Dimeric Assembly of Enterocyte Brush Border Enzymes†

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ABSTRACT: The noncovalent, dimeric assembly of small intestinal brush border enzymes was studied by sedimentation analysis in density gradients of extracts of pulse-labeled pig jejunal mucosal explants. Like aminopeptidase N (EC 3.4.11.2), sucrase-isomaltase (EC 3.2.1.48–10), aminopeptidase A (EC 3.4.11.7), and dipeptidyl peptidase IV (EC 3.4.14.5) were all observed to dimerize predominantly prior to the Golgi-associated complex glycosylation, i.e., in the endoplasmic reticulum or in an intermediate compartment between this organelle and the Golgi complex. However, small amounts of monomeric complex-glycosylated forms, in particular of sucrase-isomaltase, were detectable. This indicates that homodimerization cannot be an absolute requirement for transport to, and through, the Golgi complex although our data suggest that dimeric assembly may increase the rate of intracellular transport. Culture at low temperature (20 °C) reduced the rate of, but did not prevent, dimerization. Maltase-glucoamylase (EC 3.2.1.20) only appeared as a dimer when extracted and analyzed under low salt conditions, suggesting a weak association between the two subunits. This finding is consistent with the electronmicroscopic appearance of the liposome-reconstituted enzyme [Norén et al. (1986) *J. Biol. Chem. 261,* 12306–12309], showing only the inner, membrane-anchored domains of the monomers to be in close contact with one another while the outer domains are far apart. In contrast to the other brush border enzymes studied, lactase-phlorizin hydrolase (EC 3.2.1.23-62) was found to occur predominantly as a monomer in its transient, high mannose-glycosylated state. Dimerization mainly took place after complex glycosylation but before, or simultaneously with, the proteolytic cleavage(s) generating the 160-kDa subunit of the mature enzyme.

Plasmamembrane glycoproteins in general are oligomers when residing at the cell surface, and, in many cases studied, the quaternary structure is not only essential for the proper functioning of the mature protein but also a prerequisite for the intracellular transport of the newly synthesized protein molecules [for reviews, see Rose and Doms (1988) and Hurtley and Helenius (1989)]. The endoplasmic reticulum has often been found to be the compartment where not only polypeptide folding but also oligomeric assembly takes place, and it has been proposed to house a degradatory "quality control" which only permits "transport competent" proteins to enter the secretory pathway. Thus, oligomerization has the potential of being an event for control of expression at a posttranslational stage, as examplified by the regulation of transport of immunoglobulin light chains by assembly with corresponding heavy chains.

One of the main characteristics of the intestinal enterocytic differentiation is the massive expression of digestive peptidases and glycosidases in the apical brush border membrane (Semenza, 1986; Norén et al., 1986a; Louvard et al., 1992). As judged by electronmicroscopy of liposome-reconstituted, purified enzymes, aminopeptidase N (EC 3.4.11.2), dipeptidyl peptidase IV (EC 3.4.14.5), sucrase-isomaltase (EC 3.2.1.48-10), and maltase-glucoamylase (EC 3.2.1.20) all appear as homodimers (Hussain et al., 1981; Hussain, 1985; Cowell et al., 1986; Norén et al., 1986b), suggesting a gross similarity in quaternary structure among these enzymes of the brush border membrane. However, subsequent studies in turn have not supported the idea of dimerization being a common theme in the biosynthesis of brush border enzymes. PABA-peptide hydrolase was found by Sterchi et al. (1988) to form disulfide-linked homodimers while still being in its high mannose-glycosylated form; similarly, we observed aminopeptidase N to undergo (noncovalent) homodimerization before the Golgi-associated complex glycosylation takes place (Danielsen, 1990). On the other hand, lactase-phlorizin hydrolase (EC 3.2.1.23-62) (Danielsen, 1990) and dipeptidyl peptidase IV (Jascur et al., 1991) have both been reported to assemble into homodimers at a later stage, either in the Golgi complex or during post-Golgi transport. Finally, maltase-glucoamylase (Naim et al., 1988), sucrase-isomaltase (Jascur et al., 1991), and angiotensin-converting enzyme (Naim, 1992) have been reported to remain monomeric also in their mature, complex-glycosylated form.

In the present work, we extended our earlier studies on dimerization of aminopeptidase N and lactase-phlorizin hydrolase to include as well sucrase-isomaltase, maltase-glucoamylase, dipeptidyl peptidase IV, and aminopeptidase A (EC 3.4.11.7). The results of the work indicate that five of the enzymes largely assemble into homodimers before they enter the Golgi complex while one, lactase-phlorizin hydrolase, only dimerizes at a later stage during the intracellular transport or possibly at the cell surface.

EXPERIMENTAL PROCEDURES

Materials. Equipment for performing organ culture, including Trowell's T-8 medium, culture dishes with grids, and [^{35}S]methionine (specific radioactivity > 1000 Ci/mmol) was obtained as previously described (Danielsen et al., 1982). Fluorophenylalanine was purchased from Sigma (St. Louis, MO), leupeptin from Boehringer (Mannheim, Germany), and aprotinin from Novo-Nordisk (Bagsværd, Denmark).

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Organ Culture of Mucosal Explants. Mucosal explants of about 100 mg were excised from segments of surgically removed pig jejunum and cultured at 37 or 20 °C (Danielsen et al., 1982). After 1 h of preincubation, the explants were labeled with [^{35}S]methionine (0.2–0.5 mCi/mL) for periods of 10 min to 20 h. In experiments with fluorophenylalanine, the amino acid analog was added to the culture medium at a concentration of 5 mM. After culture, labeled explants were quickly frozen and kept at −20 °C until further processing.

