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Transport to cell surface of intestinal sucrase–isomaltase is blocked in the Golgi apparatus in a patient with congenital sucrase–isomaltase deficiency

(intracellular traffic/microvillar hydrolases/immunoelectron microscopy/monoclonal antibodies)

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ABSTRACT **A case of congenital sucrase–isomaltase deficiency in man was investigated. An intestinal biopsy sample from a 5-year-old girl lacked sucrase but possessed low residual isomaltase activity. Immunoelectron microscopy with monoclonal antibodies to sucrase–isomaltase in biopsy samples from healthy subjects revealed that sucrase–isomaltase was confined predominantly to the microvillus membrane of enterocytes and there was minimal labeling of the Golgi apparatus. In the patient immunoreactive sucrase–isomaltase was found almost exclusively in about three *trans*-Golgi cisternae and associated vesicular structures, while no specific labeling was associated with the microvillus membrane. Immunoprecipitation experiments with iodinated mucosal homogenates and a mixture of four monoclonal antibodies to sucrase–isomaltase revealed absence of enzyme subunits in the patient but presence of a M_r 210,000 protein that was also expressed in normal control biopsy specimens. This protein presumably is the high-mannose precursor of sucrase–isomaltase. Additional proteins of M_r 160,000–200,000 found in the patient but not in normal subjects might correspond to the crossreacting material found in the Golgi apparatus of the patient. Overall, the findings suggest that in the patient sucrase–isomaltase is synthesized and transported to the Golgi apparatus, where further transport is interrupted. The data imply that signals in sucrase–isomaltase that mediate its transfer from the endoplasmic reticulum to the Golgi apparatus differ from those mediating its transport from the Golgi apparatus to the cell surface.**

Plasma membrane proteins of the eukaryotic cell are synthesized and assembled in the rough endoplasmic reticulum (1), pass through the Golgi apparatus (2), and are subsequently transferred to the cell surface. While the precise pathways followed are presently being studied in great detail, mainly by the use of enveloped viruses as tools (3–5), little is known on the molecular mechanisms underlying intracellular protein transport. Current thinking assumes that this transport is mediated by receptor-like proteins recognizing signals (2, 6) in the proteins to be sorted out and that membrane vesicles are involved in the intercompartmental traffic (7). Among the approaches used for dissecting the sequence of events and gene products that act at discrete steps, the analysis of naturally occurring and induced mutant phenotypes is particularly promising (8).

We use sucrase–isomaltase, a heterodimeric enzyme complex, to study the biosynthetic route of a typical microvillar membrane protein (9). In the normal intestine sucrase–isomaltase is synthesized as a single chain precursor protein (pro-sucrase–isomaltase). When arriving at the microvillus membrane, pro-sucrase–isomaltase is cleaved into its mature subunits, sucrase and isomaltase, by pancreatic proteases (10). The present communication reports on a case of congenital sucrase–isomaltase deficiency in which sucrase–isomaltase is synthesized but accumulates in the Golgi apparatus and apparently cannot be transported to the cell surface.

The expression of at least one other microvillar hydrolase and the mucosal ultrastructure were found to be normal, suggesting that the transport defect was most probably restricted to sucrase–isomaltase itself rather than due to a general deficiency of the transport machinery for the Golgi-to-cell-surface pathway. It is currently unknown if this phenotype is common among children with sucrase–isomaltase deficiency, as no other previous case has been analyzed by immunoelectron microscopy.

MATERIAL AND METHODS

Patient and Processing of Biopsy Samples. The patient investigated is a 5-year-old girl with congenital sucrase–isomaltase deficiency who suffers from watery diarrhea after the ingestion of sucrose-containing food. A sucrose challenge test showed no increase of blood sugar. Intestinal biopsy specimens taken at the ages of 8 months and 5 years revealed a structurally normal mucosa having very low or absent sucrase activities (0.07 and 0.00 international unit/g of wet weight, respectively). Biopsy specimens from five children used for control purposes had normal levels of microvillar enzymes and a normal mucosal ultrastructure. Each biopsy sample was cut into three pieces approximately equal in size. One piece was used for the analysis of disaccharidase activities, a second piece was frozen for immunological analysis, and the third fragment was processed for immunoelectron microscopy.

