo-β-*N*-acetylglucosaminidase H further strengthened this view (Danielsen *et al.*, 1981*b*).

Organ culture is a widely used technique to maintain intestinal explants for periods up to 24 h (Browning & Trier, 1969). We have applied the system to pig intestinal explants, which were kept viable for 24 h, during which period the microvillar enzymes were radioactively labelled (Danielsen *et al.*, 1982*a*). In the present work pulse–chase labelling experiments were performed with pig intestinal explants in organ culture in order to study the biogenesis of aminopeptidase N and sucrase–isomaltase.

### Materials and methods

#### *Materials*

Endo-β-*N*-acetylglucosaminidase H [EC 3.2.1.96,



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from *Streptomyces griseus*; 30 units (μmol/min)/mg; free of exoglycosidase and proteinase activities] was from Seikagaku Kogyo Co., Tokyo, Japan. The sources of other chemicals, radiochemicals, media and intestines were as previously described (Sjöström *et al.*, 1978, 1980; Danielsen *et al.*, 1982*a*).

*Pulse–chase labelling of explants in organ culture*

Intestinal explants (1.5–2 mg wet wt.) were excised from an intestinal segment, taken 2–3 m from the pylorus immediately after the animal was killed. Within 15 min organ culture of the explants was started on stainless-steel grids placed in Falcon dishes, by the method of Browning & Trier (1969) as described by Danielsen *et al.* (1982*a*). Ten explants were placed on each grid and, after culture, explants from the same grid were pooled and subsequently processed as one single sample.

Before starting the pulse–chase experiments, explants were cultured in the non-radioactive medium for 0.75–1.5 h. Explants were radioactively pulse-labelled by placing them in a Falcon dish where [$^{35}$S]methionine had been added to the medium (200 μCi/ml). After 10 min of labelling, the explants (still on the grid) were blotted by gently pressing a paper tissue against the underside of the grid, then briefly washed in non-radioactive medium, dried again with paper tissue and finally placed in a Falcon dish with non-radioactive medium for various periods of time (chase period). In some experiments, the chase medium additionally contained either cycloheximide (1 mg/ml) or an increased concentration of non-radioactive methionine (2.5 mM).

The culture was stopped by washing the explants (still on the grid) with 3 × 1 ml of ice-cold Hanks' buffered salt solution. Immediately afterwards the explants were frozen at −80°C, at which temperature they were kept until further processing.

*Fractionation of pulse-labelled explants*

Unless otherwise stated, all procedures were performed at 4°C. Pulse-labelled explants were thawed in 1 ml of 12 mM-Tris/HCl, pH 7.1, containing 0.30 M-mannitol, and homogenized in a Potter–Elvehjem homogenizer by 15–20 strokes of the pestle, operated at 1700 rev./min. In some experiments, the homogenate was centrifuged at 125000 ***g*** for 1 h, to obtain a supernatant (the soluble protein fraction) and a pellet (the total membrane fraction). In other experiments, 2 ml of water was added to the homogenate, which was then centrifuged at 500 ***g*** for 10 min. The supernatant was collected and $CaCl_2$ was added (final concn. 10 mM). After 15 min incubation, the preparation was centrifuged at 2000 ***g*** for 10 min. The pellet (the $Ca^{2+}$-precipitated membrane fraction) was collected and the supernatant centrifuged at 48000 ***g*** for 1 h. The pellet (the microvillar membrane fraction) was collected (Kessler *et al.*, 1978).

The total membrane fraction, the $Ca^{2+}$-precipitated membrane fraction and the microvillar membrane fraction were each resuspended in 100 μl of 50 mM-Tris/HCl, pH 8.0, and solubilized by the addition of 100 μl of 10% (w/v) Triton X-100. After incubation for 1 h, the preparations were centrifuged at 48000 ***g*** for 1 h and the supernatants (solubilized membrane fractions) were stored at −20°C until use.

