Folding of Intestinal Brush Border Enzymes. Evidence That High-Mannose Glycosylation Is an Essential Early Event†

E. Michael Danielsen*
Department of Biochemistry C, The Panum Institute, University of Copenhagen, Copenhagen, Denmark
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ABSTRACT: A polyvalent antiserum which precipitates the native, folded, but not the denatured molecular forms of pig intestinal aminopeptidase N (EC 3.4.11.2) and sucrase–isomaltase (EC 3.2.1.48, EC 3.2.1.10) was used to determine the kinetics of polypeptide folding of the two newly synthesized brush border enzymes. In pulse-labeled mucosal explants, complete synthesis of the polypeptide chains of aminopeptidase N and sucrase–isomaltase required about 2 and 4 min, respectively, whereas maximal antiserum precipitation was acquired with half-times of 4–5 and 8 min, respectively. Fructose, which induces a defective cotranslational high-mannose glycosylation, increased the half-time of polypeptide folding to about 12 min for aminopeptidase N as well as for sucrase–isomaltase. Short-pulse experiments suggested that fructose exerts its effect by slowing the rate of glycosylation, making this partially a posttranslational process. In the presence of fructose, not only the malglycosylated forms but also the electrophoretically normal, high-mannose-glycosylated form of the brush border enzymes were retained in the endoplasmic reticulum and proteolytically degraded. The results obtained demonstrate an intimate interrelationship between glycosylation and polypeptide folding in the synthesis of membrane glycoproteins and, more specifically, indicate that the timing of these two early biosynthetic events is essential for correct polypeptide folding.

Aminopeptidase N (EC 3.4.11.2) and sucrase–isomaltase (EC 3.2.1.48, EC 3.2.1.10) are two of the major ectoenzymes of the brush border membrane of the small-intestinal enterocyte (Semenza, 1986; Norén et al., 1986). Their biological role is, in concert with the other peptidases and glycosidases of the epithelial surface, to function in the final stage of digestion of dietary protein and carbohydrate (Alpers, 1987). Whereas sucrase–isomaltase seems to be expressed solely in enterocytes, aminopeptidase N, like other brush border peptidases, is distributed rather ubiquitously in various tissues and cell types, where it may serve a wide range of biological functions (Kenny & Turner, 1987). Interestingly, cloning and sequencing of the cDNA encoding the human enzyme have shown it to be identical to the myeloid marker protein CD 13 (Olsen et al., 1988; Look et al., 1989).

Both aminopeptidase N and sucrase–isomaltase slowly undergo limited proteolysis in situ by pancreatic proteinases (Sjöström et al., 1978, 1980), but they are expressed biosynthetically in the brush border membrane as "stalked" homodimers, held together by noncovalent forces (Hussein et al., 1981; Cowell et al., 1986) and anchored to the membrane by a small hydrophobic stretch of amino acids (a permanent signal sequence) near the N-terminus (Hunziker et al., 1986; Olsen et al., 1988). For aminopeptidase N, homodimerization has been shown to occur while the enzyme is in its transient, high-mannose-glycosylated state, i.e., before transport out of the endoplasmic reticulum to the Golgi complex (Danielsen, 1990a). The relative slow kinetics of dimerization suggested this event to be the rate-limiting step for the transport, and recently, an observation of a close correlation between dimerization and Golgi-associated molecular processing indicated that dimerization of aminopeptidase N is a prerequisite for transport between the two organelles (Danielsen, 1990b). A similar subcellular site for homodimerization of another brush border enzyme, PABA–peptide hydrolase, whose subunits are held together by disulfide bonds, has earlier been reported (Sterchi et al., 1988a,b). Recently, however, the human brush border enzyme dipeptidylpeptidase IV was reported to dimerize in a late Golgi compartment in the intestinal epithelial cell line Caco-2 (Jascur et al., 1991).

The dietary sugar fructose rapidly induces a block in the expression of aminopeptidase N and sucrase–isomaltase (as well as other brush border membrane glycoproteins) (Danielsen, 1989). The underlying mechanism involves an abnormal cotranslational high-mannose glycosylation which in turn triggers a rapid proteolytic degradation of the enzyme molecules in the endoplasmic reticulum. Nothing is known, however, of exactly how cotranslational glycosylation determines whether a newly synthesized brush border enzyme can pass the "quality control" and be allowed to exit the endoplasmic reticulum or, alternatively, is doomed to instant degradation. Since the processes of high-mannose glycosylation and acquisition of tertiary structure should be closely related chronologically, the present work set out to investigate the kinetics of these two early events and to examine their possible interrelationship.

Experimental Procedures

Materials. Equipment for performing organ culture, including Trowell's T-8 medium, plastic dishes with grids, and [^{35}S]methionine (specific radioactivity >1000 Ci/mmol), was obtained as previously described (Danielsen et al., 1982). 3-Hydroxynorvaline was purchased from Sigma (St. Louis, MO), and leupeptin was a product of Boehringer (Mannheim, Germany).

Pig small intestines were kindly given by the Department of Experimental Pathology, Rigshospitalet, Copenhagen.

Organ Culture of Mucosal Explants. Pig small intestinal mucosal explants of about 100 mg wet weight were excised and cultured for periods up to 2 h as earlier described (Dan-

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* Correspondence should be addressed to this author at the Department of Biochemistry C, The Panum Institute, 3 Blegdamsvej, DK-2200 Copenhagen N, Denmark.