Monoclonal Antibodies. The hybridoma technique (11) was used to produce monoclonal antibodies to human disaccharidases, starting with purified microvillar membranes (12) as antigen (13). Cultures producing antibodies specific for sucrase–isomaltase or maltase–glucoamylase were identified by enzyme measurements of the immunoisolated antigens (13). Positive hybridomas were selected and cloned and the cells were injected into pristane-primed mice, yielding ascites fluid rich in monoclonal antibodies. For most experiments 10× concentrated culture supernatants were used. The antibodies were concentrated by precipitation with ammonium sulfate (to 50% saturation).

Immunoelectron Microscopy. Biopsy specimens of the sucrase–isomaltase-deficient patient and from five normal patients were fixed by immersion in 3% (para-)formaldehyde/0.1% glutaraldehyde in isotonic phosphate-buffered saline, pH 7.2, for 2 hr at room temperature. After several rinses in phosphate-buffered saline, free aldehyde groups were

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blocked by immersion of the specimens in 50 mM NH_4Cl in phosphate-buffered saline for 1 hr. The tissue pieces were dehydrated in ethanol at progressively lowered temperatures down to −35°C and then infiltrated with Lowicryl K4M (Chemische Werke Lowi, F.R.G.) at −35°C, which was polymerized with UV light first at −35°C for 24 hr and second at room temperature for 48 hr (14, 15). Thin sections were cut with a Reichert Ultracut ultramicrotome, mounted on Parlodion/carbon-coated nickel grids (150 mesh), and stored at room temperature until immunocytochemical staining. The staphylococcal protein A–gold technique has been published (16, 17). In the present study a monodisperse 6-nm protein A–gold complex was used. Grids with the attached thin sections were floated for 5 min on a droplet of 0.5% ovalbumin in phosphate-buffered saline and were then transferred to a droplet (15 μl) containing monoclonal antibodies to sucrase–isomaltase and incubated for 2 hr at room temperature in a moist chamber. Ascites fluid (at 1:50 to 1:150 dilution) as well as 10× concentrated culture supernatant was used. After two rinses (2 min each) with phosphate-buffered saline, the sections were incubated with affinity-purified rabbit antiserum to mouse IgG at 50 μg/ml for 1 hr at room temperature, rinsed twice (2 min each) with phosphate-buffered saline, and incubated with the protein A–gold complex (diluted with phosphate-buffered saline to give an absorption of 0.06 at 525 nm) for 1 hr at room temperature. After two rinses with phosphate-buffered saline and finally distilled water, the sections were dried and counterstained with 2% aqueous uranyl acetate (4–5 min) and lead acetate (45 sec). Thin sections were viewed at 80 kV with a Zeiss EM 10 electron microscope. Cytochemical specificity tests included (*i*) omission of the monoclonal antibody incubation step and (*ii*) incubation with the protein A–gold complex alone. Acid phosphatase was localized by the method of Novikoff (18) with cytidine 5′-monophosphate as substrate (incubation time, 40 min at 37°C).

Immunoblotting. These and the subsequent immunoisolation experiments were first carried out with normal mucosal biopsy specimens of three different children to establish the optimal experimental conditions. Thereafter a single mucosal piece from the deficient patient (approximately 1 mg wet weight) and one from an age-matched normal control (approximately 5 mg wet weight) were processed simultaneously. Each fragment was homogenized in 120 μl of distilled water in a 1-ml glass–Teflon Potter–Elvehjem homogenizer at 1100 rpm for 65 sec (20 strokes). A 20-μl sample of the homogenate was used for protein determination. Another sample of the homogenate (30 μg of protein) was subjected to $NaDodSO_4$/PAGE (19) under reducing conditions, and the separated proteins were transferred to nitrocellulose (20). The immunoreaction was carried out with monoclonal antibodies (10× concentrated culture supernatant) and the bound antibodies were visualized with ^{125}I-labeled protein A (500,000 cpm).

