Naturally Occurring Mutations in Intestinal Sucrase-Isomaltase Provide Evidence for the Existence of an Intracellular Sorting Signal in the Isomaltase Subunit

Jack A. M. Fransen, Hans-Peter Hauri,* Leo A. Ginsel, and Hassan Y. Naim‡

Laboratory for Electron Microscopy, University of Leiden, NL-2333 AA Leiden, The Netherlands; *Department of Pharmacology, Biocenter of the University of Basel, CH-4056 Basel, Switzerland; ‡Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Abstract. Mutations in the sucrase-isomaltase gene can lead to the synthesis of transport-incompetent or functionally altered enzyme in congenital sucrase-isomaltase deficiency (CSID) (Naim, H. Y., J. Roth, E. Sterchi, M. Lentze, P. Milla, J. Schmitz, and H. P. Hauri. *J. Clin. Invest.* 82:667-679). In this paper we have characterized two novel mutant phenotypes of CSID at the subcellular and protein levels. The first phenotype revealed a sucrase-isomaltase protein that is synthesized as a single chain, mannose-rich polypeptide precursor (pro-SI) and is electrophoretically indistinguishable from pro-SI in normal controls. By contrast to normal controls, however, pro-SI does not undergo terminal glycosylation in the Golgi apparatus. Subcellular localization of pro-SI by immunoelectron microscopy revealed unusual labeling of the molecule in the basolateral membrane and no labeling in the brush border membrane thus indicating that pro-SI is missorted to the basolateral membrane. Mapping of biosynthetically labeled pro-SI with four epitope- and conformation-specific monoclonal antibodies suggested that conformational and/or structural alterations in the pro-SI protein have prevented posttranslational processing of the carbohydrate chains of the mannose-rich precursor and have lead to its missorting to the basolateral membrane.

The second phenotype revealed two variants of pro-SI precursors that differ in their content of mannose-rich oligosaccharides. Conversion of these forms to a complex glycosylated polypeptide occurs at a slow rate and is incomplete. Unlike its counterpart in normal controls, pro-SI in this phenotype is intracellularly cleaved. This cleavage produces an isomaltase-like subunit that is transport competent and is correctly sorted to the brush border membrane since it could be localized in the brush border membrane by anti-isomaltase mAb. The sucrase subunit is not transported to the cell surface and is most likely degraded intracellularly. We conclude that structural features in the isomaltase region of pro-SI are required for transport and sorting of the sucrase-isomaltase complex.

SUCRASE-ISOMALTASE (SI)[1] is an integral membrane protein of the small intestinal microvillus membrane responsible for the terminal digestion of dietary sucrose and starch. It is synthesized as a two-active site, single-chain polypeptide precursor (Ghersa et al., 1986; Hauri et al., 1979, 1980, 1982; Naim et al., 1988a; Sjöström et al., 1980) which is cotranslationally N-glycosylated in the endoplasmic reticulum to a high mannose precursor (pro-SI$_h$) of M_r 210,000. During its transport to and through the Golgi apparatus, the N-linked glycans are trimmed and processed into complex-type oligosaccharides and O-linked sugars are attached (Naim et al., 1988a). From the Golgi apparatus the fully glycosylated enzyme (pro-SI$_c$, M_r 245,000) is transported to the microvillus membrane where it is cleaved into its two subunits, sucrase (M_r 145,000) and isomaltase (M_r 151,000), by pancreatic proteases (Hauri et al., 1979; Naim et al., 1988a). The enzyme is anchored in the microvillus membrane by its isomaltase subunit only, the sucrase subunit remains indirectly bound to the isomaltase subunit by noncovalent interactions (Hunziker et al., 1986). It has been shown that the enzyme is directly transported to the microvillus membrane in a signal-dependent manner, whereby sorting from other proteins occurs in the *trans*-Golgi network (Matter et al., 1990). This finding implies that structural motifs in protein molecules are recognized by intracellular sorting elements. As a consequence it can be expected that certain mutant proteins should be missorted. However,

---

1. *Abbreviations used in this paper:* CSID, congenital sucrase-isomaltase deficiency; LPH, lactase-phorizin hydrolase; SI, sucrase-isomaltase.

this has been rarely observed, possibly because such defects are often lethal during an early period of development, or are undetectable in the phenotype in many cases. Defects in brush border glycosidases are readily identifiable and mutations result in mild disease; thus, this class of proteins is ideal to investigate mutations that alter intracellular transport and sorting of these enzymes.

Congenital sucrase–isomaltase deficiency (CSID) is characterized by undetectable sucrase activity, while the isomaltase activity can vary from absent to practically normal. The molecular basis of CSID is unknown, but recent data (Hauri et al., 1985a; Lloyd and Olsen, 1987; Naim et al., 1988b) show that CSID is not due to a single molecular defect. In fact, all patients studied have shown a different phenotype. At least three different phenotypes have been found (Naim et al., 1988b): phenotype I in which a membrane associated high-mannose precursor accumulates intracellularly, probably in the ER; phenotype II in which the protein apparently accumulates in the Golgi apparatus but is degraded before complex glycosylation; and phenotype III in which catalytically altered enzyme is transported to the microvillus membrane. These results suggest that different mutations can lead to transport incompetent or functionally altered proteins.

In the present investigation we report on two novel phenotypes of CSID. In the first phenotype sucrase–isomaltase accumulates in the endoplasmic reticulum in its high-mannose form and is partially missorted to the basolateral membrane. In the other phenotype the single chain form of sucrase–isomaltase is cleaved, most likely intracellularly. The sucrase subunit is degraded, whereas the isomaltase subunit is normally transported to the brush border. Collectively, the two phenotypes show that sorting of the sucrase–isomaltase molecule is mediated by or involves signal recognition elements that are most likely located within the isomaltase portion of the enzyme complex.

Materials and Methods

Patients

CSID was established by enzyme activity measurements, sucrose tolerance tests, and breath hydrogen tests. Both patients originated from the Amsterdam area; patient 1 is a 38-yr-old male, and patient 2 a 24-yr-old male. Control biopsies were obtained from patients screened for diagnostic purposes other than CSID. All the peroral jejunal suction biopsies used were histologically normal. Four to five biopsies were obtained from each patient. One biopsy was cut into small pieces and immediately fixed for immunoelectron microscopy, one biopsy was frozen at −70°C for enzyme activity measurements, and the other two to three biopsies were placed in organ culture for the metabolic labeling experiments. The protocol according to which the patients were analyzed, was essentially the same as used by Naim et al. (1988b).

Enzyme Activities

Disaccharidase activities were measured according to Dahlqvist (1968), using sucrose, isomaltose, maltose or lactose as substrates. Protein was determined with the Bio-Rad protein assay kit (Bio-Rad Laboratories, Oxnard, CA) using protein standard I.

Antibodies

All mAbs used have been described before (Hauri et al., 1985b). They included HBB 2/614 against the sucrase subunit, HBB 3/705 against the isomaltase subunit, HBB 2/219 and HBB 1/691 against native sucrase–isomaltase, HBB 1/909 against lactase, HBB 3/153 against aminopeptidase N, and HBB 3/264/18 against angiotensin converting enzyme.

Initial characterization of the mAbs was performed by enzyme-linked immunosorbent assay and enzyme activity measurements of the immunoisolated antigens (Hauri et al., 1985b). The epitope specificities of the four anti-sucrase–isomaltase antibodies used in this study were determined by competitive enzyme-linked immunosorbent assay and by immunoblotting (Hauri et al., 1985a). These studies revealed that the antibody HBB 3/705 recognizes the isomaltase subunit and the antibody HBB 2/614 recognizes the sucrase subunit. However, since sucrase–isomaltase is synthesized as a single chain polypeptide precursor containing two subunits, i.e., uncleaved pro-SI, immunoprecipitation with either one of the two antibodies can precipitate uncleaved pro-SI forms. Hauri et al. (1985b) have shown that the antibody HBB 3/705 recognizes high-mannose but not complex glycosylated forms of pro-SI while the antibodies HBB 2/614, HBB 2/219, and HBB 1/691 immunoprecipitate both high-mannose as well as complex glycosylated pro-SI. Some of the characteristics and specificities of the anti-sucrase–isomaltase antibodies are listed in Table I.

Biosynthetic Labeling

Biopsy specimens were metabolically labeled in organ culture with 150 μCi/ml L-[^{35}S]methionine according to Naim et al. (1987) for 30 min, 4 h, and 20 h. In some experiments the biopsy samples were cultured in the presence of trypsin 1 mg/ml final concentration. After the labeling periods, the tissue was washed, homogenized, and subjected to immunoprecipitation.

Immunoprecipitation and SDS-PAGE

Immunoprecipitation was performed according to the method described by Naim et al. (1988b). Briefly, the metabolically labeled homogenates were solubilized with NP-40 and sodium deoxycholate. Sucrase–isomaltase was precipitated by a mixture of four mAbs adsorbed to protein A-Sepharose beads. Aminopeptidase N, lactase, and angiotensin converting enzyme were precipitated for control purposes by sequential immunoprecipitation of the SI-depleted extracts with mAbs coupled to CNBr-activated Sepharose. Part of the immunoprecipitates were digested with endo-β-N-acetylglucosaminidase H (endo H) (Kobata, 1979) and endo-β-N-acetylglucosaminidase F/glycopeptidase F as described (Naim et al., 1987). For the epitope mapping a portion of the solubilized homogenates was divided into four equal parts and each part was precipitated with one of the mAbs mentioned above.

SDS-PAGE was performed with 5% acrylamide gels according to Laemmli (1970). Gels were stained with Coomassie blue, destained, soaked with Enlightning (DuPont NEN, Wilmington, DE), dried, and exposed to Kodak SO-282 films (Eastman Kodak Co., Rochester, NY) at −80°C.

