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Monoclonal antibodies to sucrase/isomaltase: Probes for the study of postnatal development and biogenesis of the intestinal microvillus membrane

(fetal intestinal transplantation/enzyme precursor/immunofluorescence/cell differentiation)

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ABSTRACT **Two monoclonal antibodies, designated BB 3/34/12 and BB 5/8/40/90, have been produced to rat intestinal sucrase/isomaltase (SI) by the hybridoma technique using microvillus membranes as antigen. The BB 3/34/12 antibody was shown to be specific for the sucrase subunit. These antibodies provided new information regarding the biosynthesis and postnatal development of SI. In rat intestinal fetal transplants, SI was found exclusively in the form of an enzymatically active high molecular weight precursor, confirming our previous observations concerning the role of luminal proteases for the processing of SI in the microvillus membrane. The SI precursor, purified by affinity chromatography using the BB 3/34/12 antibody, had both sucrase and isomaltase activities, suggesting that a single precursor protein generates both sucrase and isomaltase subunits by proteolytic cleavage. The initial appearance of SI during normal postnatal development in the rat intestine was found to be confined to the cells present at the base of the villi. The same localization was observed after precocious induction of SI by cortisone acetate. In both cases, no immunofluorescence was observed in the crypts, suggesting that only the differentiated enterocyte is capable of synthesizing this enzyme. Even at the earliest times of appearance, newly synthesized SI was found almost completely split into its subunits, suggesting that the protease(s) responsible for the processing of the precursor in the microvillus membrane develop(s) in parallel with SI or earlier.**

The sucrase/isomaltase complex (SI) is one of the most thoroughly characterized integral proteins of the intestinal microvillus membrane (MVM) (1, 2). It has been the marker of choice in most studies of normal maturation and differentiation of the enterocyte (3, 4) and of intestinal diseases (5, 6). Sucrase and isomaltase activities are absent from the small intestine of suckling mammals except man; they appear during weaning (3). Both enzyme activities can be induced precociously by steroids, thyroxine, or insulin (3, 7). Based on immunochemical evidence, the site of initial appearance of the SI, during both normal development and precocious induction by steroids, has been ascribed to the crypt and lower villus cells (8, 9). However, because the crypt cells do not express sucrase and isomaltase activities, the presence of an inactive precursor of the SI in these cells has been postulated (9).

The structure of the SI has been extensively studied (see ref. 2 for a review). Its association with the microvillus membrane has been found to be mediated via interaction of a hydrophobic portion of the isomaltase subunit with the membrane bilayer, with the sucrase subunit occupying a more peripheral position and protruding into the intestinal lumen (10). Part of the mechanism whereby such an association of subunits is accomplished by the enterocyte during biosynthesis has been elucidated recently by the finding that sucrase and isomaltase are syntheiszed as a high molecular weight precursor which is subsequently split into the two subunits in the MVM; it has been suggested that pancreatic elastase might be responsible for this cleavage (11). Unlike other membrane proteins that are anchored to the cell membrane by a COOH-terminal amino acid sequence (12), the SI has its anchor near the NH_2 terminus of the isomaltase (10).

In the present communication we describe the preparation of monoclonal antibodies to the rat SI. These highly specific probes have been used to study the mechanisms of biosynthesis and postnatal development of the SI.

MATERIALS AND METHODS

Preparation of Monoclonal Antibodies. Monoclonal antibodies to rat MVM proteins were prepared by the hybridoma technique (13) as described (14). Briefly, BALB/c mice were immunized by intraperitoneal injection of MVMs purified according to Hopfer *et al.* (15); 200 μg of membrane protein in 200 μl of phosphate-buffered saline (P_i/NaCl) mixed with 200 μl of complete Freund's adjuvant was administered to each mouse. Subsequently, two injections, without adjuvant, were given intraperitoneally at 3-week intervals. Four days after the last booster, the animals were killed and 10^8 spleen cells were fused with 10^7 myeloma cells (P3 – NSI/1-Ag 4-1 cells) as described by Galfre *et al.* (13). Hybrids, grown in 48-well Costar plates, were isolated with selective HAT medium (16). At 10–14 days after fusion the cultures were tested for the production of specific antibodies binding to intact brush borders by using a radioimmunoassay (14). Positive cultures were then tested for the production of antibodies to SI by the following enzyme immunoassay. Rabbit anti-mouse-IgG (10 mg), affinity-purified on a mouse IgG-Sepharose 4B column, was coupled to 3 g of CNBr-activated Sepharose 4B; 100-μl aliquots of a 12.5% suspension of coupled beads in Tris-buffered saline containing albumin (TBS/A; 1 mM $CaCl_2$/5 mM KCl/0.5 mM $MgCl_2$/0.136 M NaCl/0.7 mM Na_2HPO_4/25 mM Tris/0.05% NaN_3/0.2% bovine serum albumin, pH 7.4) were added to 5-ml plastic tubes and washed with 1 ml of TBS/A. After centrifugation (at 4000 × *g*, 5 min), the supernatant was removed and the beads were incubated with 100 μl of hybridoma culture medium for 2–3 hr at room temperature. Thereafter, the beads were washed