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ielsen et al., 1982). In labeling experiments performed in the presence of fructose (50 mM), or 3-hydroxynorvaline (5 mM), the explants were preincubated for 1 h prior to the addition of radioactive methionine (with control explants cultured in parallel). Unless otherwise stated, all culture media contained 50 μg/mL leupeptin. After culture, the explants were rapidly frozen and kept at −20 °C until further processing.

Fractionation of Explants and Immunoprecipitation of Aminopeptidase N, Sucrase–Isomaltase, and Apolipoprotein A-1 (Apo A-1).[1] Cultured explants were thawed and homogenized in 400 μL of 25 mM Tris-HCl, pH 7.5, 75 mM NaCl, 5% Triton X-100, and 50 μg/mL leupeptin. The homogenates were centrifuged at 20000*g*, 5 min, and supernatants of total, detergent-extracted mucosal proteins were mixed with 200 μL of brush border antiserum (Danielsen, 1989) and 200 μL of antiserum to apo A-1 (Danielsen, 1990b) and incubated overnight at 4 °C. Immunoprecipitates were sedimented by centrifugation at 5000*g*, 5 min, and washed once in the above buffer before analysis by SDS/PAGE.

Electrophoretic Methods. Rocket immunoelectrophoresis was performed as described in Axelsen et al. (1973). SDS/PAGE in 10% gels (under reducing conditions) was performed according to Laemmli (1975) and fluorography as described by Bonner and Laskey (1974). X-ray films of gel tracks were scanned in an LKB Ultroscan XL densitometer (Pharmacia LKB, Bromma, Sweden). Western blotting on nitrocellulose paper, using the brush border antiserum as primary antibody and a peroxidase-conjugated mouse anti-rabbit IgG as secondary antibody, was performed essentially as described by Bjerrum et al. (1983).

Results

Characterization of the Precipitating Antiserum. A polyvalent brush border antiserum that predominantly precipitates aminopeptidase N and sucrase–isomaltase (Danielsen, 1987, 1989) was used in the present work to immunopurify simultaneously the two brush border enzymes from pulse-labeled mucosal explants. Immunogenic glycoproteins the size of aminopeptidase N and sucrase–isomaltase harbor several epitopes of which some react in Western blotting, following heat-denaturation and SDS/PAGE. This is shown in Figure 1A, where A, B, and C subunits of mature aminopeptidase N (fragmented in vivo by pancreatic proteases) as well as both the sucrase (140 kDa) and isomaltase (150 kDa) subunits of mature sucrase–isomaltase were recognized by the antiserum. However, as shown in Figure 1B, the ability of the antiserum to precipitate (without the use of a secondary antibody) aminopeptidase N as well as sucrase–isomaltase was perturbed by denaturation of the brush border enzymes. Thus, heating a Triton X-100 solubilized microvillar fraction at 55 °C for 5 min in the presence of 4 M urea greatly reduced the titer of the antiserum toward both brush border enzymes as evidenced by the faint and enlarged precipitates. Boiling the antigen for 5 min in the same solvent completely abolished immunoprecipitation of both brush border enzymes. Since it has earlier been shown that heat-denatured and SDS/PAGE-separated polypeptides of both aminopeptidase N and sucrase–isomaltase can be precipitated in immunoelectrophoresis against antibodies raised to the denatured brush border enzymes (Danielsen et al., 1981), these experiments show that both aminopeptidase N and sucrase–isomaltase need to be nondenatured in order to be effectively precipitated by the antiserum raised to the native brush border enzymes.