Immunoelectron Microscopy

Biopsies were cut into small pieces and fixed by immersion in a mixture of 2% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% glutaraldehyde for 1 h at room temperature, and stored in 2% formaldehyde until use. Cryosectioning and immunolabeling were performed as described previously (Fransen et al., 1985).

Results

The CSID of the two patients was initially assessed by the breath hydrogen test and has been confirmed by enzyme activity measurements in mucosal homogenates. In both pa-

Table I. Characterization and specificities of the mAbs against Sucrase–Isomaltase Used in This Study

| Antibody | Reaction with | | | | |
| | pro-SI$_c$ | pro-SI$_h$ | Subunits | | Reaction on Western blot |
			Sucrase	Isomaltase	
HBB 1/691	+	+	+	+	−
HBB 2/219	+ +	+ +	+	+	−
HBB 2/614	+ +	+	+	−	+
HBB 3/705	+	−	−	+	+

tients sucrase activity was undetectable (<1 U, normal range 40–136 U). Isomaltase was undetectable in patient I (<1 U) but displayed normal levels of activity in the other (42 U, normal range 35–123 U). Maltase–glucoamylase was 10-fold reduced in patient I and almost 4-fold in patient II. The fact that sucrase–isomaltase is responsible for ∼90% of the maltase–glucoamylase activity provides an explanation for the low levels of maltase–glucoamylase obtained with these two patients. Lactase-phlorizin hydrolase activity was ∼40% reduced in patient I and ∼80% reduced in patient II. It should be mentioned that the significant decline in the activity of this enzyme in patient II is not associated with any clinical symptoms similar to those observed in lactose malabsorption. More importantly, the biosynthesis and maturation of the enzyme was found to be similar to that in normal controls (see Fig. 11 b). The activities of other brush border membrane enzymes like aminopeptidase N, dipeptidylpeptidase IV, alkaline phosphatase and angiotensin converting enzyme were measured fluorometrically and were found to lie in the normal range. These data pointed to a defect in both sucrase and isomaltase in case I and an isolated enzyme defect associated with sucrase in case II. To investigate possible alterations associated with these defects at the subcellular and protein level we analyzed biopsy specimens from these two patients according to the experimental protocols described

previously by Naim et al. (1988b) and attempted to compare the data obtained with established phenotypes of CSID.

The biosynthetic labeling experiments with the mucosa of the first patient showed that after a 30-min pulse, sucrase-isomaltase was synthesized as an electrophoretically normal, endo H sensitive, pro-SI$_h$ (Fig. 1, lane 1). The molecule persists in the endo H sensitive high mannose form after the 4- and the 20-h labeling (Fig. 1, lanes 3–6). However, the intensity of the band in the 20-h labeling was much less than in the 30-min and 4-h samples. In contrast, the glycoprotein studied as a control, aminopeptidase N, showed an increased amount of labeled polypeptides in the 20-h labeled biopsy sample in comparison with the 30 min and 4-h labeled tissue (Fig. 1, lanes 7–9). The results indicate that sucrase–isomaltase in the patient's biopsy sample undergoes intracellular degradation during the prolonged labeling, or has a higher turnover than the molecule in normal controls. To assess whether the mutated pro-SI molecule has undergone possible gross structural alterations, immunoprecipitation experiments were performed with the mAbs HBB 2/614, HBB 2/219, HBB 1/691, and HBB 3/705 that are directed against four different epitopes of sucrase–isomaltase. Antibodies HBB 2/219 and HBB 1/691, which have high affinity for the native sucrase–isomaltase protein (Matter and Hauri, 1991), failed to immunoprecipitate pro-SI$_h$ (Fig. 2 a, lanes 2 and
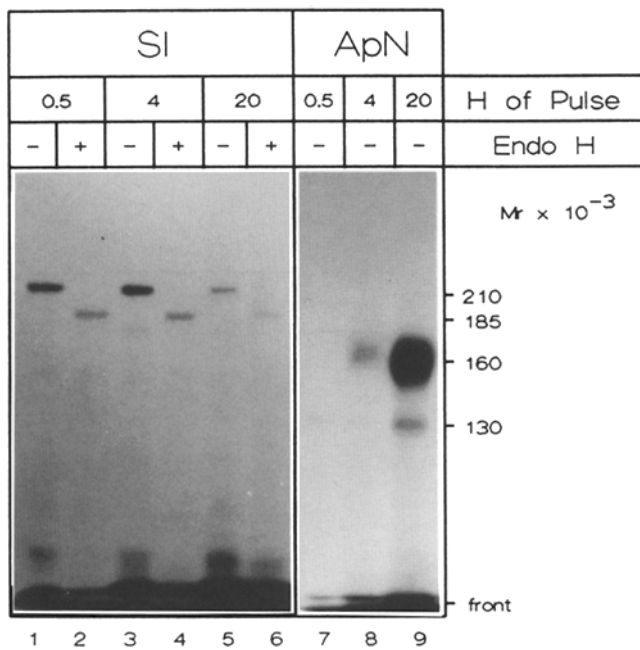

Figure 1. Metabolic labeling of sucrase-isomaltase (SI) and aminopeptidase N (ApN) in intestinal mucosa of patient I. Biopsies were cultured for the indicated time periods in the presence of [^{35}S]methionine. Immunoprecipitation was carried out with a mixture of four mAbs against SI. One of two identical immunoprecipitates was treated with endo H to probe for high-mannose and complex-glycosylated forms. The bands near the front of the gel in lanes 1–6 most likely represent pro-SI-degradation products, whose proportion increases with increasing pulse time. This is suggested by the observation that similar bands could not be detected in the protein pattern of ApN (lanes 7–9). The double front is probably due to the presence of excess SDS that we use to elute proteins from the immunoprecipitates.
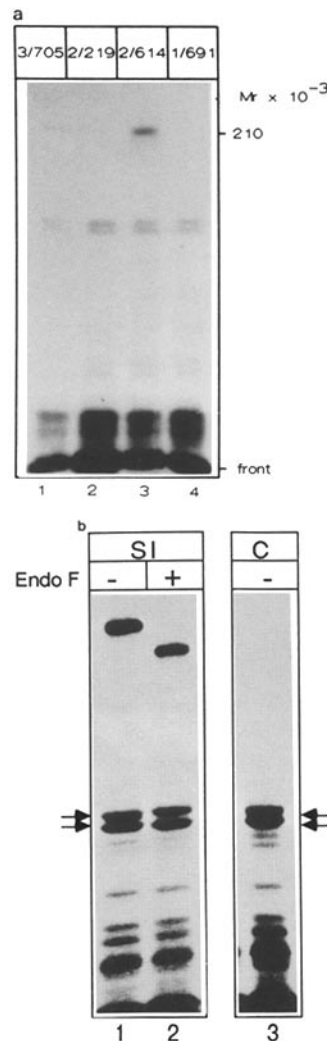

Figure 2. Immunoprecipitation with four mAbs against different epitopes of sucrase–isomaltase. (a) A biopsy of patient I was cultured for 4 h with [^{35}S]methionine and subjected to the immunoprecipitation procedures described in Materials and Methods. The additional bands in the middle and at the front of the gel originate from repeated freezing and thawing of the samples. The doublet at the center of the gel is not heterodimeric sucrase-isomaltase (see b). (b) Detergent extracts of the labeled biopsy sample in a were thawed out and precleared with nonimmune mouse immunoglobulin bound to protein A–Sepharose (lane 3). The samples were then immunoprecipitated with a mixture of the four anti-sucrase–isomaltase antibodies (lanes 1 and 2). Part of the immunoprecipitate was treated with endo F/GF (lane 2) and part was untreated (lane 1). The fluorogram shows that the doublet is also found in the precipitate with the nonimmune serum (lane 3). Further, this doublet does not represent glycosylated components since it is not sensitive to Endo F/GF.

4). This suggests that the corresponding two epitopes did not mature properly and hence were altered. Antibodies HBB 3/705 (Fig. 2 a, lane 1, better visible after longer exposure of the gel, not shown) and HBB 2/614 (Fig. 2 a, lane 3) precipitated pro-SI$_h$, with a similar intensity as observed in control experiments (Naim et al., 1988b). It is important to note that the doublet around the center of the gel is not the mature sucrase–isomaltase heterodimer that is generated in the intestinal lumen by cleavage of pro-SI by pancreatic secretions. As shown in Fig. 2 b, these two bands are found in the control with the nonimmune serum (Fig. 2 b, lane 3) as well as in the immunoprecipitate of pro-SI with specific anti-SI antibodies (Fig. 2 b, lanes 1 and 2). Further, these two bands are in contrast to sucrase and isomaltase unglycosylated, since treatment with endo F/glycopeptidase F did not affect their electrophoretic mobility at all, while a clear shift in the size of pro-SI is observed (Fig. 2 b, lanes 1 and 2). Together, these results clearly demonstrate that the doublet represents nonspecific binding of the detergent extracts to the monoclonal antibodies. This nonspecific binding is most likely generated by repeated freezing and thawing of the detergent extracts of the biopsy.

Iodination of the proteins in biopsy homogenates followed by immunoprecipitation of the detergent extracts with the anti-SI mixture (Naim et al., 1988b) supported and extended the data obtained by the biosynthetic labeling experiments. One single band was revealed of M_r 210,000, which displayed similar labeling intensity as the pro-SI$_h$ species in normal controls (not shown). Other polypeptides were not discerned. Collectively, the data indicate that in this case pro-SI$_h$ does not undergo posttranslational processing in the Golgi apparatus since no intermediates or mature forms of pro-SI were found.