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Abbreviations: SI, sucrase/isomaltase complex; MVM, microvillus membrane; P_i/NaCl, phosphate-buffered saline (0.01 M phosphate, pH 7.2/0.154 M NaCl); TBS/A, Tris-buffered saline containing 0.2% bovine serum albumin (1 mM $CaCl_2$, 5 mM KCl, 0.5 mM $MgCl_2$, 0.136 M NaCl, 0.7 mM Na_2HPO_4, 25 mM Tris, 0.05% NaN_3, 0.2% bovine serum albumin); FITC, fluorescein isothiocyanate.

three times with 2.5 ml of TBS/A and incubated at 4°C for 90 min with 100 μl of solubilized MVM proteins, prepared as follows. Purified MVM (17) (approximately 10 mg of protein) was incubated with 10 ml of 10 mM sodium phosphate/pH 7.0/100 mM NaCl/0.5% Triton X-100 for 90 min on ice, and then spun at 100,000 × *g* for 1 hr; the supernatant was used as "solubilized MVM." After this second incubation the beads were washed three times with 2.5 ml of the above Triton X-100-containing buffer, and bound SI was detected enzymatically by overnight incubation with sucrose or palatinose (a substrate for isomaltase) (18). Positive hybridoma cultures were cloned by limiting dilution plating (half a cell per well) in 96-well Costar plates containing mitomycin c-treated (19) 3T3 cells (50,000 cells per well). Cultures with the highest titer in the solid-phase enzyme immunoassay were selected and used for antibody characterization and large-scale antibody production in ascites in mice (14).

Purification of SI and Its Precursor. Ascites fluid containing 20–30 mg of immunoglobulin per ml was filtered, centrifuged, and precipitated with ammonium sulfate (to 50% saturation). The precipitate was redissolved in 0.14 M sodium phosphate buffer (pH 8.0) and the antibody (in both cases, of the IgG1 class) was purified by affinity chromatography on a protein A-Sepharose 4B column (20). Purification of SI and its precursor by affinity chromatography on a BB 3/34/12 monoclonal antibody-Sepharose 4B column was performed as follows. Solubilized MVM obtained from normal rat intestines (for SI purification) or from fetal intestinal transplants (for the purification of the precursor) were applied to the affinity column. After extensive washing of the column with the same buffer, the bound protein was eluted with 5 M guanidine HCl; the eluate was immediately neutralized and dialyzed against P_i/NaCl at pH 7.2. Partially active SI and precursor were obtained by eluting with 0.5% acetic acid into tubes containing 0.5 M borate buffer at pH 8.0 (1 vol of 0.5% acetic acid added to 1 vol of borate buffer, resulting in a pH of approximately 7.0). However, this procedure released only about 60% of the bound enzyme from the column.