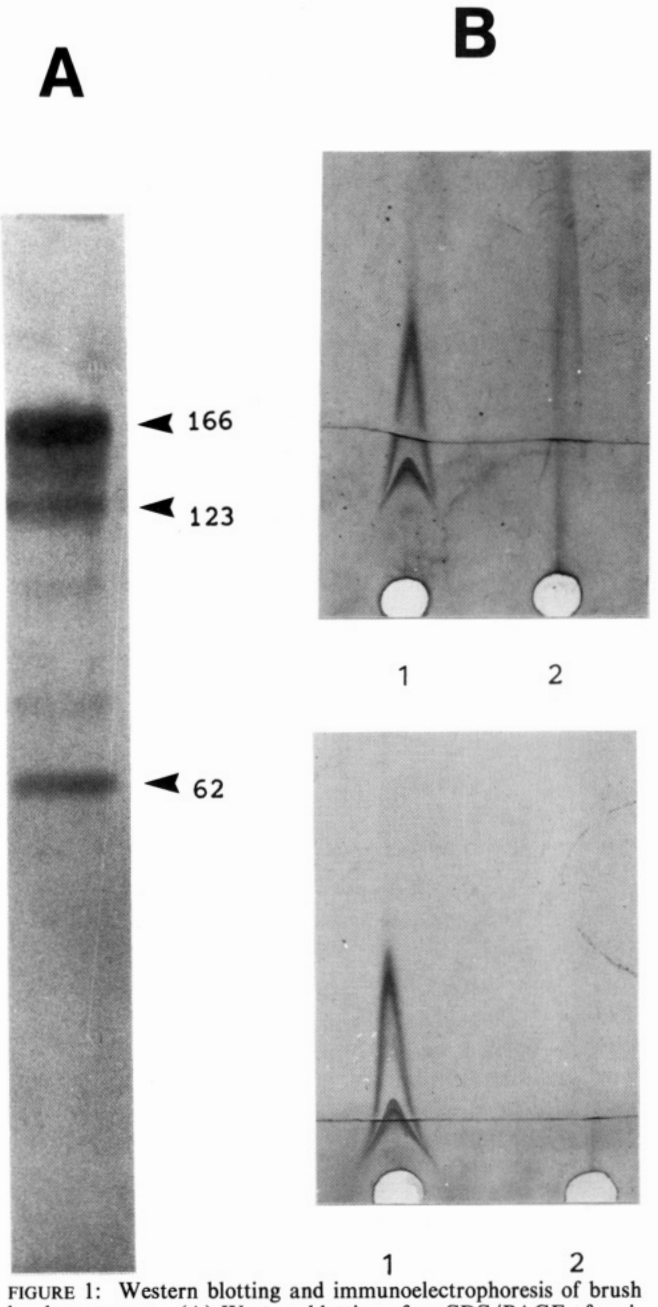

FIGURE 1: Western blotting and immunoelectrophoresis of brush border enzymes. (A) Western blotting of an SDS/PAGE of aminopeptidase N and sucrase–isomaltase immunoprecipitated from Triton X-100 solubilized mucosa as described under Experimental Procedures. The immunoprecipitate was boiled for 5 min in the presence of 1% SDS prior to electrophoresis. After electrophoresis and transfer to nitrocellulose paper, the polypeptides of the two brush border enzymes were visualized by Western blotting, using the precipitating antiserum as primary antibody. The molecular weights of the A, B, and C subunits of mature aminopeptidase N (166K, 123K, and 62K, respectively) are indicated. (B) A Triton X-100 extract of total intestinal mucosa in 50 mM Tris-HCl and 150 mM NaCl, pH 7.5, was mixed with an equal volume of 8 M urea and heated to 55 °C (upper panel) or boiled (lower panel) for 5 min. Together with controls, the samples were subjected to rocket immunoelectrophoresis against the brush border antiserum. After electrophoresis, the precipitates of aminopeptidase N (lower precipitate) and sucrase–isomaltase (upper precipitate) were visualized by staining with Coomassie brilliant blue. 1, controls; 2, denatured samples.

However, as polyvalent antisera of this type are able to precipitate malglycosylated aminopeptidase N and sucrase–isomaltase (Danielsen, 1989) as well as enzymes deficient in intracellular transport and dimerization (aminopeptidase N) (Danielsen, 1990b), precipitation with the antiserum is not a

[1] Abbreviations: SDS/PAGE, sodium dodecyl sulfate/polyacrylamide gel electrophoresis; apo A-1, apolipoprotein A-1.

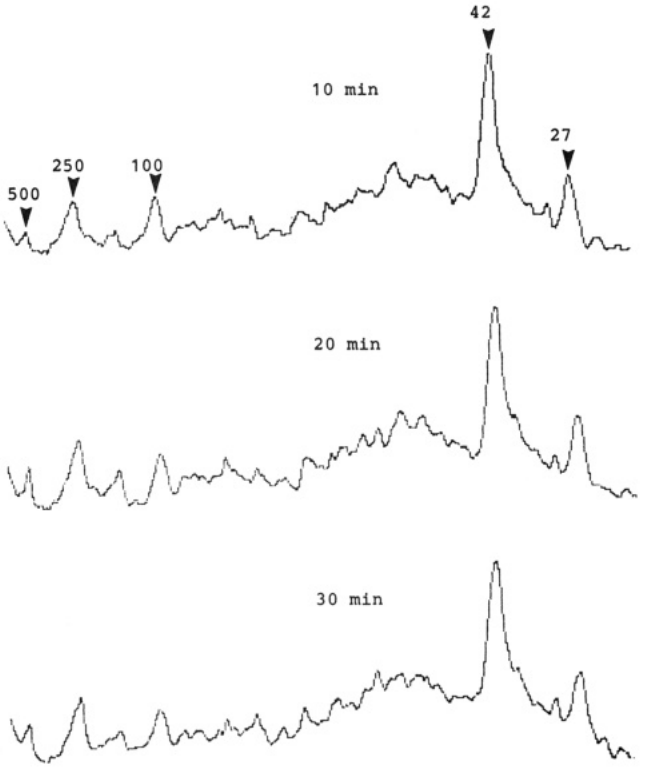

FIGURE 2: Rate of polypeptide synthesis. Mucosal explants were labeled continuously for periods of 10, 20, and 30 min with 1 mCi/mL [^{35}S]methionine. Fifty-microliter samples of mucosal extracts were subjected to SDS/PAGE, and after electrophoresis and fluorography, the gel tracks were densitometrically scanned. The relative areas of the peaks of 500, 250, and 100 kDa were determined and related to that of actin (42 kDa). The molecular weights of the major peaks ($\times 10^{-3}$) are indicated.

criterion of a native conformation. The antiserum is thus a useful tool for recording early folding stages common for both normal glycosylated as well as malglycosylated brush border enzymes during their biosynthesis in the enterocyte, whereas late, fine-tuning events in their conformational maturation are unlikely to be recognized.