In fact, the immunoelectron microscopical data obtained with antibodies HBB 2/614 and HBB 3/705 showed that sucrase–isomaltase could only be localised in the endoplasmic reticulum and, interestingly, in the basolateral membrane (Fig. 3, a–c). In control biopsies these structures are always negative (Fig. 4, see also Fransen et al., 1985). These results indicate that in this patient sucrase–isomaltase is synthesized as an electrophoretically normal pro-SI$_h$, which is partially retained in the endoplasmic reticulum and missorted to the basolateral membrane.

The biosynthetic labeling experiments with samples of the mucosa of the second patient revealed after a pulse period of 30 min, a sucrase–isomaltase protein that comprised two polypeptides (Fig. 5 a, lane 1). The upper of these two polypeptides migrated on SDS-PAGE with the same mobility as the pro-SI$_h$ in normal controls (Naim et al., 1988b) and has therefore a similar apparent molecular weight of 210,000 daltons. The lower polypeptide of the doublet migrates slightly faster with a M_r ~207,000 D. Treatment of these bands with endo H, which removes glycan units of the high mannose type, converted both of them to a single band migrating at M_r 185,000 (Fig. 5 a, lane 2) that corresponds to the size of endo H digested pro-SI$_h$ in normal controls (Fig. 6, lane 2). These data clearly indicate that the SI-doublet in the patient's biopsy sample was a consequence of variation in the glycosylation pattern of the polypeptide backbone and the band migrating with M_r 207,000 was not a truncated form of the M_r-210,000 polypeptide. After a 4-h

labeling period the doublet SI protein previously identified was still present. In addition a polypeptide of M_r 226,000 was detected (Fig. 5 a, lane 4) which was not susceptible to endo H treatment (Fig. 5 a, lane 5) indicating that it corresponds to a complex glycosylated form of mutated pro-SI. Under similar labeling conditions a polypeptide of M_r 245,000 can be detected in normal controls (Fig. 6, lanes 1) which is endo H resistant. It is known that brush border components carry ABO blood group antigenic determinants that could generate slight molecular weight variations reflecting heterogeniety of O-linked chains (Bernadac et al., 1984; Kelly and Alpers, 1973; Triadou et al., 1983). In fact, as shown in Fig. 5 b, pro-sucrase–isomaltase, sucrase and isomaltase purified from brush border membranes of individuals carrying blood group A determinants (Fig. 5 B, lane 2) have slightly higher apparent molecular weights than their counterparts in mucosal membranes of individuals with blood group O determinants (Fig. 5 b, lane 1). However, the difference observed in the apparent molecular weight between the complex glycosylated forms of wild type pro-SI (Fig. 6, lane 1) and mutated pro-SI in the patient's biopsy sample (Fig. 6, lane 3) of ~19,000 can not be attributed to differences in blood group determinants since the patient as well as the control have the same blood group. After a 20-h pulse labeling the early doublet form (M_r 210,000 and 207,000) and the M_r 226,000 form were still detected in the patient's biopsy sample. An additional strongly labeled band of M_r 149,000 was observed (Fig. 5 a, lane 7). A polypeptide of similar apparent molecular weight was absent in the control biopsy specimen that was labeled for the same period of time (Fig. 6, lanes 5 and 6) and was never observed in biopsy samples that were labeled under similar conditions (Sterchi et al., 1990, and quoted in Naim et al., 1988a,b).

The glycosylation pattern of the M_r 149,000 polypeptide was assessed by endo H and endo F/glycopeptidase F (GF) treatments. Fig. 5 a shows that the M_r 149,000 (as well as the mutated pro-SI$_c$) was resistant to endo H treatment since no shift in its apparent molecular weight was detected, while the mannose-rich pro-SI precursor (serving here as internal control) was sensitive to the enzyme (Fig. 5 a, lanes 7 and 8). Upon digestion of the M_r 149,000 with endo F/GF a substantial decrease in its apparent molecular weight was observed (Fig. 5 c, lanes 1 and 2). These results clearly indicate that the M_r 149,000 polypeptide is a complex glycosylated molecule.