Immunoisolation of Sucrase–Isomaltase. Twenty-four micrograms of protein of each homogenate was solubilized in 300 μl of 100 mM Na_2HPO_4, pH 8.0, containing 1% Triton X-100 and phenylmethylsulfonyl fluoride at 40 μg/ml for 1 hr on ice. Nonsolubilized particles were removed by centrifugation for 1 hr at 100,000 × *g*. The solubilized proteins were radioiodinated by the lactoperoxidase method as used by Weiser *et al.* (21) and stored in aliquots at −20°C in the presence of 0.1% bovine serum albumin. The specific radioactivity obtained was identical in both samples. Aliquots containing 5×10^7 cpm per tube were preadsorbed to protein A-Sepharose and the resulting supernatants were immunoprecipitated with monoclonal antibodies adsorbed to protein A-Sepharose (13) at pH 8.0. The washed immunoprecipitates were separated by $NaDodSO_4$/PAGE, and radioactive proteins were detected by autoradiography of the dried gel.

Enzyme Assays. Disaccharidase activities were measured by using the glucose oxidase method (22). An international unit of enzyme activity catalyzes production of 1 μmol of glucose per min at 37°C. Alkaline phosphatase and aminopeptidase N were measured by standard methods (23, 24).

RESULTS

Properties of Monoclonal Antibodies. See Table 1. All the antibodies to sucrase–isomaltase specifically immunoprecipitated sucrase and isomaltase activities from Triton X-100-solubilized microvillar membranes. Immunoisolated sucrase–isomaltase had the following apparent M_r on $NaDodSO_4$ gels: 146,000 (sucrase subunit), 151,000 (isomaltase subunit), and 234,000 (pro-sucrase-isomaltase). Analysis by immunoblotting showed that antibody HBB3/705/60 bound to the isomaltase- and antibody HBB2/614/88 to the sucrase subunit (Fig. 1*a*). Furthermore, it was established by competitive ELISA that antibodies HBB2/614/88, HBB2/219/20, and HBB1/691/79 recognize different epitopes. Subunit specificity of the latter two antibodies is unknown. The antibody HBB2/143/17 against maltase–glucoamylase precipitated a single M_r 355,000 protein.

Patient Has Low Residual Isomaltase Activity. Enzyme measurements in the mucosal homogenate of the patient at the age of 5 years showed complete lack of sucrase but residual albeit low isomaltase activities (Table 2). This activity was 1/8th that of an age-matched normal control and less than 1/12th normal values reported in the literature (Table 2). It is not known if sucrase–isomaltase is the only microvillar enzyme hydrolyzing isomaltase. We found that immunoisolated human maltase–glucoamylase, another disaccharidase that, like sucrase–isomaltase, is believed to be involved in the digestion of limit α-dextrins was unable to metabolize isomaltose. Furthermore, >95% of isomaltase activity, together with >95% of sucrase activity, was immunoprecipitable from Triton X-100-solubilized microvillus membranes or mucosal homogenates with the four monoclonal antibodies to sucrase–isomaltase. These results argue against the importance of an enzyme other than sucrase–isomaltase in hydrolyzing isomaltose. Thus, it appears likely that the low isomaltase activity of the patient's mucosa was indeed due to residual sucrase–isomaltase. It is not surprising that maltase activity was also reduced, since we found that in the normal intestine the sucrase–isomaltase contributes 70–90% of the total microvillar maltase activity. However, it is important to note that lactase activity was normal, demonstrating that the patient was most probably affected by an isolated sucrase–isomaltase deficiency.

Immunoelectron Microscopy. To localize residual sucrase–isomaltase in the patient we undertook an immunolabeling study. When the different monoclonal antibodies against sucrase–isomaltase were tested by the protein

Table 1. Properties of monoclonal antibodies against human disaccharidases

Antibody code	Specificity	Immunoglobulin class	Reaction on immunoblots
HBB2/614/88	Sucrase	IgG1	+
HBB3/705/60	Isomaltase	IgG1	+
HBB2/219/20	Sucrase–isomaltase*	IgG2b	−
HBB1/691/79	Sucrase–isomaltase*	IgG1	−
HBB2/143/17	Maltase–glucoamylase	IgG1	−

*Subunit specificity unknown.