In one experiment, a total extract of explant protein was prepared as follows. Explants were homogenized and solubilized in 200 μl of 25 mM-Tris/HCl, pH 7.3, containing 5% (w/v) Triton X-100, by ultrasonication (40 kHz; instrument from Firma Goof, Hørsholm, Denmark) for 30 s. After incubation for 1 h, the homogenate was centrifuged for 5 min in a Beckman Microfuge and the supernatant collected and immediately used in the immunopurification of the enzymes.

*Immunopurification of the enzymes*

Aminopeptidase N and sucrase–isomaltase from the soluble protein fraction and the solubilized membrane fractions were purified by line immunoelectrophoresis against specific antisera raised to the respective microvillar enzymes. To samples of the soluble protein fraction and the solubilized $Ca^{2+}$ precipitated membrane fraction were added 5 μl (approx. 10 μg) of Triton X-100-solubilized, but not radioactively labelled, microvillar membranes before the immunopurification in order to obtain immunoprecipitates detectable by protein staining. This was performed as described by Danielsen *et al.* (1982*a*) with the following modification: after immunoelectrophoresis, the precipitates were stained with Coomassie Brilliant Blue R250 (Axelsen *et al.*, 1973). The agarose gel was then partly re-swollen by immersing it in water for 5 min and the precipitates were excised. This modification of the immunopurification procedure allowed accurate excision of precipitates obtained from samples with small contents of the enzymes. The presence in the excised immunoprecipitate of Coomassie Brilliant Blue R250 in the subsequent polyacrylamide-gel electrophoresis did not influence the mobility of the polypeptides, as the dye was dissociated from the protein during the denaturation of the samples and therefore migrated at the front.

In the experiments with endo-β-*N*-acetylglucosaminidase H, aminopeptidase N and sucrase–isomaltase were purified by protein-A precipitation as follows. To 100 μl samples of solubilized total membrane fraction in 50 mM-Tris/HCl, pH 8.0, containing 150 mM-NaCl, was added 25 μl (about 150 μg of immunoglobulin G) of specific anti-

(aminopeptidase N) or anti-(sucrase–isomaltase) immunoglobulin G. After incubation at 20°C for 1h, 50μl of a 1:1 suspension of protein A–Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was added and the samples were further incubated for 1h at 4°C with continuous gentle shaking. Protein A–Sepharose was then collected by centrifugation for 1min in a Beckman Microfuge. Washing of the protein A–Sepharose with 1ml of 50mM-Tris/HCl, pH8.0, containing 0.5M-NaCl and 1% Triton X-100 was repeated four times, followed by a washing with 1ml of 0.1M-sodium citrate, pH5.2, the buffer used in experiments with endo-β-*N*-acetylglucosaminidase H. The protein A–Sepharose was finally resuspended in 100μl of this buffer.

*Polyacrylamide-gel electrophoresis*

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis in 10%-acrylamide slab gels was performed as described by Laemmli (1970). Before electrophoresis, samples were denatured by boiling for 5min in the presence of 1% sodium dodecyl sulphate and 2.5% 2-mercaptoethanol. As $M_r$ indicators we used the polypeptides of $Ca^{2+}$-pellet sucrase–isomaltase ($M_r$ 265000 and 240000) and $Ca^{2+}$-pellet aminopeptidase N ($M_r$ 166000, 142000 and 137000) (Danielsen *et al.*, 1981*a*).

After electrophoresis, the $M_r$ indicators were stained for protein by Coomassie Brilliant Blue R250 and the rest of the gel was prepared for fluorography by the method of Bonner & Laskey (1974). X-ray films (Kodak X-Omat) were exposed at −80°C.

*Treatment with endo-β-N-acetylglucosaminidase H*

Samples (100μl) of purified aminopeptidase and sucrase–isomaltase (bound to protein A–Sepharose) in 0.1M-sodium citrate, pH5.2, were boiled for 3min and incubated with 4 munits of endo-β-*N*-acetylglucosaminidase H at 37°C for 24h. After incubation, the samples were either directly used in electrophoresis or frozen at −20°C until use. Control samples without addition of glycosidase were incubated in parallel.