Velocity Sedimentation Analysis and Immunoprecipitation of Brush Border Enzymes. Labeled explants were thawed and extracted in 0.5 mL of ice-cold 25 mM Tris-HCl and 75 mM NaCl, pH 7.4, containing 5% (w/v) Triton X-100 and 10 μg/mL leupeptin and aprotinin. The extracts were centrifuged at 20000*g* for 5 min and the supernatants layered on top of 12-mL linear 7.5–25% sucrose gradients, made up in 50 mM Tris-HCl, 150 mM NaCl, and 0.1% Triton X-100, pH 7.4, or, in some experiments, in 10 mM Tris-HCl and 0.1% Triton X-100, pH 7.4. The gradients were centrifuged at 28 000 rpm (g_{av} = 97 400) in a SW40 Ti-rotor (Beckman Instruments, Palo Alto, CA) for about 20 h at 4 °C. After centrifugation, the gradients were fractionated into 15 samples of about 0.85 mL. Gradient buffer (0.5 mL) (containing 10 μg/mL aprotinin) and 30–50 μL of antiserum to a brush border enzyme was added to each fraction. The rabbit antisera used were those previously used for immunoprecipitation of brush border enzymes in biosynthesis studies (Danielsen, 1982; Danielsen et al., 1983, 1984). Aminopeptidase N or, in some cases, sucrase-isomaltase, was immunoprecipitated from fractions of all experiments and used as markers for the other brush border enzymes. After incubation overnight at 4 °C, immunoprecipitates were pelleted by centrifugation at 3000*g* for 10 min and analyzed by SDS–PAGE.

In experiments with unlabeled tissue (Figure 1), a microvillar vesicle fraction was prepared by the divalent cation technique and solubilized by 1% Triton X-100 as earlier described (Danielsen, 1982) and analyzed by gradient centrifugation as described above.

Electrophoresis. Prior to electrophoresis, all samples were denatured under reducing conditions by boiling for 3 min in the presence of 1% SDS and 10 mM dithiothreitol. SDS–PAGE in 8% gels was performed according to Laemmli (1970). Radioactively labeled samples were visualized by fluorography of the gels as described by Bonner and Laskey (1974). In SDS–PAGE of unlabeled tissue (Figure 1), the brush border enzymes were visualized by Western blotting on nitrocellulose paper, using an antiserum to aminopeptidase N, sucrase-isomaltase, or lactase-phlorizin hydrolase as the primary antibody and a peroxidase-conjugated mouse anti-rabbit IgG as secondary antibody, was performed essentially as described by Bjerrum et al. (1983).

Enzyme Activity. Aminopeptidase activity was determined using alanyl-*p*-nitroanilide as substrate, as previously described by Sjöström et al. (1978).

RESULTS

Sedimentation Analysis of Brush Border Enzymes. Velocity sedimentation in density gradients is an established technique for monitoring protein oligomerization (Hurtley & Helenius, 1989) and has previously been used in dimerization studies on brush border enzymes (Naim et al., 1988; Jascur et al., 1991; Naim, 1992). In the present work, sedimentation analysis of Triton X-100-solubilized microvillar vesicles was performed initially to select the experimental conditions required for resolving the monomeric and dimeric forms of the brush border enzymes (Figure 1). Mature, intestinal microvillar aminopeptidase N from pig, human, and rabbit has been reported to be a dimer by a number of investigators (Hussain et al., 1981; Danielsen, 1990; Gorvel et al., 1989; Naim et al., 1988; Jascur et al., 1991). As can be seen (Figure 1A), dimeric aminopeptidase N [the 166-kDa mature, complex-glycosylated form and its proteolytic fragments of 123 and 62 kDa; also termed "A", "B", and "C" subunits, respectively (Sjöström et al., 1978)] was confined essentially to fractions 5 and 6 of the gradient, coinciding with the sedimentation of the aminopeptidase activity (Figure 1C). The sucrase and isomaltase subunits of sucrase-isomaltase (140 and 150 kDa, respectively) sedimented mainly in fractions 7–10 of the gradient, in agreement with a quaternary structure of the mature enzyme being composed of two sucrase and two isomaltase subunits or (less visible on the gel) a dimer of sucrase-isomaltase precursor polypeptides (265 kDa) (Sjöström et al., 1980; Cowell et al., 1986). Figure 1B shows that the 160-kDa polypeptide of mature lactase-phlorizin hydrolase sedimented around fraction 5, indicating a dimeric quaternary structure, as previously observed by chemical cross-linking (Danielsen, 1990).

In conclusion, the distribution in the density gradient of the mature, microvillar form of these three pig brush border enzymes was found to be in accordance with previous reports on their quaternary structure and thus indicated that no artifactual dimer dissociation of any of the enzymes had occurred during extraction and analysis. The above distribution of dimers of the respective enzymes was subsequently used as a marker in the analyses of the biosynthetic dimerization of the newly synthesized enzymes.

Dimerization of Aminopeptidase N, Sucrase-Isomaltase, Aminopeptidase A, and Dipeptidyl Peptidase IV. As shown in Figure 2, the transient, high mannose-glycosylated 140-kDa form of aminopeptidase N obtained from mucosal explants labeled for 10 min was mainly found in fractions 3–4, consistent with our earlier finding that the newly synthesized enzyme is predominantly monomeric at this stage (Danielsen, 1990). Similarly, the 240-kDa form of the high mannose-glycosylated sucrase-isomaltase was found in fractions 4–6, indicating a monomeric state of the precursor polypeptide. However, dimers of aminopeptidase N (fractions 5–6) as well as sucrase-isomaltase (fractions 7–8) were also seen.