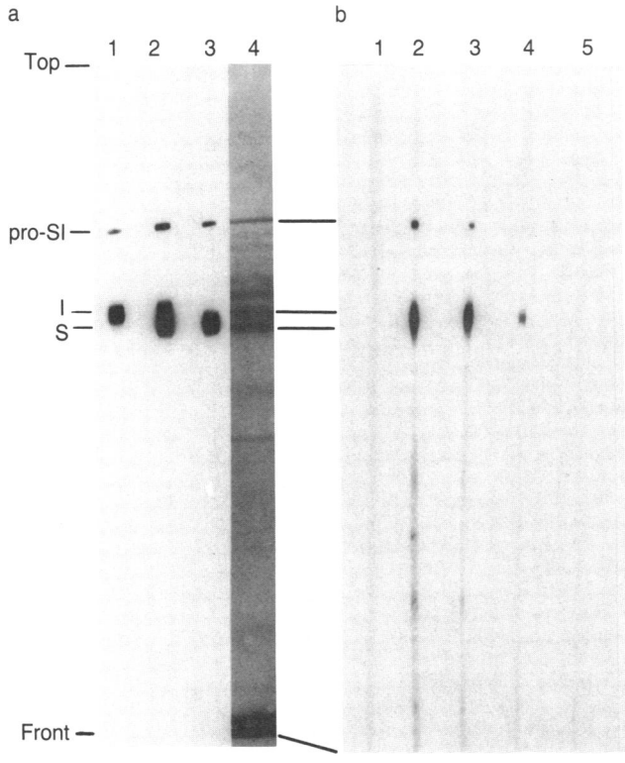

FIG. 1. Immunoblotting with monoclonal antibodies to human sucrase–isomaltase. (*a*) Purified intestinal microvillus membranes (15 μg of protein per lane) were separated by $NaDodSO_4$/PAGE and transferred to nitrocellulose, and individual strips were stained with amido black (lane 4) or incubated with antibody HBB3/705/60 (lane 1), antibody HBB2/614/88 (lane 3), or an equal mixture of both antibodies (lane 2). Bound antibody was visualized by ^{125}I-protein A (autoradiogram). I, isomaltase; S, sucrase; pro-SI, pro-sucrase–isomaltase. (*b*) Mucosal homogenates of the patient (30 μg of protein, lane 1) or of a normal control (30 μg, lane 2, 15 μg, lane 3; 7.5 μg, lane 4; 3.8 μg, lane 5) were separated by $NaDodSO_4$/PAGE, and immunoblotting was carried out with antibody HBB3/705/60 as above (autoradiogram).

A–gold technique with thin sections from normal individuals, only antibody HBB3/705/60 gave intense labeling, whereas the others gave weak or no specific staining. Labeling was associated with the microvilli of absorptive enterocytes (Fig. 2*a*), membrane invaginations at the base of the microvilli, small (presumptive endocytotic) vesicles, and multivesicular bodies in the apical cytoplasm. Over the Golgi apparatus negligible labeling was found (Fig. 2*b*), probably due to the limited sensitivity of the technique. On sections from biopsy samples of the patient no specific immunolabeling with any of the antibodies was found over the microvilli, apical plasma membrane invaginations or vesicles, multivesicular bodies, or basolateral plasma membrane (Fig. 2*c*). However, antibody HBB3/705/60 gave an intense labeling of the Golgi apparatus (Figs. 2 *d* and *e*). The gold particles were preferentially concentrated over about three *trans* Golgi cisternae and associated vesicular structures, whereas cisternae at the *cis* side of the Golgi apparatus and the cytidine-5′-monophosphatase-positive *trans*-most Golgi cisternae showed a labeling comparable to that found over mitochondria and nuclei.

Table 2. Disaccharidase activities in mucosal homogenates

Enzyme	Specific activity, international units/g of protein	
	Patient	Normal values*
Sucrase	0	28–80
Isomaltase	3	36–93
Maltase	83	114–274
Lactase	76	16–49

*Ref. 25.

Immunochemical Experiments. In a first experiment we attempted to visualize sucrase–isomaltase of the patient by immunoblotting with antibody HBB3/705/60. For the control biopsy specimen sucrase–isomaltase was detectable down to 7.5 μg of protein per lane, corresponding to approximately 10–15 ng of sucrase–isomaltase (Fig. 1*b*). No band of immunoreactivity was found with 30 μg of protein of the patient's mucosal homogenate, suggesting that the amount of immunoreactive sucrase–isomaltase in the patient was less than 1/4th of that in the normal control.