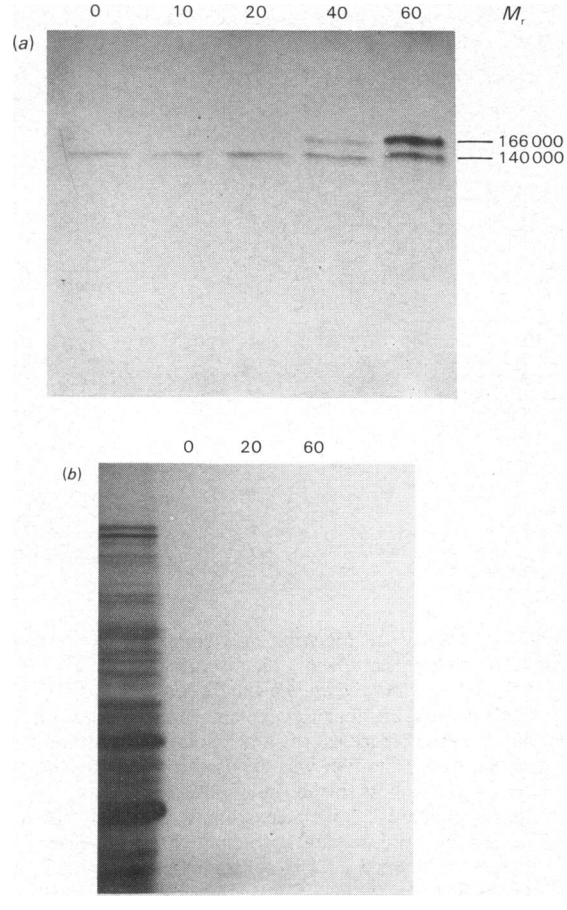


Fig. 1. *Pulse–chase labelling of aminopeptidase N, purified from the solubilized total membrane fraction (a) and from the soluble fraction (b)*

After 10min of labelling, explants were chased for the indicated periods of time (min). In (*b*) the furthest-left lane shows the labelling of the total soluble protein fraction after 20min of chase. The film was exposed for 7 days in both (*a*) and (*b*).

## Results

Fig. 1(*a*) shows the result of a pulse–chase labelling experiment showing the labelling of aminopeptidase N isolated from the solubilized total membrane fraction of cultured explants. A polypeptide of $M_r$ 140000 was immediately labelled after the 10min pulse period, and a second polypeptide of $M_r$ 166000 became visible for 40min of chase. Aminopeptidase *N* was also purified from the soluble fraction of the same cultured explants; as Fig. 1(*b*) shows, no radioactively labelled enzyme could be detected in this fraction within a period of 60min chase.

A similar experiment was performed with sucrase–isomaltase. When the enzyme was isolated from the solubilized total membrane fraction, a polypeptide of $M_r$ 240000 was clearly visible after 40min of chase and a second of $M_r$ 265000 appeared after 60min of chase. No radioactively labelled polypeptides of sucrase–isomaltase were present in the soluble fraction within a period of 0–30min of chase (results not shown).

Fig. 2 shows the appearance in the solubilized $Ca^{2+}$-precipitated membrane fraction of radio-

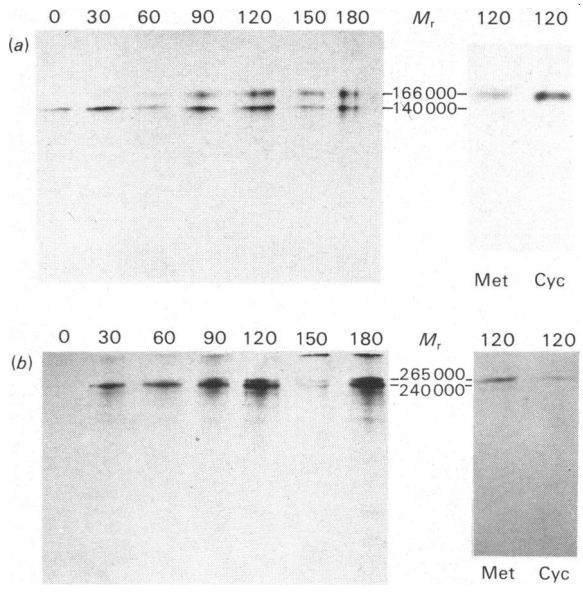


Fig. 2. *Pulse–chase labelling of aminopeptidase N and sucrase–isomaltase from the solubilized $Ca^{2+}$-precipitated membrane fraction*