Immunoprecipitation experiments with four epitope-specific mAbs showed that the pro-sucrase–isomaltase protein was identified by each of these antibodies (Fig. 7). Interesting in this respect are the variations in the band patterns of the immunoprecipitates. Thus, the antibodies HBB 2/219 and HBB 1/691 precipitated the M_r-207,000 component of the high-mannose doublet and the endo H-resistant M_r 226,000 species (Fig. 7, lanes 3 and 4, respectively); the antibody HBB 3/705 recognized both forms of the high-mannose doublet (Fig. 7, lane 1); finally the HBB 2/614 reacted with all three forms of pro-SI, although to a lesser extent with the M_r 226,000 component (Fig. 7, lane 2). The results indicate that these antibodies recognize specific conformations that the pro-SI molecule assumes during its maturation. In fact, recent observations have shown that antibodies HBB 2/219 and HBB 1/691 have higher affinities towards mature forms
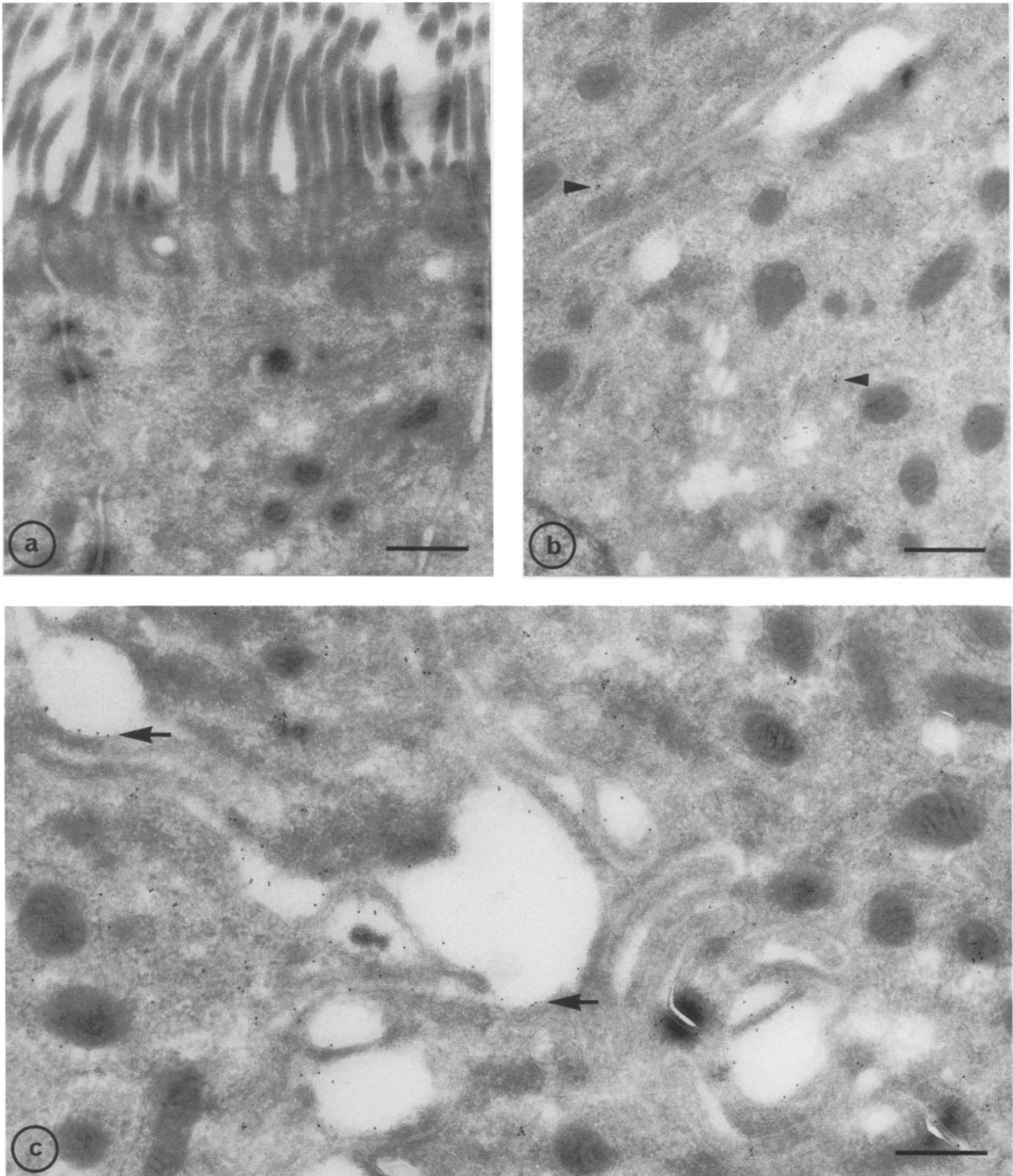
Figure 3. Immunolocalization of sucrase–isomaltase in a biopsy of patient I, with antibody HBB 2/614. (*a* and *b*) No labeling is observed over the brush border and the Golgi apparatus. The rough ER showed some labeling (*arrowheads*), which was never observed in control biopsies. (*c*) In addition a clear labeling of the basolateral membrane is observed (*arrows*), in contrast to control biopsies (see also Fig. 4). Bar, 0.5 μm.
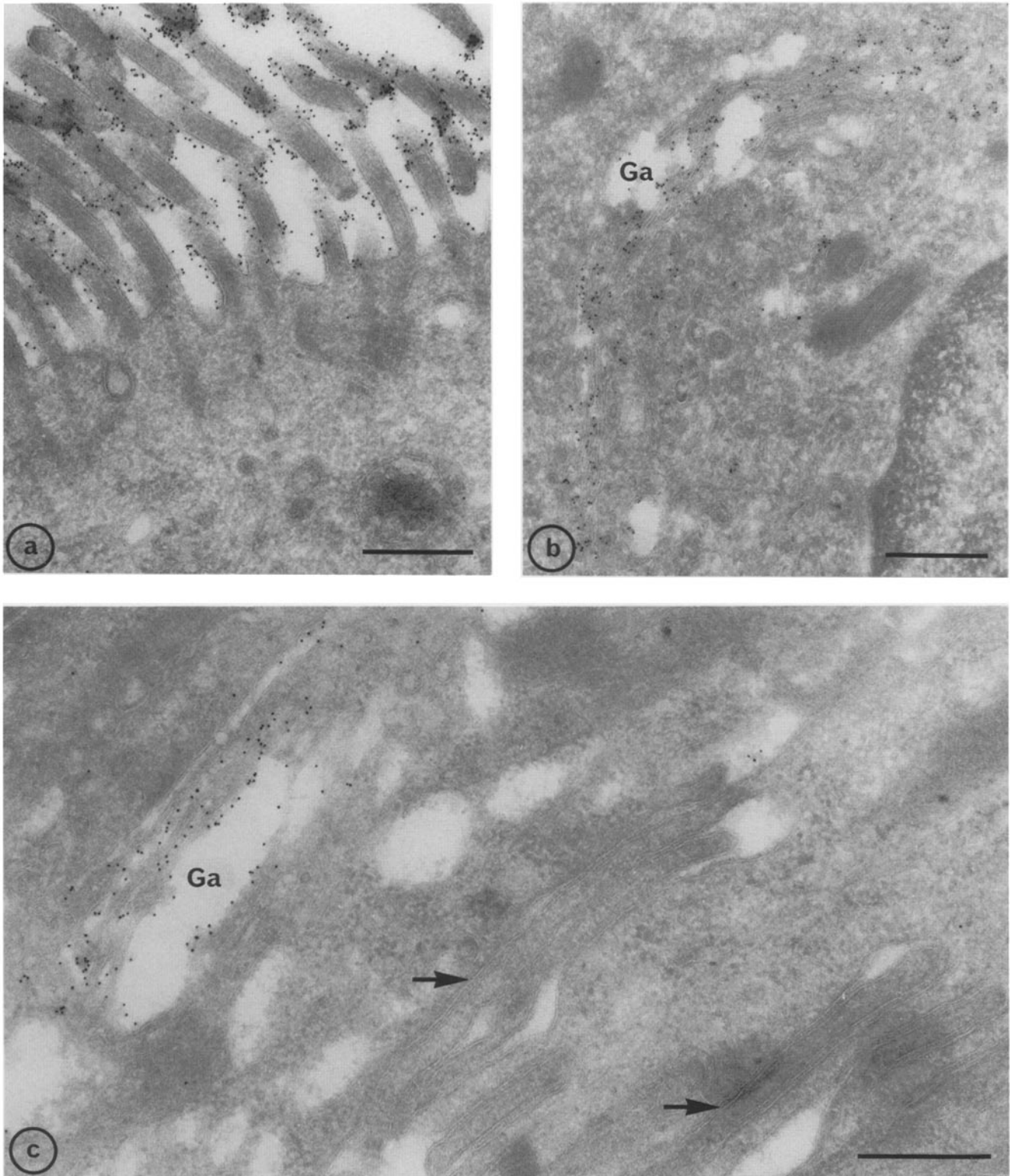
Figure 4. Immunolocalization of sucrase–isomaltase in a control biopsy, with antibody HBB 2/614. (*a*) Labeling pattern observed over the brush border. (*b*) Labeling pattern observed over the Golgi apparatus. Both structures are always strongly labeled. (*c*) However, the basolateral membrane (*arrows*) is always negative for sucrase–isomaltase. Bar, 0.5 μm.
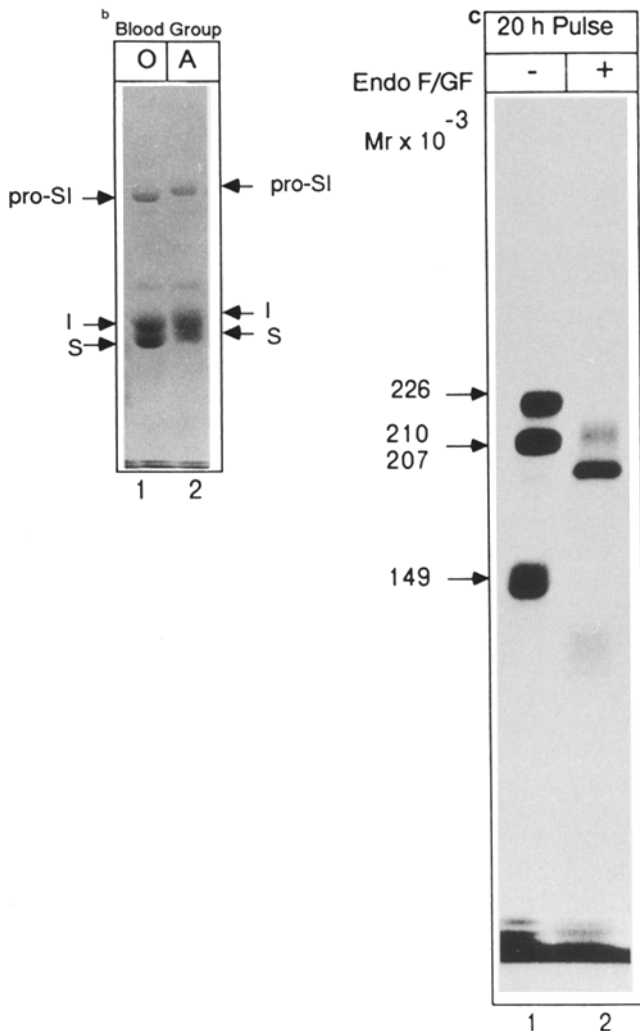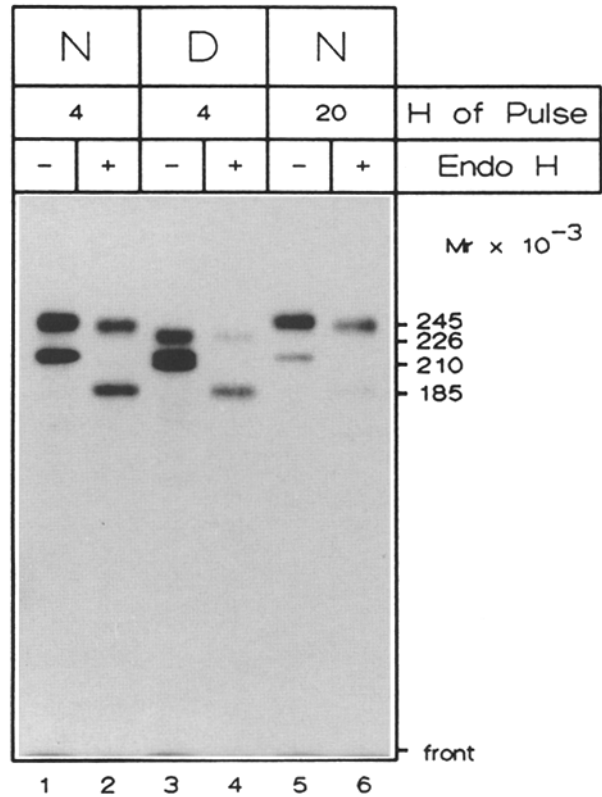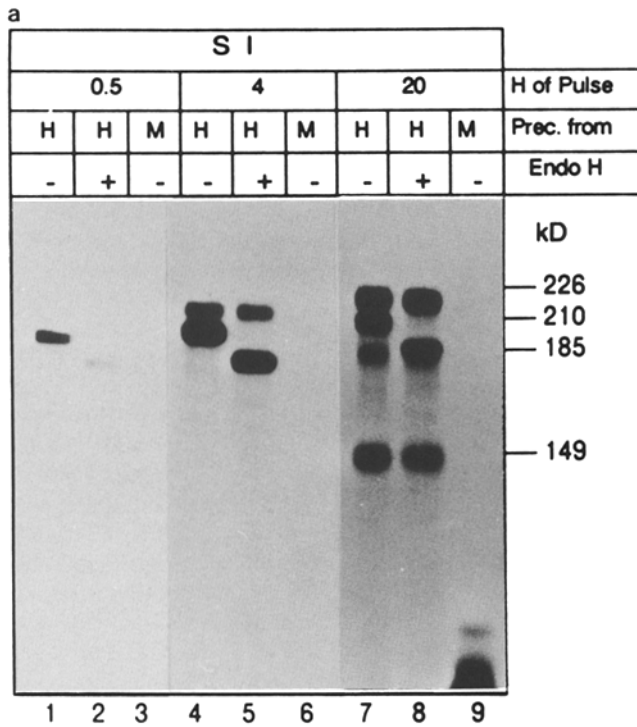
a

	S I									
	0.5		4			20			H of Pulse
H	H	M	H	H	M	H	H	M	Prec. from
−	+	−	−	+	−	−	+	−	Endo H

kD
— 226
— 210
— 185

— 149

1 2 3 4 5 6 7 8 9

N		D		N		
4		4		20		H of Pulse
−	+	−	+	−	+	Endo H

$M_r \times 10^{-3}$

— 245
— 226
— 210
— 185

front

1 2 3 4 5 6

Figure 6. Comparison of the molecular weight of pro-SI_c in patient II (*D*) with that observed in a normal control (*N*). Biopsy specimens were labeled for 4 h with [^{35}S]methionine (lanes *1–4*) or 20 h (lanes *5* and *6*) and sucrase–isomaltase was precipitated with a mixture of four mAbs against SI. The immunoprecipitates were also probed for high-mannose and complex-glycosylated forms by endo H treatment (lanes *2*, *4*, and *6*). Note that a cleaved band of M_r 149,000 similar to that observed in Fig. 5 *a*, lanes *7* and *8* is not observed in the 20-h labeled sample of the normal control (lanes *5* and *6*).