Subunit Specificity of the BB 3/34/12 Antibody. Approximately 20 mg of MVM protein (17) in 0.9 ml of P_i/NaCl was iodinated with 5 mCi (1 Ci = 3.7×10^{10} becquerels) of carrier-free $Na^{125}I$ (17 Ci/mg, New England Nuclear) by the lactoperoxidase technique (21) for 10 min at room temperature. The labeled membranes were washed seven times in 20 ml of P_i/NaCl containing 1% NaI (30,000 × *g*, 25 min) and finally resuspended in 2.5 ml of 50 mM sodium phosphate buffer (pH 8.0). The membranes were treated eight times with 10 μl of citraconic anhydride (10% in absolute dioxane) in order to split the SI into subunits (22). After completion of the reaction, the suspension was spun at 100,000 × *g* for 60 min and 1.5 ml of the supernatant was applied to a 1.5 × 88 cm Bio-Gel P-300 column equilibrated and eluted with 10 mM sodium phosphate buffer (pH 6.8) (22). Essentially all of the radioactivity eluted in two peaks, one shortly after the void volume and the other one close to the total volume of the column. The first peak, containing all the isomaltase and sucrase activities, was pooled and concentrated and its pH was adjusted to 8.0 with NaOH; NaCl (0.1 M final concentration) and Triton X-100 (1% final concentration) were added. This concentrated pool was passed through a protein A-Sepharose 4B affinity column on which the BB 3/34/12 antibody had been bound at pH 8.0 (20). After extensive washing of the column with the above 1% Triton X-100 buffer, the antibody was eluted with 0.1 M citrate buffer (pH 5.0) (20). This eluate contained all the sucrase but no isomaltase activity, indicating that the BB 3/34/12 antibody is specific for the sucrase subunit.

The pH 5.0 eluate was dialyzed against P_i/NaCl and concentrated by Amicon filtration. Triton X-100 was removed by chromatography on a BioBead SM-2 column (23), and the eluate was dialyzed against H_2O, lyophilized, resuspended in electrophoresis sample buffer, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis under reducing conditions (11). Autoradiography of the dried gel was performed with Kodak blue-sensitive-single-coated x-ray film.

Fetal Intestinal Transplantation. Whole small intestines from 16 to 20-day rat fetuses (Fisher strain) were transplanted subcutaneously on the back of syngeneic 50-g rats (24). Under these conditions the transplants undergo a maturation process comparable to the postnatal development *in situ* (24) and exhibit activities of various MVM enzymes after a few weeks (unpublished data); 5–6 weeks after transplantation the hosts were killed and the MVM were isolated from the transplants (17).

Postnatal Development and Precocious Induction of SI. The appearance of the SI during normal intestinal development was visualized by an indirect immunofluorescence technique using the two monoclonal antibodies and rat intestinal cryosections (25). Monoclonal antibodies (ascites form) were used at a 1:1000 dilution. The second antibody was a fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (Behring Diagnostics; molar ratio of fluorescein to protein, 4.0–5.0) diluted 1:25. The small intestines of 9 to 20-day-old suckling rats were cut into three segments of identical length, and the proximal 3 cm of the middle third was used for the immunocytochemical study. The remaining portions of each intestine were separately homogenized, and MVM (17) were purified from each homogenate. Sucrase and isomaltase activities were assayed in all homogenates as well as in purified MVM. SI was purified from solubilized MVM by incubation with protein A-Sepharose 4B beads to which antibody BB 3/34/12 had been bound, analogously to the solid-phase immunoassay described above. The washed beads were resuspended in electrophoresis sample buffer containing 50 mM dithiothreitol, heated to 100°C for 3 min, and, after 2 min at room temperature, spun in an Eppendorf centrifuge; the supernatants were subjected to $NaDodSO_4$ gel electrophoresis (11).

Precocious appearance of SI was induced by a single subcutaneous injection of cortisone acetate (50 μg/g of body weight) in the back of 9-day-old suckling rats. The development of SI was analyzed 21, 45, and 69 hr after cortisone injection by immunofluorescence and by gel electrophoresis of the purified enzyme as described above.

RESULTS

A total of 43 hybridoma cultures producing antibodies specific for brush border membrane antigens were produced in four independent hybridizations; two of these hybridomas were positive in the solid-phase enzyme immunoassay and were therefore producing antibodies directed against SI. These two hybridomas were cloned; all clones obtained from both cell lines were positive in solid-phase enzyme immunoassay, suggesting that each of the original cultures was derived from a single fusion event. The antibodies produced by the final clones, BB 3/34/12 and BB 5/8/40/90, were characterized by immunodiffusion and immunoelectrophoresis using heavy chain specific rabbit antibodies. Both monoclonal antibodies were of the IgG1 class, did not inhibit sucrase or isomaltase activities upon binding to the antigen, and bound strongly to intestinal cryosections (as detected by immunofluorescence) and to isolated brush borders (in a radioimmunoassay). Binding of the BB 5/8/40/90 antibody to solubilized SI (in the solid-phase enzyme immunoassay), however, was strongly inhibited by Triton X-

100. The BB 3/34/12 antibody, which did not show a similar inhibition, was therefore used for affinity chromatographic purification of SI and its precursor. [^{14}C]Lysine-labeled monoclonal antibodies were purified by affinity chromatography on a protein A-Sepharose 4B column and analyzed by two-dimensional gel electrophoresis (26). The two monoclonal antibodies showed a similar pattern, consisting of a single heavy and a single light chain each.