Subsequent experiments were performed with labeling periods of 1–3 h, which is a relevant time interval for monitoring dimerization that takes place during intracellular transport of the newly synthesized brush border enzymes. As shown in Figure 3A, a substantial proportion of the 140-kDa form of aminopeptidase N was dimeric (fractions 5–6) by 1 h of labeling. In addition, the mature, complex-glycosylated form of the enzyme (166 kDa) was almost entirely in a dimeric state with only minute amounts present as monomer (fraction 4). Thus, although most aminopeptidase N assembles into dimers while still in the high mannose-glycosylated form (pre-Golgi) as earlier proposed (Danielsen, 1990, 1991), small amounts may not dimerize until after the carbohydrate processing in the Golgi complex. In the same experiment, sucrase-isomaltase was found to behave similarly to aminopeptidase N; a large proportion of the high mannose-glycosylated 240-kDa form and most of the complex-glycosylated 265-kDa form was in a dimeric state (fractions 7–10), indicating that also this brush border enzyme in the enterocyte assembles into dimers mainly before reaching the

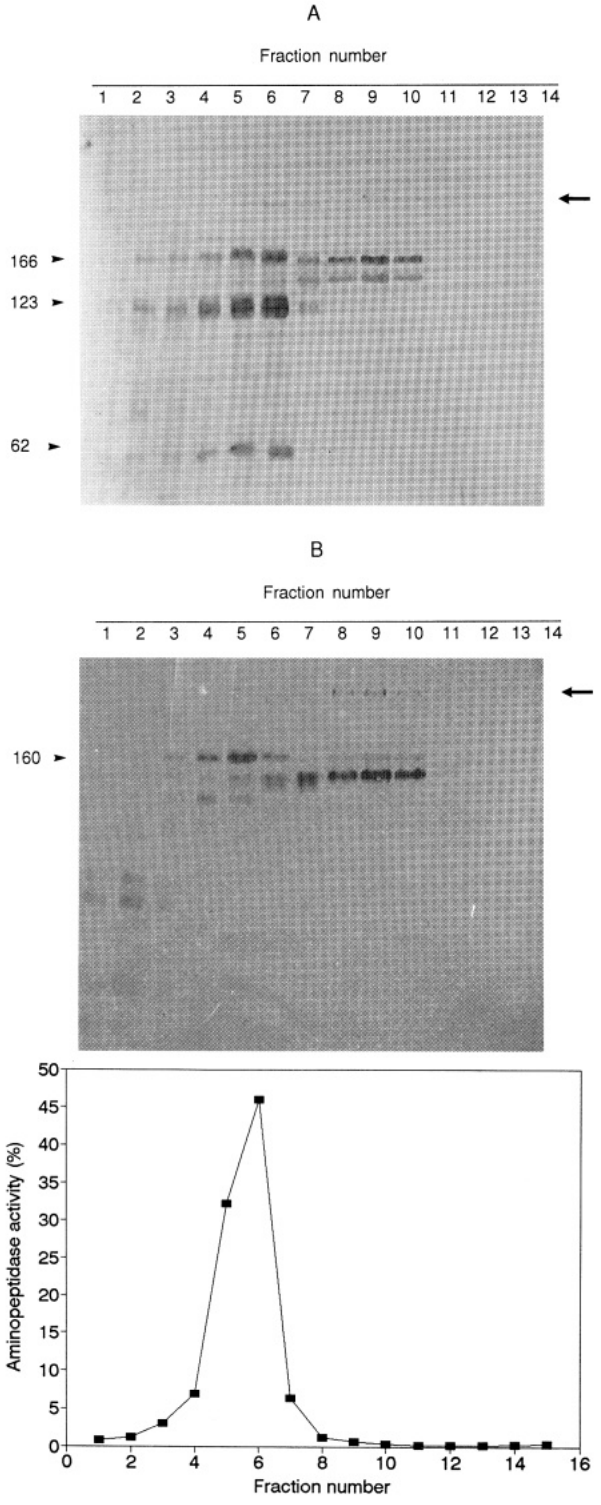

FIGURE 1: Sedimentation analysis of microvillar aminopeptidase N, sucrase-isomaltase, and lactase-phlorizin hydrolase. A small-intestinal microvillar vesicle fraction was prepared by using the divalent cation precipitation method and solubilized by 1% Triton X-100 as previously described (Danielsen, 1982). Microvillar detergent extract (0.5 mL) was layered on top of a 7.5–25% sucrose gradient and centrifuged as described under Experimental Procedures. After centrifugation, samples of 25 μL of each fraction were subjected to SDS–PAGE, and the gel was analyzed by Western blotting after electrotransfer onto nitrocellulose paper. (A) Primary antibodies to aminopeptidase N and sucrase-isomaltase. (B) Primary antibodies to sucrase-isomaltase and lactase-phlorizin hydrolase. (The position of the sucrase-isomaltase precursor polypeptide is indicated by an arrow.) In this and in all subsequent experiments, fraction no. 1 refers to the top of the gradient. (C) Distribution of aminopeptidase (Ala-*p*-nitroanilide hydrolyzing) activity in the same sucrose gradient.