b Blood Group

O	A
1	2

pro-SI → ← pro-SI

I → ← I
S → ← S

c 20 h Pulse

Endo F/GF | − | + |

$M_r \times 10^{-3}$

226 →
210 →
207

149 →

1 2

Figure 5. (*a*) Metabolic labeling of sucrase-isomaltase (*SI*) in intestinal mucosa of patient II. Biopsies were cultured for the indicated time periods in the presence of [^{35}S]methionine. Immunoprecipitation was called out with a mixture of four mAbs against SI from homogenates (*H*) or the culture medium (*M*). One of two identical immunoprecipitates was treated with endo H to probe for high-mannose and complex-glycosylated forms. (*b*) Brush border sucrase-isomaltase carries blood group determinants. Brush border membranes prepared from mucosa of individuals having blood groups O (lane *1*) and A (lane *2*) were solubilized and immunoprecipitated with anti-sucrase-isomaltase antibodies. The gel was stained with Coomassie blue. As shown, slight variations in the size of pro-SI, sucrase (*s*), and isomaltase (*I*) of both blood groups are discerned. The bands around the center of the gel correspond to the immunoglobulin molecule (M_r 150,000). (*c*) Treatment of sucrase-isomaltase with endo F/glycopeptidase F (endo F/GF). Detergent extracts of the 20-h labeled biopsy sample were immunoprecipitated with a mixture of four anti-sucrase-isomaltase antibodies. The immunoprecipitates were analyzed by SDS-PAGE without (lane *1*) or with (lane *2*) endo F/GF treatment.
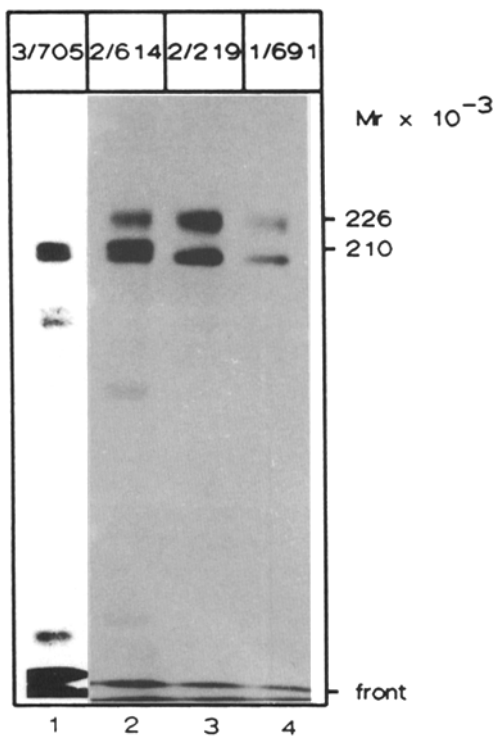
Figure 7. Immunoprecipitation with four mAbs against different epitopes of sucrase–isomaltase. A biopsy of patient II was cultured for 4 h with [^{35}S]methionine and subjected to the immunoprecipitation procedures described in Materials and Methods.

of the protein, while antibody HBB 3/705 recognizes more efficiently the newly synthesized forms. This suggests that the M_r-207,000 component is a processed form of the M_r-210,000 polypeptide. In this respect, pulse-chase experiments are required to support this hypothesis. The processing of the M_r-210,000 form to the M_r-207,000 form may be related to the second conformational change before complex glycosylation of the protein (Matter and Hauri, 1991). Therefore, our results are compatible with the identification of at least three conformations of pro-SI (corresponding to the M_r 207,000, 210,000, and 226,000 forms) that vary in their glycosylation pattern.

Immunoelectron microscopy with antibody HBB 3/705 showed similar labeling patterns in controls as in the second patient's biopsy, both in the brush border (Fig. 8, a and c) and in the Golgi apparatus (Fig. 8, b and d). It should be mentioned that antibody HBB 3/705 recognizes the complex glycosylated pro-SI in ultrathin cryosections more efficiently then in detergent-solubilized membranes. In contrast, no labeling of the sucrase subunit with antibody HBB 2/614 was observed in the brush border and only little labeling in the Golgi apparatus (Fig. 9, a and b), which are heavily labeled in the control biopsies (Fig. 4, see also Fransen et al., 1985). This suggests that the M_r-149,000 is in all likelihood isomaltase that is transported to the brush border membrane. These data are in accordance with the enzyme activity measurements. The observation that the anti-sucrase antibody HBB 2/614 precipitates the M_r-149,000 in immunoprecipitation experiments, can be explained by an association of this subunit with the uncleaved precursor, and not to a direct

binding of the antibody to this subunit. In normal brush border membranes the sucrase and isomaltase subunits remain associated via non-covalent interactions (Hunziker et al., 1986). Therefore, this subunit has most likely coprecipitated with the uncleaved precursor by antibody HBB 2/614.

The following lines of evidence and arguments strongly favor the possibility that the M_r-149,000 subunit is generated by intracellular cleavage of the mutated pro-SI$_c$ and not by extracellular cleavage by putative brush border endopeptidases or proteases: (a) It is obtained in the organ culture of a biopsy sample that was not exposed to any source of pancreatic secretions; a cleaved polypeptide was never observed in normal control biopsy samples (see Fig. 6; Sterchi et al., 1990; and quoted in Naim et al., 1988a,b). (b) Assuming that pro-SI is cleaved extracellularly, then sucrase–isomaltase must have passed the Golgi apparatus in its uncleaved pro-SI form. In view of the fact that normal levels of pro-SI are synthesized in our patient's biopsy sample, immunolabeling of the Golgi apparatus with anti-sucrase antibodies (HBB 2/614) should be as intense as in normal controls. Figs. 4 and 9 show that this was not the case. In fact, immunolabeling of the Golgi apparatus in the control biopsy sample with anti-sucrase antibodies (Fig. 4, b and c) was many fold stronger than the Golgi labeling of the patient's biopsy sample with the same antibody (Fig. 9 b).

(c) Since the immunolabeling of the isomaltase subunit was comparable to that in normal controls, one would also expect to find some label in the brush border membrane with the anti-sucrase antibody even if an extracellular cleavage of pro-SI takes place. This is supported by the observation that at steady state, at least 20% of the sucrase–isomaltase protein persists as an uncleaved precursor (pro-SI) in the brush border membrane (Naim et al., 1988a). Since normal levels of pro-SI are synthesized in the patient's sample, one would also expect to find at least 20% of sucrase–isomaltase as an uncleaved precursor assuming that pro-SI is cleaved extracellularly. This amount of protein should be detected immunocytochemically. Here again, no labeling of the brush border membrane was revealed (Fig. 9 a). It should be emphasized that the absence of label was not due to an altered epitope, since epitope mapping of pro-SI with the four mAbs revealed immunoprecipitable pro-SI in all cases (see Fig. 7).

(d) Should pro-SI be cleaved extracellularly, then the sucrase subunit must be either found associated with the isomaltase subunit as it is the case in the in vivo situation in normal mucosa (Hauri et al., 1979; Naim et al., 1988a; for a review see Semenza, 1986) or found in the culture medium since it does not possess a membrane anchor. We did not detect a sucrase subunit associated with the M_r-149,000 species (isomaltase) (Fig. 5 a). More importantly, immunoprecipitation of the 100,000g supernatants of the culture media with anti-sucrase antibodies did not reveal any band corresponding to sucrase (Fig. 5 a, lanes 3, 6, and 9 and Fig. 10, lane 3). By contrast, angiotensin converting enzyme, an intestinal secretory glycoprotein (Naim, H. Y., unpublished), could be immunoprecipitated from the supernatant (Fig. 10, lane 6).