The subunit specificity of the BB 3/34/12 antibody was established by splitting SI from ^{125}I-labeled MVM into free subunits by using citraconic anhydride (22), followed by affinity chromatography of the solubilized protein fraction on a protein-A-Sepharose 4B column to which the BB 3/34/12 antibody had been bound. In this way the bound antigen could be eluted with a pH 5.0 buffer (20), with full preservation of the sucrase and isomaltase activities. After citraconylation, the ratio of sucrase to isomaltase activities (measured with sucrose and palatinose, respectively, as substrates) was 14, higher therefore than the ratio 4–5 found in the MVM: this difference reflects the preferential solubilization of the sucrase over the isomaltase subunit with citraconic anhydride (22). The pH 5.0 eluate from the BB 3/34/12-protein A-Sepharose 4B column had sucrase activity but isomaltase activity was undetectable, suggesting that only the isolated sacrase subunit was bound to the antibody; $NaDodSO_4$/polyacrylamide electrophoretic analysis of this eluate revealed a single radioactive band comigrating with the small subunit of SI (Fig. 1, lane 8). It is concluded, therefore, that antibody BB 3/34/12 is specific for the sucrase subunit and that the faster-moving band of SI on the gel represents this subunit, as found for rabbit SI (22).

Immunofluorescent staining of frozen sections of rat intestine with both monoclonal antibodies was confined exclusively to the luminal membrane (MVM) of the differentiated enterocytes present on the intestinal villi; no specific fluorescence was detected in the crypts.

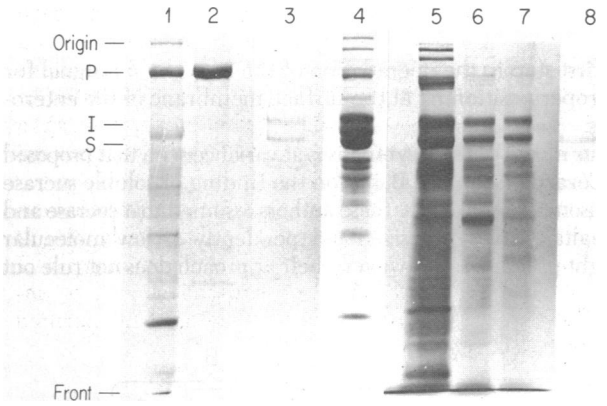

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis (autoradiogram) of MVM and SI purified from normal adult and transplanted fetal intestines, showing subunit specificity of BB 3/34/12 monoclonal antibody. Lanes: 1, protein pattern of MVM (17) from fetal intestines 5 weeks after subcutaneous transplantation; 2, SI precursor (P) from fetal intestinal transplants after purification by BB 3/34/12-Sepharose 4B affinity chromatography; 3, SI from normal adult intestine after purification by BB 3/34/12-Sepharose 4B affinity chromatography; 4, protein pattern of MVM (17) from normal adult intestine; 5–7, attempt to purify SI from normal adult intestine by BB 5/8/40/90-Sepharose 4B-affinity chromatography as follows. MVM (17) were solubilized with 1% Triton X-100/10 mM sodium phosphate, pH 8.0/0.1 M NaCl. After removal of Triton by BioBead SM-2 chromatography (23), the sample was applied to affinity column and eluted stepwise with 0.01%, 0.1%, and 1% Triton X100 in the same buffer. Fractions containing sucrase activity were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis; 80% of sucrase activity eluted with 0.01% Triton (lane 6), 20% eluted with 0.1% Triton (lane 7), and none was found after elution with 1% Triton (not shown). Lane 5, Triton X-100-solubilized MVM prior to affinity chromatography; lane 8, subunit specificity of BB 3/34/12 monoclonal antibody.