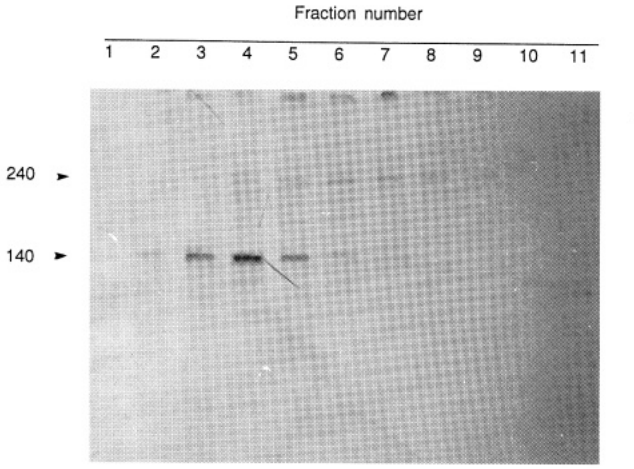

FIGURE 2: Sedimentation of 10-min pulse-labeled aminopeptidase N and sucrase-isomaltase. Fluorograph of an analysis of the high mannose-glycosylated, transient forms of aminopeptidase N (140 kDa) and sucrase-isomaltase (240 kDa) from mucosal explants, labeled for 10 min.

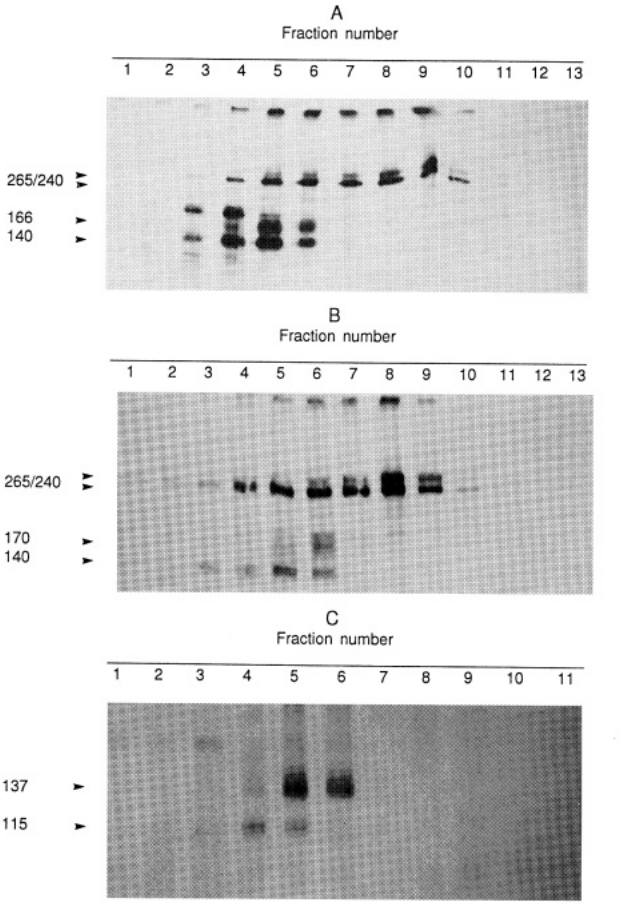

FIGURE 3: Dimerization of aminopeptidase N, sucrase-isomaltase, aminopeptidase A, and dipeptidyl peptidase IV. Fluorographs of (A) dimerization of aminopeptidase N (140 and 166 kDa) and sucrase-isomaltase (240 and 265 kDa) from explants, labeled for 1 h. (B) Aminopeptidase A (140 and 170 kDa) (and sucrase-isomaltase) from explants, labeled for 1 h. (C) Dipeptidyl peptidase IV (115 and 137 kDa) from explants, labeled for 1 h, followed by 1 h of chase.

Golgi complex. The blur extending between the 240-kDa form and the 265-kDa form in fractions 5–6 represents monomeric sucrase-isomaltase in Golgi-associated stages of "intermediate" glycosylation between the high mannose- and complex-glycosylated forms and thus shows the proportion of the enzyme "caught" in transit through the Golgi cisternae in a monomeric state. The fact that this blurred band of

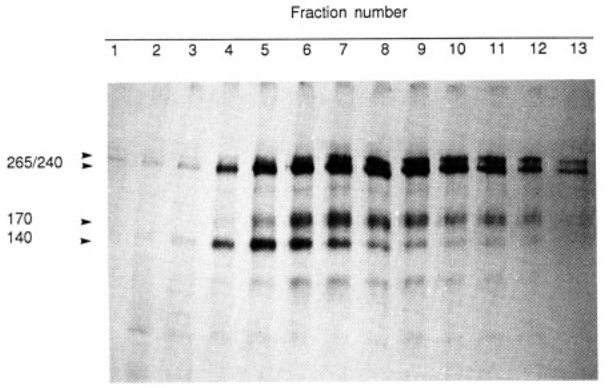

FIGURE 4: Aggregation of aminopeptidase A and sucrase-isomaltase in the absence of detergent. Fluorograph of aminopeptidase A and sucrase-isomaltase extracted from explants, labeled for 2 h, and sedimented in a density gradient in the absence of detergent.

"intermediate"-glycosylated sucrase-isomaltase was not seen as a dimer (i.e., in fractions 7–10) indicates that dimers must pass through the Golgi complex more rapidly than monomers, resulting in an intra-Golgi concentration too low to be detected. (This phenomenon was consistently observed in all experiments with sucrase-isomaltase, as shown in Figure 9.)