After 10 min of labelling, explants were chased for the indicated periods of time (min). (*a*) Aminopeptidase N. The film was exposed for 5 days. The two lanes furthest to the right show the labelling of aminopeptidase N, isolated from a Triton X-100 extract of total explant protein, when the chase medium contained 2.5 mM non-radioactive methionine (Met) or 1 mg of cycloheximide/ml (Cyc) (exposure time 2 days). (*b*) Sucrase–isomaltase. The film was exposed for 4 days. The two lanes furthest to the right show the labelling of sucrase–isomaltase, isolated from a Triton X-100 extract of total explant protein, when the chase medium contained 2.5 mM non-radioactive methionine (Met) or 1 mg of cycloheximide/ml (Cyc) (exposure time 8 days).

actively labelled aminopeptidase N and sucrase–isomaltase. For aminopeptidase, the polypeptide of $M_r$ 140000 became labelled immediately after the 10 min pulse period, followed by the polypeptide of $M_r$ 166000 after 30–60 min of chase (Fig. 2*a*). For sucrase–isomaltase, a polypeptide of $M_r$ 240000 was clearly visible after 30 min of chase; after 60–90 min of chase, a polypeptide of $M_r$ 265000 appeared (Fig. 2*b*). (In addition to the polypeptides of sucrase–isomaltase, some contaminating faint bands with lower $M_r$ could be seen. The radioactivity not entering the separation gel probably represents immunoprecipitated sucrase–isomaltase not dissolved by the pre-treatment.) On long exposure of the fluorogram, the $M_r$ 240000 polypeptide of sucrase–isomaltase could be detected immediately after the 10 min pulse period (results not shown).

Fig. 2 also shows the effect of the presence in the chase medium of either cycloheximide or an increased concentration of non-radioactive methionine on the two labelled polypeptides of aminopeptidase N and sucrase–isomaltase. For both enzymes, only the polypeptide of higher $M_r$ (166000 and 265000 for aminopeptidase N and sucrase–isomaltase, respectively) persisted in a total explant extract after 2 h of chase.

The appearance of aminopeptidase N and sucrase–isomaltase in the microvillar membrane fraction is shown in Fig. 3. For aminopeptidase N, the polypeptide of $M_r$ 166000 occurred in this membrane fraction after about 60–90 min of chase; the polypeptide of $M_r$ 140000 could not be detected for periods up to 180 min of chase (Fig. 3*a*). Similarly, for sucrase–isomaltase, only the polypeptide of $M_r$ 265000 appeared in the microvillar

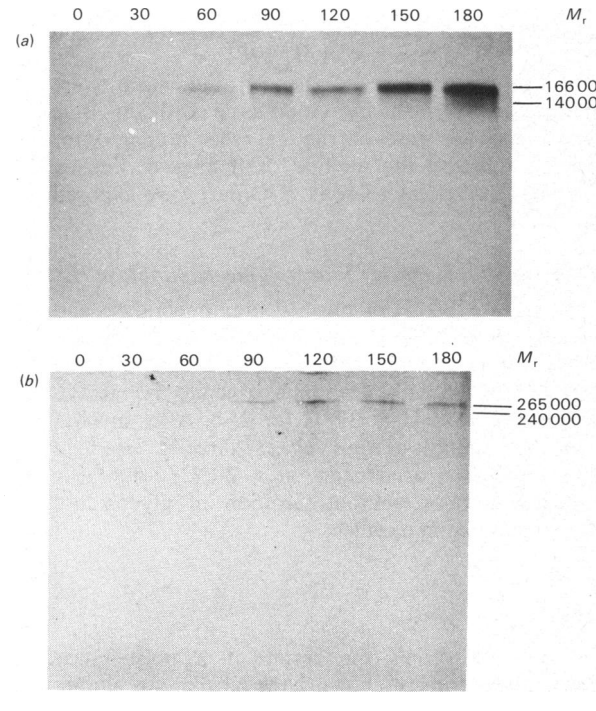


Fig. 3. *Pulse–chase labelling of aminopeptidase N and sucrase–isomaltase from the solubilized microvillar membrane fraction*

After 10 min of labelling, explants were chased for the indicated periods of time (min). (*a*) Aminopeptidase N. The film was exposed for 4 days. (*b*) Sucrase–isomaltase. The film was exposed for 22 days.