Purification of SI by Affinity Chromatography. SI was purified from adult rat small intestines by affinity chromatography on a BB 3/34/12-Sepharose 4B column. On $NaDodSO_4$ slab gel electrophoresis, the purified enzyme was resolved into two major protein bands corresponding to the two subunits (Fig. 1, lane 3); two minor bands, corresponding to proteins with smaller apparent molecular weights were observed. These two bands were also present on gels run with solubilized MVM immunoprecipitated with a rabbit anti-rat SI antiserum, but they were never found labeled with [^{3}H]-fucose (11), suggesting that they may represent either degradation products of SI or SI subunits lacking carbohydrate chains and consequently moving faster on $NaDodSO_4$/polyacrylamide gels.

Presence of an Active SI Precursor in Fetal Intestinal Transplants. At 2–3 weeks after transplantation of fetal rat intestines to the back of adult syngeneic rats, their overall morphologic features and complement of enzymatic activities typical of mature enterocytes are similar to those of normal adult intestine. When MVM were purified from fetal intestinal transplants, they were found to possess sucrase and isomaltase specific activities comparable to those of MVM purified from adult rat intestines. After solubilization of MVM prepared from the intestinal transplants, we purified SI by affinity chromatography on a BB 3/34/12-Sepharose 4B column. $NaDodSO_4$ slab gel electrophoresis of the purified enzyme revealed a single protein band with a mobility much lower than that of the subunits (Fig. 1, lane 2) but similar to that of the [^{3}H]fucose-labeled SI precursor previously observed in Golgi and lateral basal membranes prepared from intestinal villus cells (11). When eluted from the affinity column with 0.5% acetic acid, the precursor was partially active, possessing both sucrase and isomaltase activities. Because the BB 3/34/12 antibody is specific for the sucrase subunit, we conclude that there is a single precursor present in the fetal intestinal transplants comprising both the sucrase and the isomaltase subunit.

Normal Postnatal Intestinal Development and Appearance of SI. The postnatal appearance of SI in rat intestine was studied by the indirect immunofluorescence technique using both monoclonal antibodies; they gave identical results. SI antigen appeared, on day 19 after birth, at the base of the villi (Fig. 2 *b* and *c*). However, the crypts showed no immunofluorescence (Fig. 2 *b* and *d*), as observed in adult rat intestine. Injection of cortisone acetate at day 9 after birth elicited a precocious appearance of SI antigen after 45 hr (Fig. 3 *b* and *c*) but not after 21 hr (Fig. 3*a*). The pattern of immunofluorescence after 45 hr was identical to that observed at day 19 of normal development, with the crypts again completely negative. To determine if SI is already processed into subunits when it appears for the first time at the base of the villi, we purified the enzyme with BB 3/34/12-protein A-Sepharose 4B beads and analyzed the bound SI by $NaDodSO_4$ slab gel electrophoresis (Fig. 4). Apparently, SI is cleaved as soon as it appears, on day 19 (Fig. 4, lane 7). Similarly, most of the SI was found in the processed form 45 hr after cortisone injection (Fig. 4, lane 1); however, the precursor was slightly more abundant at that time than at 69 hr after cortisone injection (Fig. 4, lane 3). It is noteworthy that the sucrase activity in the homogenate, immunofluorescence on cryosections, and sucrase protein bands on the $NaDodSO_4$/polyacrylamide slab gel appeared simultaneously, both during normal postnatal development and after cortisone administration.