Figure 3B shows an analysis of aminopeptidase A from 1-h labeled mucosal explants. Again, the majority of the 140-kDa high mannose-glycosylated form as well as the broad 170-kDa complex-glycosylated form (Danielsen et al., 1983) sedimented as a dimer (fractions 5–6). Only scarce amounts of the 170-kDa form appeared as monomer (fractions 3–4). Finally, Figure 3C shows dipeptidyl peptidase IV after 1-h labeling followed by a 1-h chase; as can be seen, the mature, complex-glycosylated 137-kDa form (Danielsen et al., 1983) was almost entirely in a dimeric state (fractions 5–6), whereas both monomeric (fraction 4) and dimeric states of the remaining 115-kDa high mannose-glycosylated form were visible.

When detergent was omitted from the density gradient, sucrase-isomaltase and aminopeptidase A formed aggregates (fractions 11–13) during the sedimentation (Figure 4), demonstrating that both enzymes remained amphiphilic during the centrifugation. Since such aggregation was never observed in the presence of detergent for any of the brush border enzymes studied, unspecific aggregation of monomers cannot be considered a likely occurrence. Aminopeptidase N and maltase-glucoamylase were observed to behave in a similar way in the absence of detergent (data not shown).

In conclusion, aminopeptidase N, sucrase-isomaltase, aminopeptidase A, and dipeptidyl peptidase IV all assemble into dimers during intracellular transport in the enterocyte. For all four enzymes, the major subcellular site of dimerization is the endoplasmic reticulum (or, possibly, an intermediate compartment between the endoplasmic reticulum and the Golgi complex), but small amounts of monomeric, Golgi-associated forms of the enzymes, in particular in case of sucrase-isomaltase, were detectable. This indicates that dimerization is not exclusively a pre-Golgi event.

Dimerization of Maltase-Glucoamylase. When maltase-glucoamylase was extracted and analyzed under experimental conditions identical to those used for the four brush border enzymes described above, it invariably appeared exclusively as a monomer; as shown in Figure 5A the broad band, comprising both the 225-kDa (high mannose-glycosylated) form and the 245-kDa (complex-glycosylated) form sedimented with a velocity similarly to that of aminopeptidase N (fractions 5–6), when obtained from explants labeled for 1 h

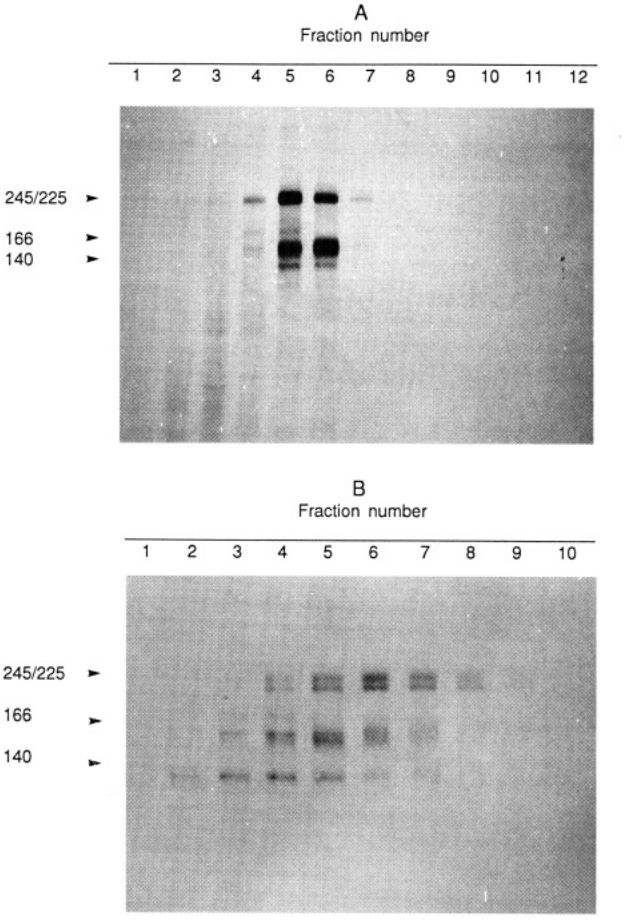

FIGURE 5: Dimerization of maltase-glucoamylase. Fluorographs of maltase-glucoamylase (225 and 245 kDa) (and aminopeptidase N) from explants, labeled for 1 h followed by 1 h of chase (A) or labeled continuously for 3 h (B). (A) Extraction and sedimentation analysis performed in "normal" salt, as described under Experimental Procedures; (B) The explant was extracted in 0.5 mL of 5 mM Tris-HCl, pH 7.4, containing 5% Triton X-100 and layered on top of a 7.5–25% sucrose gradient, made up in 10 mM Tris-HCl, pH 7.4, containing 0.1% Triton X-100.

and chased for 1 h. Essentially the same result was obtained with maltase-glucoamylase from explants, labeled continuously for 20 h or when Tween 20 or *n*-octylglucoside was used instead of Triton X-100 as solubilizing agent (data not shown). However, when extraction and sedimentation analysis was performed under low salt conditions, dimers of both 225- and 245-kDa forms of maltase-glucoamylase, obtained from explants labeled continuously for 3 h, could be seen (Figure 5B, fractions 7–9) (as shown, the sedimentation profile of aminopeptidase N was not markedly affected by this change in ionic strength). Even in low salt, however, both high mannose-glycosylated and complex-glycosylated forms of maltase-glucoamylase remained predominantly monomeric (fractions 4–6). This observation indicates that maltase-glucoamylase in detergent solution may exist either as a monomer or dimer, depending on the ionic strength. From this experiment alone, we therefore cannot predict whether the enzyme is monomeric or dimeric when residing in a lipid bilayer; it is thus conceivable that the low salt conditions induce artifactual dimerization. However, a dimeric rather than monomeric structure of the native, membrane-anchored pig maltase-glucoamylase was visualized by electron microscopy of the liposome-reconstituted enzyme (Norén et al., 1986b). This would indicate that dimer dissociation rather than monomer association in the present work is artifactual. The considerable dilution occurring during tissue extraction and