Rate of Polypeptide Synthesis in the Enterocyte. Both newly synthesized aminopeptidase N and sucrase–isomaltase are composed of sizable polypeptides (140 and 240 kDa, respectively, including carbohydrate). A determination of the synthesis time required for completion of these polypeptides was therefore necessary before data on the kinetics of polypeptide folding could be interpreted. Figure 2 shows densitometrically scanned gel tracks of total mucosal extracts of explants, labeled continuously for 10, 20, and 30 min. In such extracts, actin (42 kDa) is the most abundant polypeptide component synthesized and is therefore a suitable reference to which polypeptide bands of higher molecular weight can be related. The largest polypeptide synthesized by mucosal explants and detectable in total extracts was of 500 kDa. Relative to actin, the 500-kDa peak had achieved its maximal size by 20 min, but only about 50% of that size by 10 min. Assuming that the 500-kDa peak and actin during these short labeling periods are not significantly turned over, this indicates that the time required for completion of the 500-kDa polypeptide is about 10 min. The second largest peak of the gel tracks is of approximately 250 kDa and includes sucrase–isomaltase. This peak was 80% of maximal size by 10 min, indicating a synthesis time of 4–5 min. Finally, a conspicuous peak of about 100 kDa was virtually maximally labeled already by 10 min, indicating a synthesis time $\ll$10 min. From these data, it can be estimated that the enterocyte synthesizes polypeptides at a rate of approximately 50 kDa/min and that full-length synthesis of the polypeptides of sucrase–isomaltase and aminopeptidase N requires about 4 and 2 min, respectively. A similar experiment was performed using mucosal explants, labeled in the presence of fructose and leupeptin. No effect on the rate of polypeptide synthesis of these reagents could be detected (data not shown).

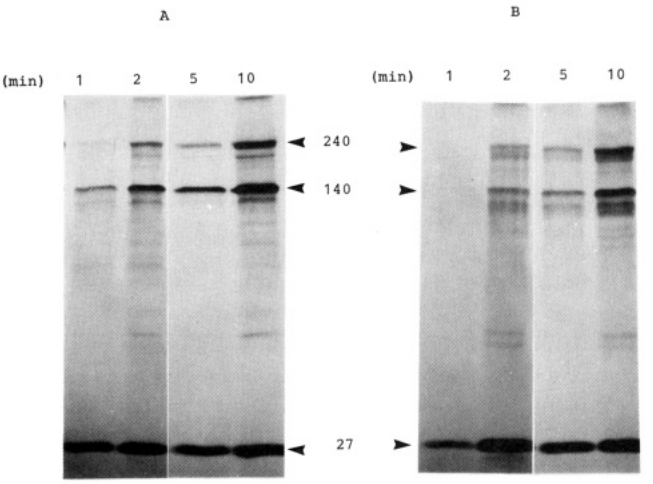

FIGURE 3: Short-pulse labeling of aminopeptidase N and sucrase–isomaltase. Mucosal explants were labeled continuously for 1–10 min with 1 mCi/mL [^{35}S]methionine in the absence (A) or presence (B) of 50 mM fructose. Aminopeptidase N (140 kDa), sucrase–isomaltase (240 kDa), and apo A-1 (27 kDa) were immunoprecipitated and run in SDS/PAGE. Molecular weights ($\times 10^{-3}$) are indicated.

Short-Pulse Labeling of Aminopeptidase N and Sucrase–Isomaltase. Figure 3A shows immunoprecipitation of the transient, high-mannose-glycosylated forms of aminopeptidase N and sucrase–isomaltase together with apo A-1 from mucosal explants, labeled continuously from 1 to 10 min. The 140-kDa polypeptide of aminopeptidase N was clearly visible at 1 min at which time the 240-kDa polypeptide of sucrase–isomaltase was barely detectable; 2 min of labeling was required to produce a clearly visible band of this large brush border enzyme. In the presence of 50 mM fructose, neither the transient form of aminopeptidase N nor sucrase–isomaltase was clearly visible until after 2 min of labeling (Figure 3B). In addition, a broad blur of malglycosylated polypeptides was seen extending below the position of the normal high-mannose-glycosylated form of both enzymes. In a previous work, the electrophoretically normal high-mannose form and also the malglycosylated forms, seen in the presence of fructose, were all fully susceptible to endoglycosidase H and migrated in the position of the nonglycosylated brush border enzymes after treatment with this glycosidase (Danielsen, 1989).

In this and subsequent experiments, the secretory protein apo A-1 was coprecipitated with the two brush border enzymes as a marker to which the amounts of immunoprecipitated aminopeptidase N and sucrase–isomaltase were related. Apo A-1 was chosen as such a marker because it is abundantly synthesized by enterocytes (the major peak of 27 kDa in Figure 2 represents apo A-1) and can thus be reliably quantitated by densitometry. In addition, its precipitation with antiserum was quantitative at labeling periods as short as 1 min (data not shown). Futhermore, apo A-1 is not a glycoprotein and hence its molecular size not affected by fructose, as evidenced by Figure 3. Its quantitative precipitation with antiserum was also not affected by fructose (data not shown).