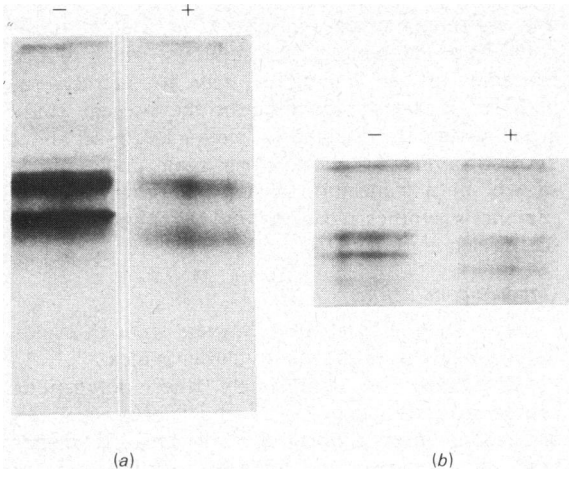


Fig. 4. *Susceptibility of pulse-labelled aminopeptidase N (a) and sucrase–isomaltase (b) to treatment with endo-β-N-acetylglucosaminidase H*

After 10 min of labelling, the explants were chased for 50 min (*a*) or 100 min (*b*): (+) and (−) indicate endo-β-*N*-glucosaminidase-H-treated and control samples respectively. The film was exposed for 7 days (*a*) or 5 days (*b*).

membrane fraction after 60–90 min of chase (Fig. 3*b*). The polypeptide of $M_r$ 240000 could not be seen even after 180 min of chase.

The susceptibility of the pulse-labelled aminopeptidase N and sucrase–isomaltase to treatment with endo-β-*N*-acetylglucosaminidase H is shown in Fig. 4. For both enzymes, the polypeptide of higher $M_r$ was only slightly affected by the action of endo-β-*N*-acetylglucosaminidase H, whereas the polypeptide of lower $M_r$ had its mobility in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis significantly increased by the treatment.

## Discussion

The fact that pulse-labelled aminopeptidase N and sucrase–isomaltase, for periods up to 60 and 80 min of chase respectively, were both found in association with membranes rather than in a soluble state strongly indicates that the enzymes become inserted into the membrane during or immediately after polypeptide synthesis. This finding makes it reasonable to suggest that aminopeptidase N and sucrase–isomaltase are synthesized on membrane-bound ribosomes rather than on free polyribosomes. This agrees well with the observation of Hauri *et al.* (1979), who found newly synthesized sucrase–isomaltase associated with the membranes of the Golgi apparatus, but strongly contrasts with the results of Cezard *et al.* (1979) and Maze & Gray (1980), who suggested the occurrence of soluble cytosol precursors of sucrase–isomaltase and aminopeptidase N.

The $Ca^{2+}$-precipitation method is a convenient and widely used way of preparing microvillar membrane fractions (Kessler *et al.*, 1978). $Ca^{2+}$ aggregates intracellular and basolateral membranes, but has little or no effect on microvillar membranes (Schmitz *et al.*, 1973; Booth & Kenny, 1974). In the present work, the $Ca^{2+}$-precipitation method was used in order to obtain two cell fractions: one containing aminopeptidase N and sucrase–isomaltase during their cellular transport ($Ca^{2+}$-precipitated membrane fraction), and one containing the two enzymes at their final location (microvillar membrane fraction). Aminopeptidase N and sucrase–isomaltase obtained from the solubilized $Ca^{2+}$-precipitated membrane fraction became labelled earlier than the corresponding enzymes from the solubilized microvillar fraction. The 10 min pulse period was long enough to produce easily detectable amounts of aminopeptidase N, indicating that the time required for the polypeptide synthesis for this enzyme is at least comparable in length with the pulse period. As mentioned above, prolonged exposure of the fluorogram was needed to detect sucrase–isomaltase immediately after the 10 min pulse period; this may indicate that the synthesis of the large polypeptide of this enzyme takes a longer time. Furthermore, if the methionine residues of sucrase–isomaltase are situated near the *N*-terminal region of the polypeptide, the chains completed during the 10 min pulse period would be non-radioactive.