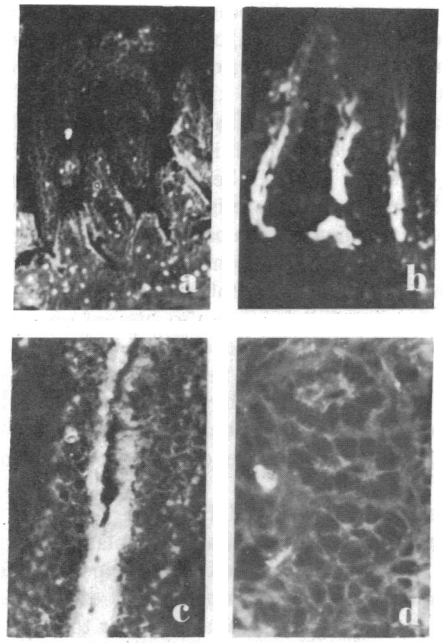

FIG. 2. Normal postnatal appearance of SI visualized by the indirect immunofluorescence technique with BB 3/34/12 monoclonal antibody. (*a*) Lack of specific staining of intestinal cryosection from 18-day-old rat. (*b*) Specific fluorescent staining of the luminal membrane of the epithelial cells in the lower half of the villi in intestinal cryosection from 19-day-old rat. (*c*) Higher magnification of a region approximately halfway between two villi in cryosection from 19-day-old rat: note the weakening of the fluorescence toward the top of the villi (top of the figure). (*d*) Intestinal crypts in 19-day-old rat intestine at higher magnification, illustrating the total absence of specific fluorescence. (*a* and *b*, ×100; *c* and *d*, ×450.)

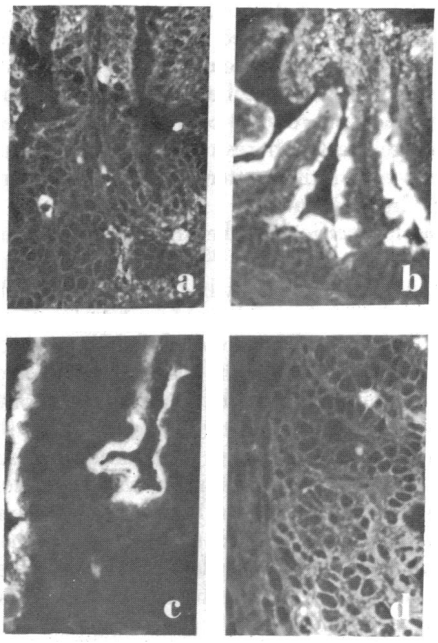

FIG. 3. Precocious induction of SI by cortisone acetate administration to 9-day-old rats, visualized by the indirect immunofluorescent technique with the BB 3/34/12 monoclonal antibody. At 21, 45, and 69 hr after a single subcutaneous cortisone injection, one injected and one control rat were sacrificed. (*a*) No specific fluorescence is present in specimen from 10-day-old rat taken 21 hr after cortisone injection. (*b*, *c*, and *d*) At 45 hr after cortisone injection in 11-day-old rat, SI has appeared at the luminal membrane of the epithelial cells present in the lower third of the villi; no specific fluorescence is present in the crypts. No specific fluorescence was detected in the intestines of the control animals (cortisone not injected) of the same age. (*a* and *b*, ×100; *c* and *d*, ×450.)

DISCUSSION

Earlier we described (11) a putative high molecular weight precursor of SI, in the Golgi membranes purified from mature enterocytes, that is rapidly cleaved into the mature subunits after its incorporation into the MVM. We suggested that pancreatic elastase might be responsible for this late posttranslational processing of SI. The results obtained in the present work using fetal intestinal transplants and monoclonal antibodies to rat SI confirm and extend our previous observations.

The fetal intestinal transplants represent a model system in which normal postnatal intestinal development is reproduced in the absence of extra intestinal luminal contents such as food and pancreatic secretions. Under these conditions we have shown that the SI is present in the MVM of the enterocytes exclusively as an uncleaved precursor, similar in mobility on $NaDodSO_4$ gels to the Golgi precursor we have previously described. Furthermore, the precursor from the intestinal transplants can be split by elastase into two fragments with mobilities on $NaDodSO_4$ gels similar to those of the SI subunits (unpublished observations). The finding that the precursor purified from the intestinal transplants has both sucrase and isomaltase activities conclusively demonstrates its identity with the SI and suggests that the splitting of the precursor into the two subunits has no apparent effect on its enzymatic activity. Because the monoclonal antibody used for the affinity purification of the precursor is specific for the sucrase subunit, it can be concluded that the SI is synthesized as a single polypeptide chain comprising both sucrase and isomaltase subunits. The physiological effect of the splitting of the precursor into the two subunits in the MVM of normal enterocytes, presumably at a specific point of the polypeptide chain, at present is solely a matter of speculation. This later posttranslational event may possibly represent the first step in the degradation of the enzyme or a signal for its proper positioning at the surface membrane of the enterocytes.