Kinetics of Folding. Figure 4A shows the immunoprecipitation of aminopeptidase N relative to that of apo A-1 during continuous labeling periods of 1–30 min, as judged from

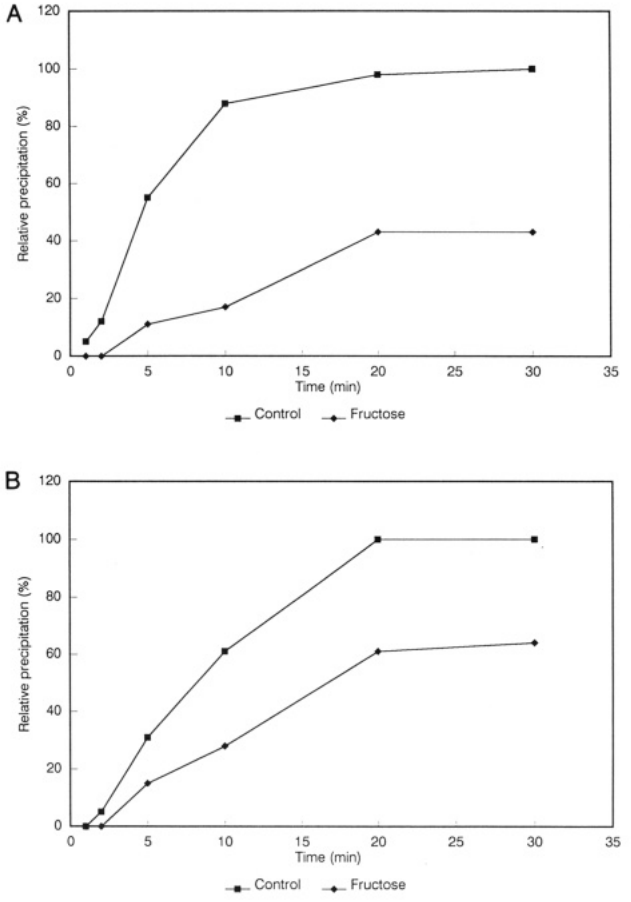

FIGURE 4: Kinetics of polypeptide folding. A labeling experiment, performed in the absence or presence of fructose as described in the legend to Figure 3. At each time point, the amounts of aminopeptidase N (A) and sucrase–isomaltase (B) (including the malglycosylated molecular forms in the presence of fructose) were determined by densitometric scanning of the gel tracks and related to that of apo A-1. For both brush border enzymes, the highest value (=100%) was obtained by 30 min of labeling in the absence of fructose.

densitometric scanning of gel tracks. Maximal immunoprecipitation was only achieved after 20–30 min; the half-time for achieving maximal precipitation was 4–5 min. Taking into account the time required for full-length synthesis of the polypeptide, these kinetics indicate that the acquisition of a tertiary conformation of newly made aminopeptidase N occurs with a half-time of 2–3 min.

Fructose markedly reduced the maximal immunoprecipitation of aminopeptidase N, relative to that of apo A-1 (Figure 4A). Furthermore, fructose increased the half-time for achieving this reduced maximal precipitation to 12 min. Corrected for the time required for polypeptide synthesis, this indicates a half-time of folding of about 10 min in the presence of fructose, i.e., almost a 5-fold increase in time compared to the control. In addition, this experiment confirmed the result, shown in Figure 3B, that aminopeptidase N in the presence of fructose is undetectable at 1 min and only barely detectable after 2 min of synthesis.

Figure 4B shows a similar immunoprecipitation curve of sucrase–isomaltase relative to apo A-1. The half-time for maximal immunoprecipitation was 8 min; corrected for the synthesis of this large polypeptide, it indicates a half-time of folding of about 4 min, i.e., almost twice as much as for aminopeptidase N. As with aminopeptidase N, fructose also reduced the maximal precipitation of sucrase–isomaltase; the half-time for achieving this reduced level of immunoprecipitation was increased to 12 min. Again, corrected for polypeptide synthesis, this corresponds to a half-time of folding of about 8 min, that is, twice as long as in the absence of fructose.

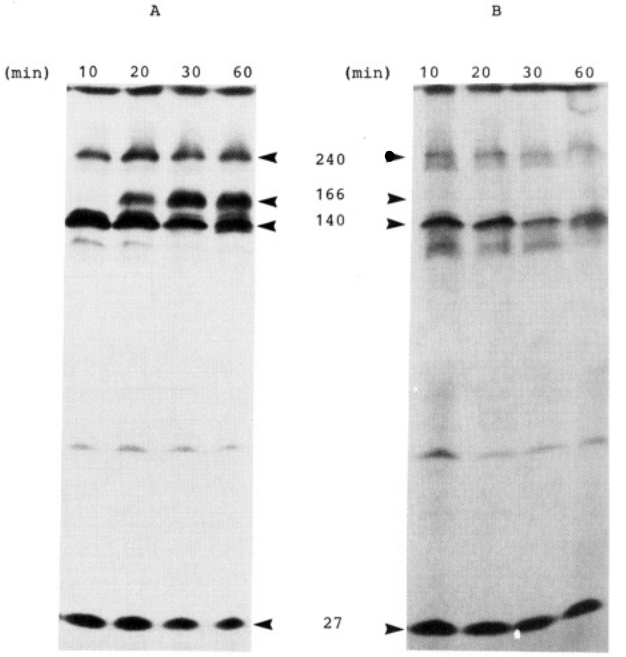

FIGURE 5: Pulse–chase labeling of aminopeptidase N and sucrase–isomaltase. Mucosal explants were labeled for 10 min with 0.5 mCi/mL [^{35}S]methionine in the absence (A) or presence (B) of fructose and chased with medium, containing nonradioactive methionine, for the indicated periods of time. Aminopeptidase N, sucrase–isomaltase, and apo A-1 were immunoprecipitated and subjected to SDS/PAGE. Molecular weights ($\times 10^{-3}$) are indicated.