When the Triton X-100-solubilized radioiodinated homogenate of the patient's biopsy specimen was immunoprecipitated with an equal mixture of the four antibodies against sucrase–isomaltase and the immunoprecipitates were analyzed by $NaDodSO_4$/PAGE, a major band of M_r 210,000 was found on the autoradiogram (Fig. 3, lane 2). Three to four additional bands appeared in a M_r 160,000–200,000 range. However, no bands were apparent in the position of the subunits of sucrase–isomaltase (Fig. 3, compare lanes 2 and 5). This pattern was reproducibly obtained in three independent immunoprecipitations with the same sample.

In the normal control biopsy samples the M_r 210,000 protein was also expressed (Fig. 3, lane 3), together with a M_r 234,000 protein that migrated with the fully glycosylated pro-sucrase–isomaltase of purified microvillus membranes and proteins of M_r 145,000–155,000, representing the subunits of sucrase–isomaltase (Fig. 3, lanes 5 and 6). However, no bands appeared on the gels of biopsy specimens from normal individuals in the M_r 160,000–200,000 range (Fig. 3, lane 5). The M_r 210,000 protein does not appear to result from nonspecific precipitation, since controls performed with the monoclonal antibody to human maltase–glucoamylase did not precipitate this protein (Fig. 3, lanes 1 and 4). Furthermore, a single monoclonal antibody (HBB2/614/88) to the sucrase subunit also recognized this protein in samples of both the patient and the normal control, albeit with lower efficiency (not shown). The protein had a molecular weight identical to that of the endoglycosidase H-sensitive high-mannose pro-sucrase–isomaltase synthesized in the adenocarcinoma cell line Caco 2 (unpublished data) and it was not expressed in purified microvillus membranes (Fig. 3, lane 6).

DISCUSSION

In this report the microvillar hydrolase sucrase–isomaltase has been localized in small-intestinal biopsy specimens obtained from normal children and from a child with congenital sucrase–isomaltase deficiency. By using an isomaltase-specific monoclonal antibody in conjunction with immunoelectron microscopy it was found that in enterocytes of a patient with sucrase–isomaltase deficiency immunoreactive enzyme was restricted to the Golgi apparatus, with apparent concentration of immunolabeling on its *trans* side and the *trans*-most Golgi cisternae being negative. All other organelles did not show any specific labeling. In the normal mucosa sucrase–isomaltase was highly concentrated in the microvillus membrane, confirming previous data obtained by subcellular fractionation (26). Furthermore, small vesicles and multivesicular bodies were also labeled, while no specific labeling was associated with the Golgi apparatus. These results suggest that in the patient sucrase–isomaltase is synthesized but on its way to the cell surface is blocked in the Golgi apparatus.