The ER in the mucosal cells of the patient's biopsy sample showed more label with antibody HBB 2/614 than in the control specimen suggesting an accumulation of pro-SI$_h$ in this organelle. This may be due to a slow transport of the precursor to the Golgi apparatus. The sucrase subunit is probably
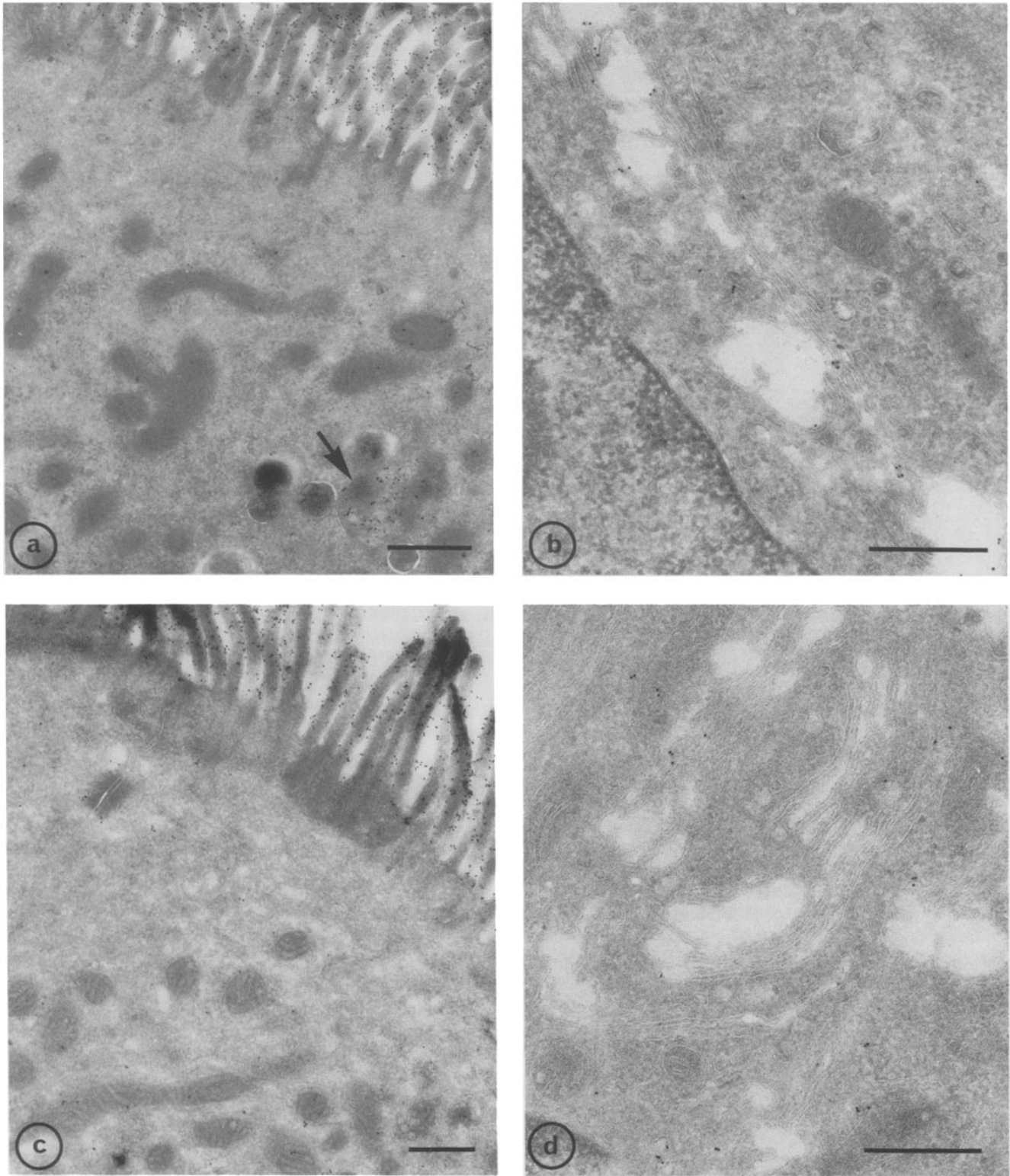
Figure 8. Ultrathin cryosections of a control biopsy and a biopsy of patient II incubated with antibody HBB 3/705 against the isomaltase subunit. (*a*) Labeling pattern observed over the brush border and lysosomes (*arrow*) of a control biopsy. (*b*) Labeling of the Golgi apparatus in a control biopsy. (*c*) Labeling pattern observed over the brush border of patient II. (*d*) Labeling of the Golgi apparatus in a biopsy of patient II. No difference in labeling pattern is observed between the patient and the control with antibody HBB 3/705, except that in the patient more label seems to be present over the rough ER (not shown). Bar, 0.5 μm.
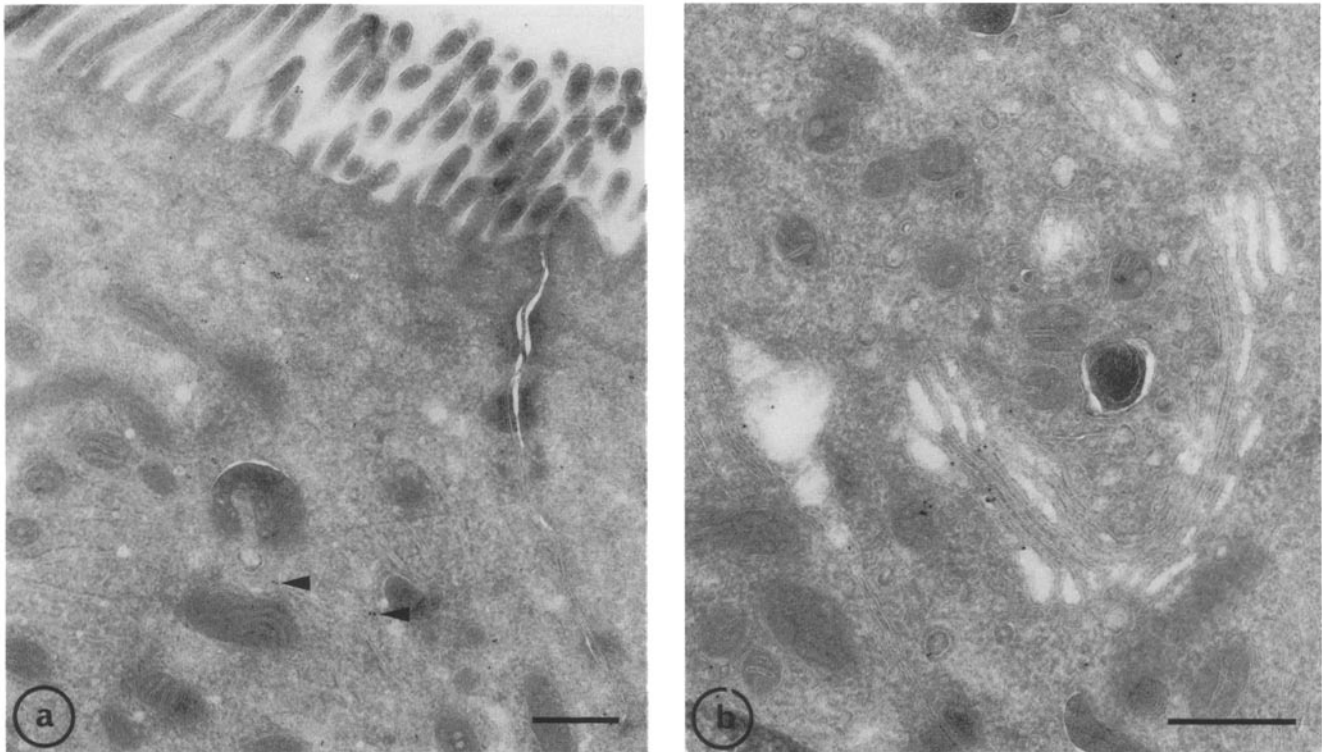
Figure 9. Immunolocalization of the sucrase subunit, with antibody HBB 2/614, in a biopsy of patient II. (*a*) Only a few gold particles can be observed in the brush border. (*b*) Also in the Golgi apparatus only a few gold particles are observed. Again more label seems to be present over the rough ER (*arrowheads*) as compared with the control biopsies. Bar, 0.5 μm.

not associated with the isomaltase subunit in the same way as in normal controls and may have undergone intracellular degradation.

To examine whether the abnormal processing of the mutated pro-SI molecule was, indeed, due to isolated CSID or due to a more general defect in glycosylation or a disturbance of protein transport, the biosynthesis of aminopeptidase N and lactase–phlorizin hydrolase was studied. Fig. 11 *a* shows that the biosynthesis and intracellular processing of aminopeptidase N followed the typical precursor–product route. Hence a M_r-130,000 component was first revealed after 30 min of pulse labeling (Fig. 11 *a*, lane *1*) which was sensitive to endo H (Fig. 11 *a*, lane *2*). After 4 h of labeling a complex glycosylated mature form was detected (Fig. 11 *a*, lane *4*) as assessed by its resistance to endo H (Fig. 11 *a*, lane *5*). The biopsy sample which was labeled for 20 h revealed similar band patterns of aminopeptidase N which, however, were labeled more strongly. To determine whether the observed decline in lactase–phlorizin hydrolase (LPH) activity is associated with abnormalities in posttranslational processing of the enzyme, we investigated the biosynthesis of LPH by immunoprecipitation and endo H treatment. Fig. 11 *b* shows that LPH in the patient's biopsy was processed in a fashion similar to LPH in normal human biopsy samples (Naim et al., 1987). Here, LPH was synthesized as a mannose-rich polypeptide that was endo H sensitive (Fig. 11 *b*, lanes *1* and *2*). Within four hours of labeling the intracellular cleaved, endo H resistant, and brush border form (M_r 160,000) was detected (Fig. 11 *b*, lanes *3* and *4*). After 20 h of labeling, the M_r-160,000 polypeptide became the predominantly labeled band (Fig. 11 *b*, lane *5*). Together, the results obtained with two control glycoproteins LPH and ApN indicate that the abnormal processing of pro-SI is not due to general cellular abnormalities along the exocytotic pathway.

Collectively, our data indicate that in this patient sucrase–isomaltase is split intracellularly, the sucrase subunit is degraded, while the isomaltase subunit reaches the brush border membrane in a normal manner. This phenotype may be similar to previously described cases in which residual isomaltase activity was observed (Skovbjerg and Krasilnikoff, 1981, 1986).

Discussion

Recent studies in an intestinal epithelial cell line, Caco-2, have suggested that proteins destined for the microvillar membrane are selectively sorted before their transport to that membrane (Hauri, 1988; Matter et al., 1990); basolateral proteins are on the other hand nonselectively sorted and reach their final destination by a default pathway (Rindler and Traber, 1988). Although the molecular mechanisms underlying these events have not been unraveled yet, an attractive hypothesis is that selective sorting is accomplished by cellular recognition elements that recognize sorting signals or specific structural motifs in the mature protein and interact with them in a ligand/receptor fashion. Proteins that are not identified by these recognition elements are then translocated to the basolateral membrane. The nature and/or structure of putative sorting signals are unknown.