Kinetics of Fructose-Induced Proteolytic Degradation. In pulse–chain experiments, both aminopeptidase N and sucrase–isomaltase, synthesized in a 10-min pulse, showed unchanged immunoprecipitation (relative to apo A-1) for periods up to 30 min (Figure 5A). (By 60 min, a substantial portion of apo A-1 has been secreted from the explant, and this protein is no longer a reliable marker to which the precipitation of the brush border enzymes can be related.) After 20 min of chase, the complex-glycosylated 166-kDa form of aminopeptidase N was visible, and by 60 min, it was the predominant molecular form. Sucrase–isomaltase, in contrast, is processed from a transient to a mature form at a much slower rate (Danielsen & Cowell, 1985; Stieger et al., 1988) and was still predominantly in its high-mannose-glycosylated form by 60 min. This indicates that newly synthesized aminopeptidase N and sucrase–isomaltase under normal conditions in the enterocyte are stable and resistant to degradation in the endoplasmic reticulum. Fructose, however, induced a degradation of aminopeptidase N as well as sucrase–isomaltase. As shown in Figure 5B, this happened not only to the malglycosylated polypeptides of the enzyme but also to the electrophoretically normal high-mannose-glycosylated brush border enzymes. The fact that the seemingly normal transient form of aminopeptidase N failed completely to undergo conversion to the Golgi-associated, complex-glycosylated form of 166 kDa even after 60 min of chase demonstrates that the proteolytic degradation occurs while the enzyme is being retained in a pre-Golgi compartment.

The quantitative immunoprecipitation of aminopeptidase N and sucrase–isomaltase (relative to apo A-1) during a chase in fructose-exposed mucosal explants is shown in Figure 6. For both brush border enzymes, maximal precipitation was achieved by 20 min of chase at which time point their relative

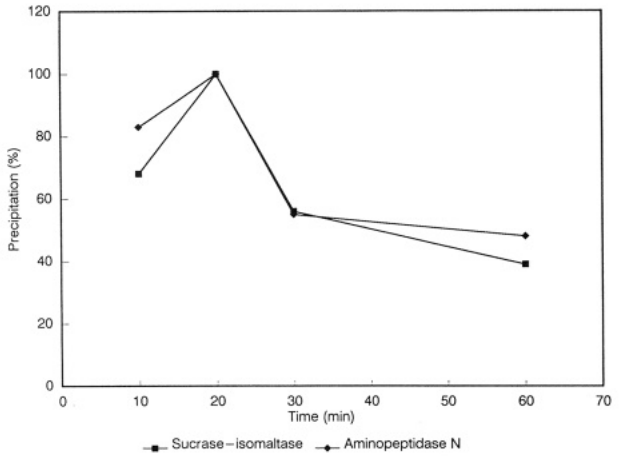

FIGURE 6: Kinetics of fructose-induced proteolytic degradation. The gel tracks of Figure 5B were densitometrically scanned, and for each time point, the amounts of aminopeptidase N and sucrase–isomaltase relative to that of apo A-1 were determined. For both enzymes a maximal value (=100%) was obtained after 20 min of chase.

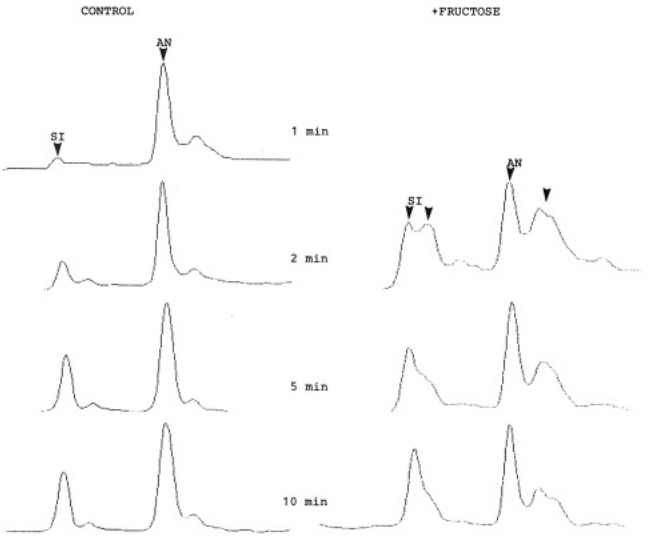

FIGURE 7: Fructose-induced delay of glycosylation. The gel tracks of aminopeptidase N and sucrase–isomaltase of Figure 3 were densitometrically scanned (in the presence of fructose, no conspicuous signal of either brush border enzyme could be obtained at the 1-min time point). Left panel, controls; right panel, fructose-exposed explants. The high-mannose-glycosylated form and malglycosylated forms of sucrase–isomaltase (SI) and aminopeptidase N (AN) are indicated by arrows.

precipitation slowly decreased, indicating a protelytic degradation occurring with a half-time of about 40 min.