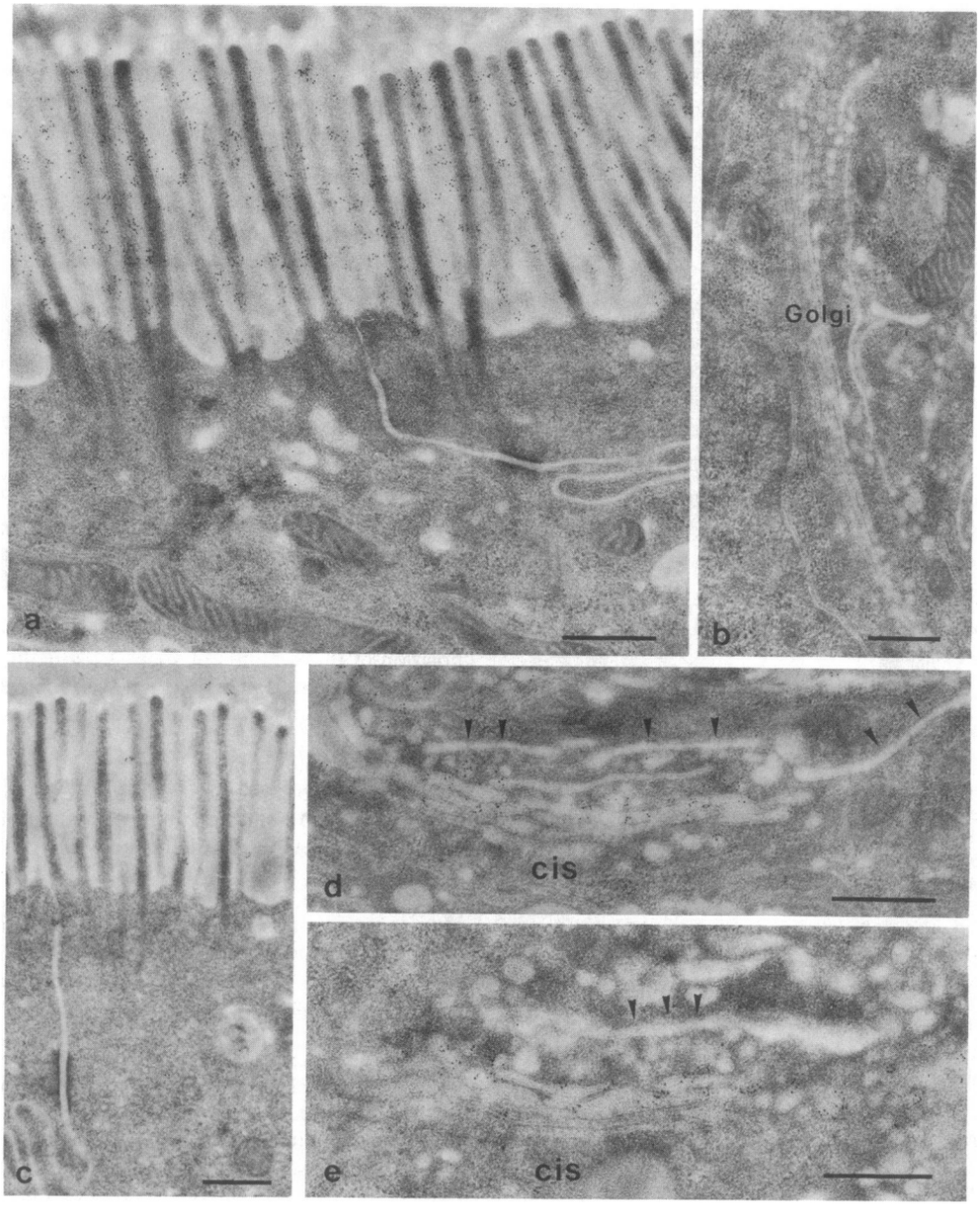

FIG. 2. Immunolocalization of sucrase–isomaltase in human duodenal biopsy specimens with the protein A–gold technique. Labeling over the microvilli is intense on thin sections of normal mucosa (*a*), whereas only negligible labeling is seen over the Golgi apparatus (*b*). In the patient no labeling is found over the microvilli (*c*). However, prominent labeling is observed over two to three *trans* Golgi cisternae (*d* and *e*), with the *cis* cisternae and the *trans*-most cisternae (arrowheads) not being labeled. (Bars = 0.5 μm.)

The biochemical studies of the residual sucrase–isomaltase confirm and extend the morphological data. In particular, they lead to the following conclusions. (*i*) As the biopsy specimen of the patient displayed low isomaltase but no sucrase activity, the residual sucrase–isomaltase is apparently partially enzymatically active.(*ii*) The transport defect in the patient is most probably restricted to sucrase–isomaltase and is not due to a general disturbance of the Golgi-to-cell-surface pathway, since the microvillar ultrastructure and the expression of lactase–phlorizine hydrolase were normal. (*iii*) Immunoprecipitation experiments with antibodies to sucrase–isomaltase revealed the absence of sucrase–isomaltase subunits from gels containing the radioiodinated sample of the patient but the presence of a M_r 210,000 protein that we consider to be the core-glycosylated precursor of sucrase–isomaltase. Furthermore, additional proteins were found in a M_r 160,000–200,000 range that were not detectable on gels of control biopsy samples. The most likely interpretation of this observation is that sucrase–isomaltase in the patient is synthesized and core-glycosylated with an apparent molecular weight that is indistinguishable from that of the normal enzyme by $NaDodSO_4$/PAGE. Since the amount of the M_r 210,000 protein did not exceed that of the normal control and since no subunits appeared, it is likely that the newly synthesized sucrase–isomaltase of the patient is intracellularly degraded. The M_r 160,000–200,000 proteins might represent degradation products of the M_r 210,000 protein. Due to the limited amount of material it was not possible to further investigate this point.