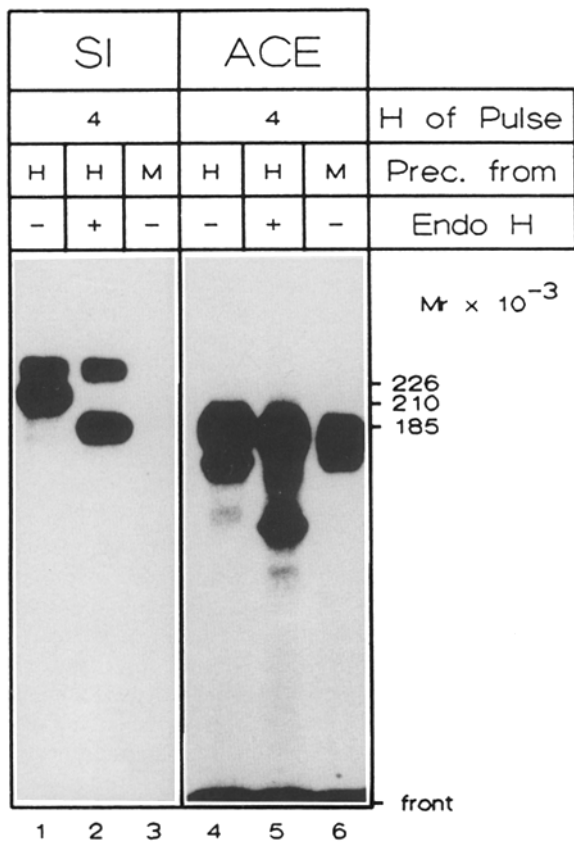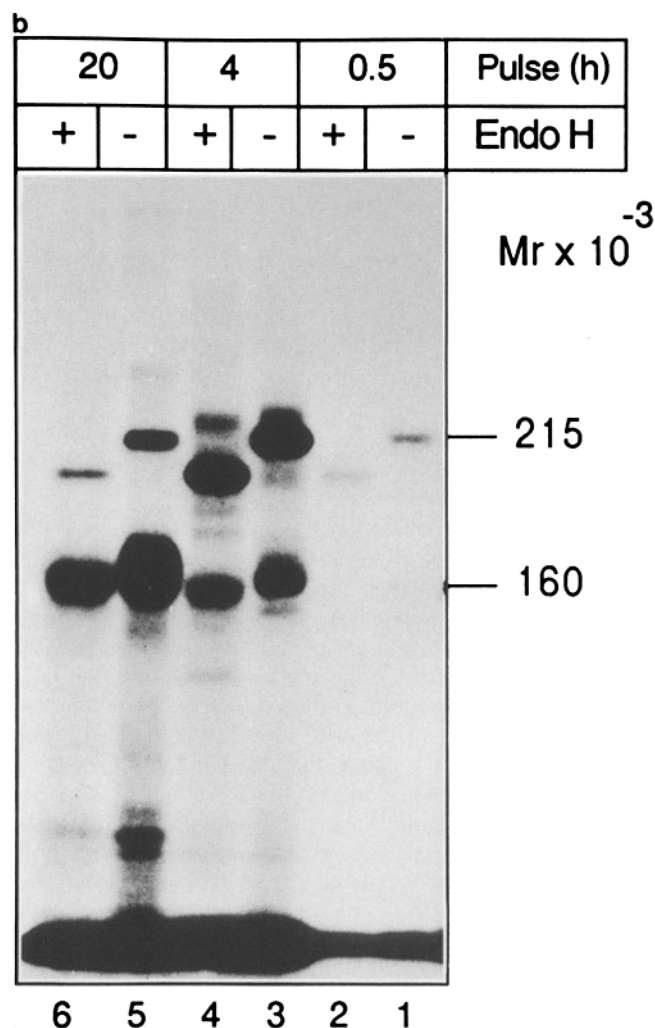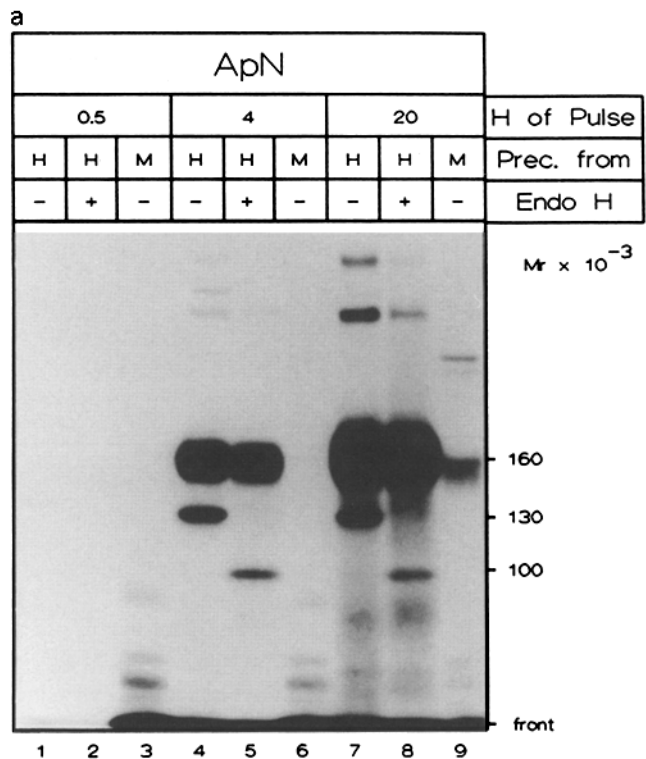In accordance with this hypothesis, it is conceivable that

54

SI		ACE			
4		4			H of Pulse
H	H	M	H	H	M	Prec. from
–	+	–	–	+	–	Endo H

Mr × 10^{-3}

226
210
185

front

1 2 3 4 5 6

Figure 10. Immunoprecipitation of sucrase–isomaltase (*SI*) with a mixture of four mAbs, and of angiotensin converting enzyme (*ACE*) with one mAb from homogenates (*H*) and the culture medium (*M*) of patient II. Biopsy specimens were cultured for 4 h with [^{35}S]methionine, and the culture medium was retained for the analysis of secreted enzyme.

slight alterations in the sorting signal of an otherwise sorted protein will disrupt its interaction with signal recognition elements leading to a protein that operates by default. Naturally occurring mutant phenotypes of proteins that are mis-sorted have been rarely observed.

We have presented in this paper two novel phenotypes (phenotype IV and phenotype V) of sucrase–isomaltase in congenital sucrase–isomaltase deficiency (CSID). One of these, phenotype IV, is the first identification of a protein that is missorted to the basolateral membrane in a naturally occurring mutant phenotype.

Figure 11. (*a*) Biosynthesis and maturation of aminopeptidase N (*ApN*) in intestinal mucosa of patient II. Biopsies were cultured for the indicated time periods in the presence of [^{35}S]methionine. Immunoprecipitation was carried out with a single mAb against ApN from homogenates (*H*) or the culture medium (*M*). One of two identical immunoprecipitates was treated with endo H to probe for high-mannose and complex-glycosylated forms. (*b*) Biosynthesis and maturation of lactase–phlorizin hydrolase (LPH) in patient II. Biopsy samples were cultured and immunoprecipitation was performed as described for *a*. The samples were immunoprecipitated with an anti-lactase–phlorizin hydrolase mAb.

a

ApN								
0.5			4			20			H of Pulse
H	H	M	H	H	M	H	H	M	Prec. from
–	+	–	–	+	–	–	+	–	Endo H

Mr × 10^{-3}

160
130
100

front

1 2 3 4 5 6 7 8 9

b

20		4		0.5		Pulse (h)
+	–	+	–	+	–	Endo H

Mr × 10^{-3}

215

160

6 5 4 3 2 1

This phenotype is characterized by the synthesis of a mannose-rich pro-sucrase–isomaltase of an apparent molecular weight practically indistinguishable from normal controls. By contrast to normal controls, however, this form remains the predominately labeled species of pro-SI. Subcellular localization of this species by immunoelectron microscopy demonstrated labeling of the basolateral membrane and of the ER. In view of the fact that in normal biopsy specimens these two organelles are not labeled we conclude that pro-SI in this case accumulates in the ER and is partially transported to the basolateral membrane. It seems therefore that a mutation in the SI-gene has generated a pro-SI phenotype that differs from its wild-type counterpart on the basis of its folding properties. In fact, mapping of the mutant SI with four epitope- and conformation-specific mAbs indicated that two antibodies have failed to immunoreact with detergent-solubilized, i.e., native forms of SI in this phenotype. Current concepts of the biosynthesis and posttranslational processing of proteins have assigned a decisive role to protein folding and oligomerization in the transport mechanisms within cellular compartments and to the cell surface (Gething et al., 1986; Hurtley and Helenius, 1989; Kreis and Lodish, 1986; Rose and Doms, 1988). Along these lines, it seems that sucrase–isomaltase in this phenotype accumulates in the ER as a consequence of improper folding. Furthermore, the alterations in the three-dimensional structure of SI have led to disruption in putative sorting elements or masking of these elements. Consequently, a proportion of the intracellular pool of misfolded SI is segregated by default, which in intestinal epithelial cells leads to the basolateral membrane (Rindler and Traber, 1988). Recently Matter et al. (1990) reported that brush border hydrolases, which are missorted to the basolateral membrane, can subsequently be transported to the brush border membrane via a correction mechanism. The putative mutation in this phenotype should have also affected structures in the pro-SI molecule that are probably involved in this correction mechanism. It is still surprising, however, that although the protein is transported to the basolateral membrane and hence has passed the Golgi apparatus does not become complex glycosylated.