Kinetics of Glycosylation. As shown in Figure 7 (left), the profiles of the peaks of immunoprecipitated aminopeptidase N and sucrase–isomaltase appeared identical in labeling periods from 2 to 10 min. At 1 min, however, the minor peak below the 140-kDa molecular form of aminopeptidase N was higher and broader than was observed at longer labeling periods. This could indicate that not all newly synthesized enzyme molecules under normal conditions receive their full complement of 10 high-mannose chains cotranslationally. In the presence of fructose, the peaks of malglycosylated molecules, extending below the normal high-mannose-glycosylated forms of both aminopeptidase N and sucrase–isomaltase, increased progressively in size (both in width and in height) with shorter labeling periods down to 2 min (Figure 7, right; as described above, no conspicuous profile of any of the enzymes could be obtained at 1 min). In theory, this could be explained by a preferential proteolytic degradation of the malglycosylated

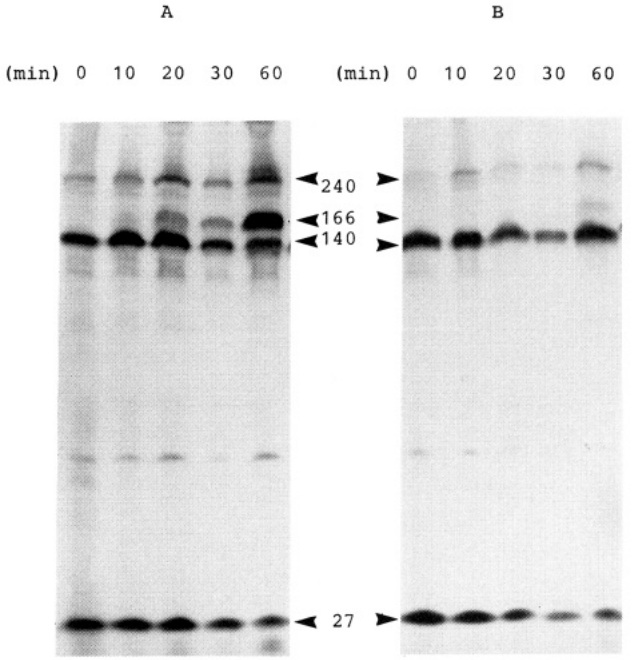

FIGURE 8: Effect of 3-hydroxynorvaline. Mucosal explants were pulsed for 10 min with 0.5 mCi/mL [^{35}S]methionine in the absence (A) or presence (B) of 3-hydroxynorvaline and chased for the indicated periods of time (in the absence of leupeptin). Aminopeptidase N, sucrase–isomaltase, and apo A-1 were immunoprecipitated from the labeled explants and run in SDS/PAGE. Molecular weights ($\times 10^{-3}$) are indicated.

molecular forms of the enzymes, but as shown in Figure 5B, this cannot be the case since the electrophoretically normal high-mannose form of aminopeptidase N as well as sucrase–isomaltase is degraded in parallel with the malglycosylated forms. In addition, the rate of degradation (half-time approximately 40 min) is too slow to account for the rapid reduction in the amount of the malglycosylated molecular form of both brush border enzymes. The most plausible explanation of this phenomenon is therefore that the rate of attachment of high-mannose chains is decreased in the presence of fructose, causing a transient pool of newly synthesized, yet not fully high-mannose-glycosylated, enzymes.

Perturbation of Biosynthesis with 3-Hydroxynorvaline. When 3-hydroxynorvaline (an analogue of threonine) is incorporated biosynthetically into aminopeptidase N, it severely inhibits the processing of the enzyme from the transient, high-mannose-glycosylated form to the mature, complex-glycosylated form as well as its transport to the apical cell surface (Danielsen, 1990b). Since molecular processing and surface expression are closely correlated with the ability of newly made aminopeptidase N polypeptides to form noncovalent homodimers, we have proposed that dimerization is a prerequisite for transport from the endoplasmic reticulum to the Golgi complex. The pulse–chase experiment, shown in Figure 8, compared the early posttranslational fate of aminopeptidase N in the presence or absence of this amino acid analogue. As can be seen, the appearance of the mature form of 166 kDa was severely retarded and almost blocked. However, the high-mannose-glycosylated form remained stable during periods up to 60 min. From this result, it can be concluded that prolonged retention in a pre-Golgi compartment does not per se trigger a rapid proteolytic degradation, as that observed in the presence of fructose. In contrast to aminopeptidase N, synthesis of sucrase–isomaltase was severely suppressed in the presence of 3-hydroxynorvaline, suggesting that the amino acid analogue in the case of this enzyme causes

the formation of an unstable and rapidly degraded molecule.

Discussion

It has long been established that folding of polypeptides into a compact, globular structure characteristic of the native and biologically active conformation is basically a process determined by the amino acid sequence and one ruled by the laws of thermodynamics (Creighton, 1990). However, in the case of membrane glycoproteins synthesized by ribosomes attached to the rough endoplasmic reticulum, a number of events may modify the nascent polypeptide and hence interfere with the folding process. These include removal of the signal sequence and attachment of N-linked, high-mannose carbohydrate. In addition, resident proteins of the endoplasmic reticulum may facilitate the folding process by reshuffling of disulfide bonds (protein disulfide isomerase) and by preventing aggregation during folding (BiP) (Hurtley & Helenius, 1989; Freedman et al., 1989).