For both enzymes, the complete susceptibility of the lower-$M_r$ polypeptides to treatment with endo-β-*N*-acetylglucosaminidase H shows that glycosylation takes place during or immediately after completion of the polypeptide chain. We suggest that these endo-β-*N*-acetylglucosaminidase-H-sensitive early-labelled polypeptides represent transition forms of aminopeptidase N and sucrase–isomaltase, being exclusively intracellularly located and present only in very minute amounts, compared with the total cellular amount of the two enzymes. This suggestion is supported by the experiment shown in Fig. 2. When the pulse period was followed by a 2 h chase during which protein synthesis was blocked by cycloheximide or non-radioactive methionine was present in a 25-fold excess, only the $M_r$-166000 polypeptide of aminopeptidase N and the $M_r$-265000 polypeptide of sucrase–isomaltase remained labelled. These were also the only bands of the two enzymes that were visibly radioactively labelled during 24 h of organ culture with continuous labelling (Danielsen *et al.*, 1982*a*), indi-

cating that the polypeptides of lower $M_r$ do not accumulate over a longer culture period.

Aminopeptidase N and sucrase–isomaltase appeared in the microvillar membrane shortly after the conversion from the lower-$M_r$ into the higher-$M_r$ polypeptide had taken place. This conversion, which, as shown by the experiment with endo-$\beta$-*N*-acetylglucosaminidase H, seems to consist, at least in part, of a reglycosylation of the polypeptides, yields the 'complex' form, which is characterstic of the final state of other plasma-membrane and secretory glycoproteins (Robbins *et al.*, 1977).

The results obtained in the present work are consistent with our previous findings of new molecular forms of aminopeptidase N and sucrase–isomaltase in the $Ca^{2+}$-precipitated membrane fraction of enterocytes (Danielsen *et al.*, 1981*a*). $Ca^{2+}$-pellet aminopeptidase N was found to consist of polypeptides, of $M_r$ 166000, 142000 and 137000, and sucrase–isomaltase of two polypeptides, of $M_r$ 265000 and 240000. For both enzymes, only the polypeptides of lower $M_r$ were susceptible to treatment with endo-$\beta$-*N*-acetylglucosaminidase H, whereas the polypeptides of higher $M_r$ were found to be sensitive to neuraminidase (Danielsen *et al.*, 1981*b*). The $Ca^{2+}$-pellet forms of the enzymes were present only in very minute amounts, constituting about 2–4% of the total activity of the respective enzymes. There are good reasons to believe that the $Ca^{2+}$-pellet forms of the enzymes which can be isolated in a preparative scale from the small intestine are in fact the precursors of the corresponding microvillar enzymes, observed in the present investigation. The non-radioactive and labelled precursors are similarly located within the cell and exhibit the same pattern of sensitivity to treatment with endo-$\beta$-*N*-acetylglocosaminidase H. Furthermore, there is good agreement between the $M_r$ of the polypeptides of the non-radioactive and labelled precursors of the corresponding enzymes. The only discrepancy was found for the lower-$M_r$ polypeptide of aminopeptidase N, which in the non-radioactive enzyme was resolved as a doublet.