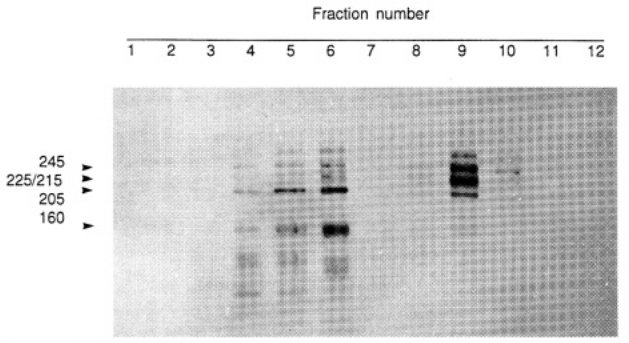

FIGURE 6: Dimerization of lactase-phlorizin hydrolase. Fluorograph of the dimerization of lactase-phlorizin hydrolase from explants, labeled for 3 h. Extraction and sedimentation analysis was performed using "low salt" conditions, as described in the legend to Figure 5. Lactase-phlorizin hydrolase is seen as a complex pattern of bands of which those of 225 (faint) and 205 kDa represent the high mannose-glycosylated precursor and a cleavage product, respectively, and the 245- and 215-kDa bands represent the complex-glycosylated precursor (and its cleavage product). The 160-kDa band represents the mature, complex-glycosylated form of the enzyme.

solubilization by detergent provides a likely reason for this. It should also be noted that the association of monomers, seen in low salt, was restricted to formation of dimers and thus different from the aggregation induced by omission of detergent from the gradients (Figure 4). The experiment shown in Figure 5B probably does not reflect a true ratio between monomers and dimers of newly synthesized maltase-glucoamylase, but the fact that a fraction of both the transient and mature forms of maltase-glucoamylase appeared dimeric in low salt indicates that dimeric assembly at least may commence before the newly made enzyme molecules reach the Golgi complex.

Dimerization of Lactase-Phlorizin Hydrolase. Figure 6 shows lactase-phlorizin hydrolase, analyzed using low salt conditions identical to the experiment depicted in Figure 5B. The 160-kDa mature form was present almost exclusively as a dimer (fractions 5–6), whereas the 225/205-kDa high mannose-glycosylated precursor (Danielsen et al., 1984) was mainly monomeric (fractions 4–6). The 245-kDa complex-glycosylated precursor of lactase-phlorizin hydrolase and its proteolytic cleavage product of 215 kDa (possibly an intermediate in the conversion to the final 160-kDa form) were predominantly dimeric (fractions 8–10), although monomers of both form were also seen (fractions 4–6). For this enzyme, formation of dimers therefore mainly occurs during or after passage through the Golgi complex, and since little if any monomeric final 160-kDa form was seen, it must be completed shortly after the proteolytic cleavages. Since the subcellular site(s) for these cleavage steps has not been ascertained, dimerization of lactase-phlorizin hydrolase may proceed in the trans-Golgi network, in post-Golgi transport vesicles, or even at the apical cell surface.

Effect of Low Temperature. Culture at low temperature is known to interfere with intracellular vesicular transport (Matlin & Simons, 1983), and delivery at the apical surface as well as posttranslational carbohydrate processing of newly made brush border enzymes in mucosal explants has earlier been shown to be inhibited by culture at 20 °C (Danielsen et al., 1989), indicating a blocked transport between the endoplasmic reticulum and the Golgi complex. Figure 7 shows sedimentation analyses (using low salt conditions) of aminopeptidase N and maltase-glucoamylase from explants, labeled continuously for 3 h at 20 °C. As should be expected, both enzymes essentially remained in their transient, high mannose-glycosylated form (small amounts of the complex-

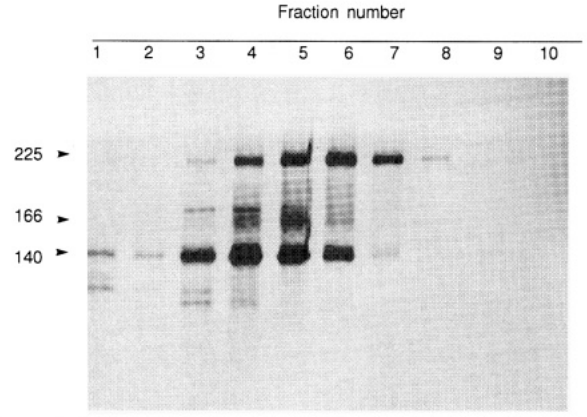

FIGURE 7: Effect of low temperature. Fluorograph of a sedimentation analysis (using low salt conditions) of maltase-glucoamylase and aminopeptidase N from explants, labeled for 3 h at 20 °C.