Whereas refolding in vitro of denatured proteins is often a time-consuming process, polypeptide folding in vivo generally occurs on the second to minute time scale (Creighton, 1990), posing obvious experimental difficulties for the researcher trying to record the event. In spite of this, short-pulse experiments have been able to demonstrate that the influenza virus hemagglutinin and the G protein of vesicular stomatitis virus undergo folding and disulfide bond formation within 1–4 min of synthesis (Yewdell et al., 1988; Machamer et al., 1990; Braakman et al., 1991).

In the present work, the kinetics of folding of aminopeptidase N and sucrase–isomaltase were monitored as a time-dependent increase in immunoprecipitability of the newly made enzyme molecules, using a polyvalent antiserum raised to the native brush border enzymes. The kinetics whereby aminopeptidase N and sucrase–isomaltase attained maximal precipitation with the antiserum clearly exceeded the time required for full-length synthesis of their respective polypeptide chains. Thus, while polypeptide folding undoubtedly commences during synthesis, its termination must clearly occur posttranslationally. For aminopeptidase N, homodimerization likewise occurs in a pre-Golgi compartment, but at a much slower rate (Danielsen, 1990a). Acquisitions of tertiary and quaternary structures are therefore sequential processes in the biosynthesis of aminopeptidase N, as has been observed for viral membrane glycoproteins (Copeland et al., 1986; Gething et al., 1986; Yewdell et al., 1988; Doms et al., 1988). The marked difference in observed half-times of folding between the two brush border enzymes may in part be due simply to their 100-kDa difference in size. However, it could also reflect the fact that sucrase–isomaltase, unlike aminopeptidase N, is composed of two homologous but separate globular domains which presumably fold independently of another (Hunziker et al., 1986).

In a recent paper (Matter & Hauri, 1991), the folding of sucrase–isomaltase and dipeptidylpeptidase IV in Caco-2 cells was studied, using precipitation with monoclonal antibodies and protease sensitivity as criteria for their conformational maturation. Here, the kinetics whereby both brush border enzymes attained maximal precipitation with antibodies, recognizing the native, folded enzyme molecules, were very slow (half-times of about 60 min, following a 15-min pulse), indicating that the final conformational maturation takes place in the Golgi complex rather than in the endoplasmic reticulum. However, during their passage through the Golgi complex, brush border enzymes generally increase their molecular weight by 20–25K, mainly due to attachment of O-linked carbohydrate. Such an extensive glycoslylation probably causes minor alterations in their conformation, but it may also by itself alter the precipitability and protease sensitivity of the brush border enzymes. The apparent discrepancy in the folding kinetics of brush border enzymes of the present work and that of Matter and Hauri (1991) therefore probably reflects that different stages of the process are being studied: The short-pulse experiments of the present work record early folding events of the nascent polypeptide chains, whereas that of Matter and Hauri (1991) focuses on later steps in the process of conformational maturation of these enzymes.

The general importance of cotranslational, N-linked high-mannose glycosylation for correct folding of growing polypeptides is well documented (Kornfeld & Kornfeld, 1985; Elbein, 1987; Paulson, 1989). Endoglycosidase H and F digestion of pig aminopeptidase N and sucrase–isomaltase indicates that they contain about 10 high-mannose chains per polypeptide (Danielsen, 1982; Danielsen & Cowell, 1984). The molecular mechanism whereby fructose (or maybe a phosphorylated derivative) generates the malglycosylated forms of the brush border enzymes is unknown, but the observation that the "fructose effect" is reversible and suppressible by mannose suggests that the dietary sugar, like some monosaccharide analogues, interferes with the synthesis of lipid-linked oligosaccharide intermediates and thereby depletes the cell of available dolichol phosphate (Elbein, 1987). In the present work, fructose was observed to affect the earliest stage of biosynthesis of both aminopeptidase N and sucrase–isomaltase in two ways. First, it induced a time-dependent change in densitometric profiles of both brush border enzymes during short-pulse labeling (Figure 7B) which is indicative of a delayed (posttranslational) glycosylation that may well result from a shortage of lipid-linked high-mannose chains. Second, fructose caused a marked increase in the half-time of polypeptide folding, in particular of aminopeptidase N, but also of sucrase–isomaltase. This delayed and aberrant polypeptide folding was also indicated by the failure of the antiserum to precipitate detectable amounts of aminopeptidase N after 1 min of labeling, and it is the only plausible explanation why the seemingly normal high-mannose-glycosylated forms of both aminopeptidase N and sucrase–isomaltase were retained and degraded in the endoplasmic reticulum together with the corresponding malglycosylated forms. The present work therefore highlights the importance of correct timing of N-linked high-mannose glycosylation in relation to protein synthesis: If the rate of glycosylation cannot keep up with the rate of polypeptide synthesis, the nascent polypeptide chains of both aminopeptidase N and sucrase–isomaltase misfold into a tertiary conformation that signals retention and subsequent degradation in a pre-Golgi compartment.

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