The high $M_r$ (240000) of the earliest labelled polypeptide of sucrase–isomaltase in the present work confirms previous findings (Hauri *et al.*, 1979; Sjöström *et al.*, 1980) that this enzyme complex is synthesized as a large single polypeptide. The absence or limited amount of pancreatic proteinases in the organ-culture system explains why the precursor polypeptide of sucrase–isomaltase occurs in the microvillar membrane in a non-split form (Danielsen *et al.*, 1982*a*). Aminopeptidase N, purified from the intestine of pigs which 3 days before death had had their pancreatic duct disconnected, has been shown to consist of two subunits of equal $M_r$ (162000) (Sjöström *et al.*, 1978). It has been speculated (Svensson, 1979) that this enzyme may be synthesized as a single large polypeptide containing both subunits, which is then processed in an analogous way to sucrase–isomaltase. However, the data of the present study argue against the existence of such a large ($M_r$ about 250000) aminopeptidase N precursor, and we have shown, in a translation system *in vitro*, that the enzyme is synthesized as a $M_r$-115000 polypeptide (Danielsen *et al.*, 1982*b*).

*General conclusions*

The results suggest the following order of events in the biogenesis of the microvillar enzymes.

(1) During or immediately after polypeptide synthesis, aminopeptidase N and sucrase–isomaltase become inserted into the membrane. This means that the enzymes are probably synthesized by ribosomes attached to the rough endoplasmic reticulum.

(2) During or immediately after completion of the polypeptide chains, the enzymes become glycosylated with an intermediate high-mannose oligosaccharide sequence, which characteristically is susceptible to treatment with endo-$\beta$-*N*-acetylglucosaminidase H.

(3) After 20–40 min and 60–90 min respectively, aminopeptidase N and sucrase–isomaltase undergo reglycosylation.

(4) The reglycosylated final aminopeptidase N and sucrase–isomaltase reach the microvillar membrane after 60–90 min. At no time during the cellular transport do any of the enzymes appear in the soluble cell fraction, indicating that the transfer of aminopeptidase N and sucrase–isomaltase between the cell organelles occurs in membranes.

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## References

Axelsen, N. H., Krøll, J. & Weeke, B. (1973) *A Manual of Quantitative Immunoelectrophoresis. Methods and Applications*, Universitetsforlaget, Oslo

Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88

Booth, A. G. & Kenny, A. J. (1974) *Biochem. J.* **142**, 575–581

Browning, T. H. & Trier, J. S. (1969) *J. Clin. Invest.* **48**, 1423–1432

Cezard, J. P., Conklin, K. A., Bijoy, C. D. & Gray, G. M. (1979) *J. Biol. Chem.* **254**, 8969–8975

Danielsen, E. M., Sjöström, H. & Norén, O. (1981*a*) *FEBS Lett.* **127**, 129–132

Danielsen, E. M., Skovbjerg, H., Norén, O. & Sjöström, H. (1981*b*) *FEBS Lett.* **132**, 197–200

Danielsen, E. M., Sjöström, H., Norén, O., Dabelsteen, E. & Bro, B. (1982*a*) *Biochem. J.* **202**, 647–654
Danielsen, E. M., Norén, O. & Sjöström, H. (1982*b*) *Biochem. J.* **204**, 323–327
Hauri, H.-P., Quaroni, A. & Isselbacher, K. J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5183–5186
Hauri, H.-P., Quaroni, A. & Isselbacher, K. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6629–6633
Kenny, A. J. & Maroux, S. (1982) *Physiol. Rev.* **62**, 91–128
Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, H. & Seminza, G. (1978) *Biochim. Biophys. Acta* **506**, 136–154
Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
Maze, M. & Gray, G. M. (1980) *Biochemistry* **19**, 2351–2358
Robbins, P. W., Hubbard, S. C., Turco, S. J. & Wirth, D. F. (1977) *Cell* **12**, 893–900
Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J. & Crane, R. K. (1973) *Biochim. Biophys. Acta* **323**, 98–112
Sjöström, H., Norén, O., Jeppesen, L., Staun, M., Svensson, B. & Christiansen, L. (1978) *Eur. J. Biochem.* **88**, 503–511
Sjöström, H., Norén, O., Christiansen, L., Wacker, H. & Semenza, G. (1980) *J. Biol. Chem.* **255**, 11332–11338
Svensson, B. (1979) *Carlsberg Res. Commun.* **44**, 417–430