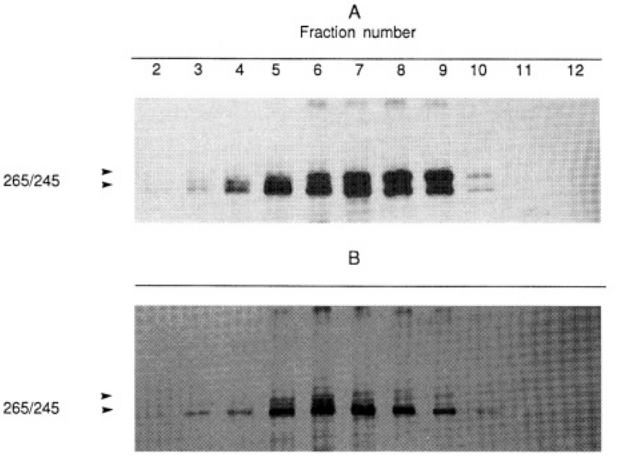

FIGURE 8: Effect of the incorporation of amino acid analogs. Fluorographs of the dimerization of sucrase-isomaltase from explants, labeled for 3 h in the absence (A) or presence of fluorophenylalanine (B).

glycosylated, 166-kDa form of aminopeptidase N were detectable). For aminopeptidase N, about equal amounts of monomers (fractions 3–4) and dimers (fractions 5–6) were seen, indicating a slower rate of dimeric assembly at the reduced temperature. Maltase-glucoamylase predominantly appeared monomeric (fractions 4–6), but a minor proportion had assembled into dimers (fractions7–8).

Effect of Biosynthetic Incorporation of Fluorophenylalanine. In a previous biosynthesis study, we used the amino acid analog fluorophenylalanine to perturb the molecular processing and intracellular transport of newly synthesized aminopeptidase N (Danielsen, 1991). In a 3-h labeling experiment, this analog only marginally inhibited the dimerization of the 140-kDa high mannose-glycosylated form of aminopeptidase A as well as its molecular processing to the 170-kDa complex-glycosylated form, indicating that the enterocyte's ability to transport and process newly synthesized membrane proteins was not generally affected (data not shown). For sucrase-isomaltase, on the other hand, much less of the 265-kDa complex-glycosylated form was generated in the presence of fluorophenylalanine as compared with the control, showing that only small amounts of newly synthesized enzyme was transported to the Golgi complex by 3 h (Figure 8). Densitometric scanning of the gel tracks of Figure 8 showed that while the dimer/monomer ratio (fractions 7–10/fractions 3–6) of the control was 1.7, the similar ratio was only 0.8 in the presence of fluorophenylalanine. Thus for sucrase-isomaltase, there seems to be a correlation between the ability

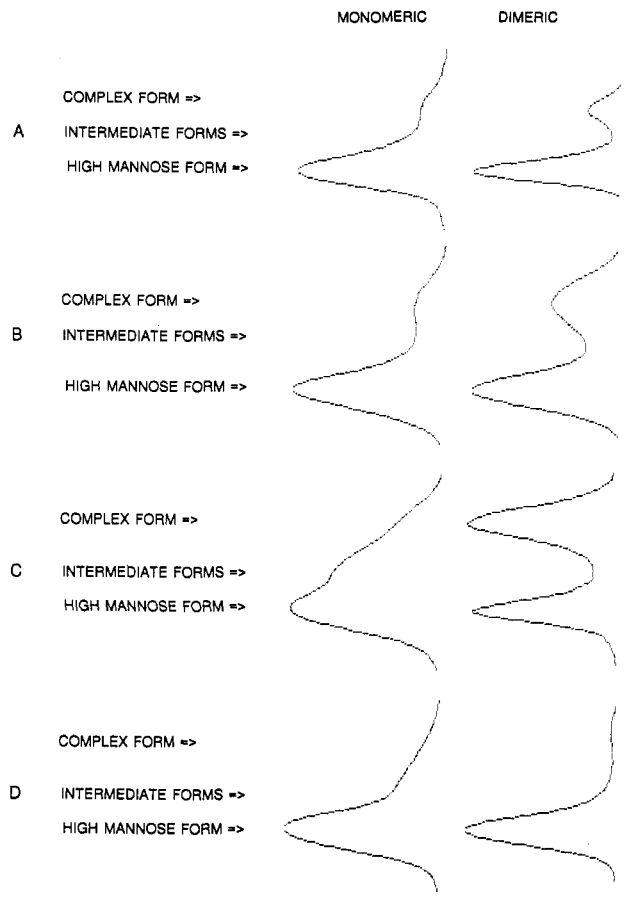

FIGURE 9: Golgi-associated intermediate forms of monomeric sucrase-isomaltase. Densitometric scanning of fluorographs of monomeric (fraction 5 or 6) and dimeric (fraction 7, 8, 9 or 10) sucrase-isomaltase from the experiments shown in Figure 3A (A), 3B (B), Figure 8A (C), and Figure 8B (D). The electrophoretic positions of the high mannose- and complex-glycosylated forms of the enzyme are indicated by arrows together with the position of the "blur" of molecules in Golgi-associated intermediate forms of glycosylation.

to dimerize and to exit the endoplasmic reticulum and pass through the Golgi complex, as we have previously observed for aminopeptidase N (Danielsen, 1991). In addition, and confirming the distribution of the molecular forms of sucrase-isomaltase shown in Figure 3, molecules bearing "intermediate" glycosylation between the high mannose-glycosylated form and the complex-glycosylated form were seen as monomers but not as dimers also in the presence of fluorophenylalanine (Figure 9). This is further evidence of the relatively slow rate of transport of monomers through the Golgi complex.

DISCUSSION

The present work was undertaken primarily to establish whether or not the enzymes of the enterocyte brush border membrane share a common theme of homodimeric assembly as an early, pre-Golgi step in their intracellular, posttranslational processing to mature enzyme molecules. Secondly, it was also the aim to examine to what extent dimerization might be required to render newly synthesized brush border enzymes "transport competent" for the intracellular transport to the apical cell surface.