What is the relationship between the immunocytochemical and the biochemical data? We propose that the proteins in the M_r 160,000–200,000 range, at least in part, correspond to the crossreacting material visualized in the Golgi complex of the patient by immunoelectron microscopy since they were not found in normal biopsy specimens. This interpretation might be weakened by the observation that these proteins did not appear to represent the majority of the immunopurified antigen on gels. However, it is important to note that the

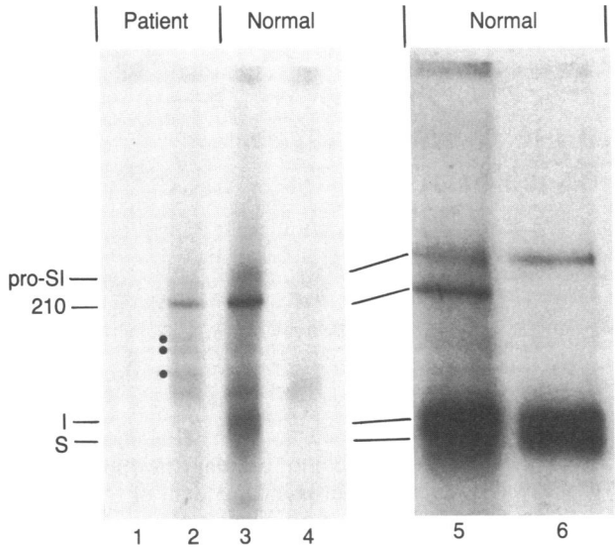

FIG. 3. Immunoprecipitation with monoclonal antibodies to sucrase–isomaltase. Mucosal homogenates of the patient (lanes 1 and 2) and of a normal individual (lanes 3, 4, and 5) were iodinated after solubilization with Triton X-100. ^{125}I-labeled proteins (5×10^7 cpm per lane) were immunoprecipitated with an equal mixture of the four antibodies to sucrase–isomaltase (lanes 2, 3, and 5) and the immunoprecipitates were separated by NaDodSO$_4$/PAGE and subjected to autoradiography. Lane 6, immunoprecipitate obtained with the four anti-sucrase–isomaltase antibodies and detergent-solubilized radioiodinated microvillus membranes (2.5×10^6 cpm). Lanes 1 and 4, control with monoclonal antibody to maltase–glucoamylase. Under these conditions not enough radiolabeled maltase-glucoamylase was present to yield a visible band at M_r 355,000. The samples of lanes 1–4 were on a gel with 3-mm slots and the samples of lanes 5 and 6 were on a gel with 7-mm slots. Dots in lane 2 indicate the M_r 160,000–200,000 proteins. Abbreviations as in Fig. 1; 210, M_r 210,000 protein.

intensities of the different radioactivity bands on autoradiograms do not necessarily reflect the actual amounts of proteins present. For instance, it is conceivable that a completely glycosylated protein is less amenable to lactoperoxidase-catalyzed iodination than its incompletely glycosylated precursor. This would explain the apparent discrepancy between immunoblots, on which only the fully glycosylated pro-sucrase–isomaltase could be visualized (Fig. 1*b*), and the autoradiograms, on which the putative high-mannose pro-sucrase–isomaltase was more pronounced than the fully glycosylated pro-sucrase–isomaltase (Fig. 3). Likewise the actual amount of the M_r 160,000–200,000 proteins might have been underestimated after radioiodination if they were fully glycosylated.

Previously described cases of congenital sucrase–isomaltase deficiency were characterized either by a complete lack of sucrase and isomaltase activities (type 1) or by residual isomaltase activity (type 2) (27). Enzymatically the case described herein can be classified as type 2. However, none of the previously reported cases has been investigated by immunoelectron microscopy, but fluorescence microscopy has suggested a genetic heterogeneity of antigen expression in a small number of sucrase–isomaltase-deficient children (28, 29).

Our findings on the localization of sucrase–isomaltase have important implications of general interest in the study of the biosynthetic route. They imply that the structural features of surface membrane proteins mediating their transport from the endoplasmic reticulum to the Golgi apparatus are different from those mediating transport from the Golgi apparatus to the cell surface.

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