Our model of SI biosynthesis is at variance with that proposed by Cézard *et al.* (27). Based on the finding of soluble sucrase and isomaltase subunits, these authors assumed that sucrase and isomaltase are synthesized independently as low molecular weight precursors. However, their approach does not rule out

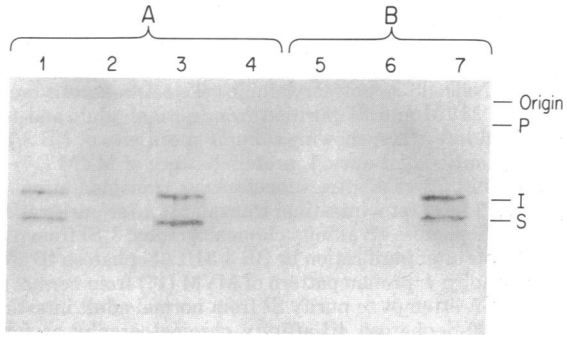

FIG. 4. $NaDodSO_4$/SI of polyacrylamide gel from intestines of suckling rats after precocious induction by cortisone acetate (*A*) and during normal development (*B*). Lanes: 1, 45 hr after a single cortisone injection to 9-day-old sucklings; 2, 11-day-old suckling without cortisone (control); 3, 69 hr after a single cortisone injection to 9 day-old suckling; 4, 12-day-old suckling without cortisone (control); 5, 6, and 7, 17-, 18-, and 19-day-old sucklings, respectively (normal development). A weak band corresponding to the precursor (p) could be detected in lanes 1 and 3 but could not be reproduced photographically.

the possibility that these soluble enzyme subunits were generated by mechanical dislodgement and proteolytic cleavage of the high molecular weight precursor we have described (11).

The localization of the SI in the rat intestine by using the monoclonal antibodies described in this work is different from that previously observed by using conventional monospecific antisera (8, 9, 25) in that no antigen could be detected in the intestinal crypts. This discrepancy is of considerable importance in understanding the mechanism underlying intestinal epithelial cell differentiation. Since the pioneering work of Kretchmer and colleagues (8), various reports have confirmed the presence of immunoreactive SI antigen in the crypts in spite of the absence of enzymatically active SI (see ref. 3 for a review). This finding has led to the suggestion that the proliferative crypt cells synthesize an inactive precursor of the SI, which is somehow transformed into the active enzyme during intestinal cell differentiation. In contrast, our results suggest that the appearance of the SI in the newly differentiated cells involves *de novo* synthesis of the enzyme.

Two possible explanations may be offered to explain this discrepancy: either the conventional antisera used in previous studies recognize degradation products of SI, adsorbed at the luminal surface of the crypt cells, which are not recognized by the more specific monoclonal antibodies, or the monoclonal antibodies, which are presumably specific for single antigenic sites of the enzyme, cannot recognize a different molecular form of the SI synthesized by the crypt cells. The former possibility is supported by two observations: (*i*) conventional antibodies to the SI show highest binding at the tip of the villus and in the crypt (3), whereas maximal enzymatic activity is found in the midvillus region (28); and (*ii*) crossreactive material from the crypts, analyzed by $NaDodSO_4$ gel electrophoresis, was found to be of low molecular weight (3) as would be expected for a degradation product. It should also be noted that the monoclonal antibodies described in this work were capable of recognizing equally well the precursor protein present in fetal intestinal transplants and the processed enzyme present in the MVM of normal enterocytes.

With our monoclonal antibodies, as found (11) with our conventional rabbit anti-rat SI antiserum, we also were unable to detect the catalytically inactive enzyme precursor observed during postnatal development in the rabbit (9). Instead, we observed a strict parallelism among the appearance of sucrase activity in intestinal homogenates, the appearance of SI subunits detected by $NaDodSO_4$/polyacrylamide gel electrophoresis, and the appearance of immunoreactive SI antigen at the base of the villi. A similar result was obtained when the SI was induced precociously by cortisone acetate injection in 9-day-old sucklings.

In conclusion, the use of monoclonal antibodies to the SI in combination with the technique of fetal intestinal transplantation has shown that (*i*) the SI is synthesized as an enzymatically active single-chain precursor protein that is not split into the subunits in the absence of pancreatic proteases and (*ii*) that apparently the differentiated villus cells, but not the proliferating crypt cells, are capable of synthesizing the SI at weaning and in adult animals.

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