The results obtained do confirm that non-covalent homodimerization is indeed a common biosynthetic event for this group of ectoenzymes. Furthermore, it can be stated that the endoplasmic reticulum is the primary organelle for the dimeric assembly; in case of four out of the six enzymes studied, this is where dimerization begins, and, for sucrase-isomaltase and the three peptidases, the major part of newly synthesized enzyme molecules have assembled into dimers by the time they enter the Golgi complex. However, as evidenced by the presence of monomeric complex-glycosylated and "intermediate"-glycosylated forms of sucrase-isomaltase, it is equally clear that dimerization cannot be an absolute requirement for transport out of the endoplasmic reticulum, as also pointed out previously by Jascur et al. (1991). Nevertheless, when an amino acid analog was used as a perturbant, a correlation between the ability to dimerize and to become complex-glycosylated was observed for sucrase-isomaltase, as it was previously seen for aminopeptidase N (Danielsen, 1990). In line with this, we have recently observed that aminopeptidase N in cultured mucosal explants of fetal small intestine dimerizes much slower than in adult tissue and likewise undergoes complex glycosylation only at a rate of about one-third of that of the "adult" enzyme (E. M. Danielsen, unpublished observation). Thus, it does seem that dimerization increases the "transport competence" of newly synthesized brush border enzymes, and different rates of dimer assembly in the endoplasmic reticulum therefore may be a contributing factor to the varying kinetics of intracellular transport among these enzymes that has been reported by several investigators (Hauri et al., 1985; Danielsen & Cowell, 1985; Gorvel et al., 1986; Stieger et al., 1988). In support of this is the presence in only the monomer fractions of "intermediate"-glycosylated molecular forms between the high mannose-glycosylated and complex-glycosylated forms of sucrase-isomaltase (Figures 3, 8, and 9). This at the least suggests that dimers pass through the Golgi cisternae more rapidly than monomers.

Our finding that dipeptidylpeptidase IV and sucrase-isomaltase predominantly dimerize in the endoplasmic reticulum is at variance with the work of Jascur et al. (1991), stating that dipeptidylpeptidase IV in the epithelial cell line Caco 2 only assembles into dimers in a late Golgi compartment after complex glycosylation and that sucrase-isomaltase remains monomeric even after complex glycosylation. Since monomoer concentration in the endoplasmic reticulum may be a determining factor for the rate of dimerization (Hurtley & Helenius, 1989), the relatively late dimerization of dipeptidyl peptidase IV in Caco 2 cells may well reflect the fact that this cell type expresses considerably less amounts of brush border enzymes in comparison with enterocytes. The somewhat surprising result that sucrase-isomaltase remains monomeric in Caco 2 cells can hardly be thus accounted for but may be ascribed to cell type-related differences in the molecular processing, for instance possible differences in O-linked glycosylation. In the present work, the stability of the dimers of sucrase-isomaltase was tested by collecting the dimeric form of the enzyme (fractions 8–9 of the sucrose gradient) and subjecting it to a second sedimentation analysis (after dialysis, concentration, and adjustment of the detergent concentration). No detectable dimer dissociation (or aggregation) occurred after such a lengthy experiment, showing that the pig sucrase-isomaltase dimer is stable in solution (data not shown).

In contrast to sucrase-isomaltase, the association between the two monomers of maltase-glucoamylase was found to be weak and salt-dependent, and this enzyme thus exemplifies the problems that may arise when studying noncovalent oligomerization *in vitro*. Consequently, the data obtained are not conclusive for this brush border enzyme. However, the ambiguous result of the present work concerning maltase-glucoamylase may reflect, and be explained by, the electron microscopic appearance of the liposome-reconstituted enzyme,

where only the inner, membrane-anchored, globular domain of each monomer is seen in direct contact with its counterpart; the outer domains protruding in separate directions and thus too far apart from each other to interact (Norén et al., 1986b). In contrast, electron microscopy of sucrase-isomaltase showed a much more compact structure with close contact both between both the inner, membrane-anchoring (isomaltase) domains as well as the outer (sucrase) domains (Cowell et al., 1986). Naim (1988) reported maltase-glucoamylase from labeled, human small intestinal biopsies to be monomeric, as judged by its inability to be cross-linked by dithiobis-(succinimidyl propionate). It is quite plausible that the human and pig enzymes differ with regard to quaternary structure, but it is also conceivable that a weak association between the monomers could account for an unsuccessful chemical cross-linking.

Lactase-phlorizin hydrolase is a unique brush border enzyme in being synthesized as a high molecular weight precursor of which about 80 kDa is removed from the N-terminal end (in one or more proteolytic cleavages) to yield the mature molecular form (Semenza & Auricchio, 1989). On the basis of cross-linking experiments using dimethyladipimidate, we have previously reported that lactase-phlorizin hydrolase only occurs as a dimer in its mature, 160-kDa form, indicating that the assembly takes place at the earliest in the Golgi complex (Danielsen, 1990). The present results confirm that the high mannose-glycosylated precursor of lactase-phlorizin hydrolase largely is monomeric but in addition provide clear evidence that the precursor dimerizes after complex glycosylation. This observation rules out the possibility that cleavage of the 80-kDa pro-piece should be required for the dimeric assembly. Of the six enzymes studied here, lactase-phlorizin hydrolase provides the clearest example that dimerization need not be required for exit from the endoplasmic reticulum, but the enzyme's remarkably slow rate of intracellular processing, notably in Caco 2 cells (Hauri et al., 1985), may reflect the poor "transport competence" of a monomeric plasmamembrane protein.

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