If Specific Structural Domains Are Involved in the Sorting of SI to the Brush Border, in Which Domain Are These Elements Located?

The fact that two mAbs did not react with SI in phenotype IV suggests that conformational and/or structural domains are involved in the sorting recognition event. The second phenotype described in this paper (phenotype V) extends this information and provides evidence for the presence of putative sorting signals of the sucrase–isomaltase complex on the isomaltase subunit.

This phenotype is characterized by the synthesis and processing of two forms of pro-SI that differ in the size of their oligomannose side chains and not in the size of the polypeptide backbone as assessed by endo H treatments. Moreover, the processing of the mannose-rich chains to a complex form proceeded at a slow rate and was incomplete, while the glycosylation pattern of the control protein, aminopeptidase N (ApN), was as in normal controls. These observations point to possible conformational alterations in the mutated pro-SI protein rendering its potential glycosylation sites less accessible to the addition of mannose-rich chains and also leading to inefficient processing of the oligosaccharide chains in the Golgi apparatus. By virtue of the importance of oligosaccharides in protecting glycoproteins from proteolytic degradation (West, 1986), it is likely that an aberrantly glycosylated pro-SI is more susceptible to intracellular proteases than normal pro-SI.

Our data have shown that pro-SI is intracellularly cleaved to generate an isomaltase-like subunit; the sucrase subunit on the other hand was neither detected at the protein level nor at the subcellular level. Furthermore, experiments using angiotensin-converting enzyme as marker of a secreted protein excluded the possibility that sucrase may be secreted in this phenotype.

Together the data presented indicate that sucrase is degraded intracellularly immediately after cleavage of pro-SI. By contrast, the isomaltase subunit is transported and segregated to the correct membrane. In fact, immunolabeling of the biopsy specimen with anti-isomaltase antibodies revealed significant labeling of the brush border membrane and no labeling of the basolateral membrane. Because that isomaltase was identified by epitope-specific antibodies and is enzymatically active, we conclude that there are no apparent conformational alterations in the isomaltase domain of the sucrase–isomaltase complex in this phenotype. Putative mutation(s) in this case may have occurred in the coding region of the sucrase subunit and have generated an improperly folded sucrase that is processed to an aberrantly glycosylated molecule prior to intracellular degradation. The selective sorting of the isomaltase subunit to the brush border membrane strongly suggests that (a) putative structural features reside in the isomaltase domain, which are involved in the sorting event and transport of the sucrase–isomaltase complex to the brush border membrane and (b) the sorting event does not necessarily require the presence of the sucrase domain. The validity of these hypotheses require, however, the analysis of mutant phenotypes at the genetic level.

References

Bernadac, A., J. P. Gorvel, H. Ferraci, and S. Maroux. 1984. Human blood group A-like determinants as marker of the intracellular pools of glycoproteins in secretory and absorbing cells of A+ rabbit jejunum. Biol. Cell. 50:31–36.

Dahlqvist, A. 1968. Assay of intestinal disaccharidases. Anal. Biochem. 22:99–107.

Fransen, J. A. M., L. A. Ginsel, H. P. Hauri, E. Sterchi, and J. Blok. 1985. Immuno-electronmicroscopical localization of a microvillus membrane disaccharidase in the human small-intestinal epithelium with monoclonal antibodies. Eur. J. Cell Biol. 38:6–15.

Gething, M. J., K. McCammon, and J. Sambrook. 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. Cell. 46:939–950.

Ghersa, P., P. Huber, G. Semenza, and H. Wacker. 1986. Cell free synthesis, membrane integration, and glycosylation of pro-sucrase-isomaltase. J. Biol. Chem. 261:7969–7974.

Hauri, H. P. 1988. Biogenesis and intracellular transport of intestinal brush border membrane hydrolases. Subcell. Biochem. 12:155–219.

Hauri, H. P., A. Quaroni, and K. J. Isselbacher. 1979. Biogenesis of intestinal

plasma membrane: posttranslational route and cleavage of sucrase-isomaltase. *Proc. Natl. Acad. Sci. USA.* 76:5183–5186.

Hauri, H. P., A. Quaroni, and K. J. Isselbacher. 1980. Monoclonal antibodies to sucrase-isomaltase: Probes for the study of postnatal development and biogenesis of the intestinal microvillus membrane. *Proc. Natl. Acad. Sci. USA.* 77:6629–6633.

Hauri, H. P., H. Wacker, E. E. Rickli, B. Bigler-Meier, A. Quaroni, and G. Semenza. 1982. Biosynthesis of sucrase-isomaltase. Purification and NH2-terminal amino acid sequence of the rat sucrase-isomaltase precursor (prosucrase-isomaltase) from fetal intestinal transplants. *J. Biol. Chem.* 257:4522–4528.

Hauri, H. P., J. Roth, E. Sterchi, and M. Lentze. 1985a. Transport to cell surface of intestinal sucrase-isomaltase is blocked in the Golgi apparatus in a patient with congenital sucrase-isomaltase deficiency. *Proc. Natl. Acad. Sci. USA.* 82:4423–4427.

Hauri, H. P., E. E. Sterchi, D. Bienz, J. A. M. Fransen, and A. Marxer. 1985b. Expression and intracellular transport of microvillus membrane hydrolases in human intestinal epithelial cells. *J. Cell Biol.* 101:838–851.

Hunziker, W., M. Spiess, G. Semenza, and H. F. Lodish. 1986. The sucrase-isomaltase complex: Primary structure, membrane orientation, and evolution of a stalked, intrinsic brush border protein. *Cell.* 46:227–234.

Hurtley, S. M., and A. Helenius. 1989. Protein oligomerization in the endoplasmic reticulum. *Annu. Rev. Cell Biol.* 5:277–307.

Kelly, J. J., and D. H. Alpers. 1973. Blood group antigenicity of purified human intestinal disaccharidases. *J. Biol. Chem.* 248:8216–8221.

Kobata, A. 1979. Use of endo- and exoglycosidases for structural studies of glycoconjugates. *Anal. Biochem.* 100:1–14.

Kreis, T., and H. Lodish. 1986. Oligomerization is essential for transport of vesicular stomatitis viral glycoprotein to the cell surface. *Cell.* 46:929–937.

Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lloyd, M. L., and W. A. Olsen. 1987. A study of the molecular pathology of sucrase-isomaltase deficiency. A defect in the intracellular processing of the enzyme. *N. Engl. J. Med.* 316:438–442.

Matter, K., and H. P. Hauri. 1991. Intracellular transport and conformational maturation of intestinal brush border hydrolases. *Biochemistry.* 30:1916–1923.

Matter, K., M. Brauchbar, K. Bucher, and H. P. Hauri. 1990. Sorting of endogenous plasma membrane proteins occurs from two sites in cultured human intestinal epithelial cells (Caco-2). *Cell.* 60:429–437.

Naim, H. Y., E. E. Sterchi, and M. J. Lentze. 1987. Biosynthesis and maturation of lactase-phlorizin hydrolase of the human small intestinal epithelial cells. *Biochem. J.* 241:427–434.

Naim, H. Y., E. E. Sterchi, and M. J. Lentze. 1988a. Biosynthesis of the human sucrase-isomaltase complex. Differential O-glycosylation of the sucrase subunit correlates with its position within the enzyme complex. *J. Biol. Chem.* 263:7242–7253.

Naim, H. Y., J. Roth, E. Sterchi, M. Lentze, P. Milla, J. Schmitz, and H. P. Hauri. 1988b. Sucrase–isomaltase deficiency in man. Different mutations disrupt intracellular transport, processing and function of an intestinal brush border enzyme. *J. Clin. Invest.* 82:667–679.

Rindler, M. J., and M. G. Traber. 1988. A specific sorting signal is not required for the polarized secretion of newly synthesized proteins from cultured intestinal epithelial cells. *J. Cell Biol.* 107:471–479.

Rose, J., and R. W. Doms. 1988. Regulation of protein export from the endoplasmic reticulum. *Annu. Rev. Cell Biol.* 4:257–288.

Semenza, G. 1986. Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. *Annu. Rev. Cell Biol.* 2:255–313.

Sjöström, H., O. Norén, L. Christiansen, H. Wacker, and G. Semenza. 1980. A fully active, two-active-site, single-chain sucrase-isomaltase from pig small intestine. Implications for the biosynthesis of a mammalian integral stalked membrane protein. *J. Biol. Chem.* 255:11332–11338.

Skovbjerg, H., and P. A. Krasilnikoff. 1981. Immunoelectrophoretic studies on human small intestinal brush border proteins. The residual isomaltase in sucrose intolerant patients. *Pediatr. Res.* 15:214–218.

Skovbjerg, H., and P. A. Krasilnikoff. 1986. Maltase-glucoamylase and residual isomaltase in sucrose intolerant patients. *J. Pediatr. Gastroenterol. Nutr.* 5:365–371.

Sterchi, E. E., P. R. Mills, J. A. M. Fransen, H. P. Hauri, M. J. Lentze, H. Y. Naim, L. Ginsel, and J. Bond. 1990. Biogenesis of intestinal lactase-phlorizin hydrolase in adults with lactose intolerance. Evidence for reduced biosynthesis and slowed-down maturation in enterocytes. *J. Clin. Invest.* 86:1329–1337.

Triadou, N., E. Audran, M. Rousset, A. Zweibaum, and R. Oriol. 1983. Relationship between the secretory status and the expression of ABH blood group antigenic determinants in human intestinal brush border membrane hydrolases. *Biochim. Biophys. Acta.* 761:231–236.

West, C. M. 1986. Current ideas on the significance of protein glycosylation. *Mol. Cell. Biochem.* 72:3–20.