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IN VITRO TRANSLATION OF INTESTINAL SUCRASE-ISOMALTASE AND GLUCOAMYLASE

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It is has been proposed that both sucrase-isomaltase and glucoamylase are initially synthesized as large single-chain precursors which are then processed to heterodimers. We have tested this hypothesis by in vitro translation of their mRNAs.

The primary translation product of sucrase-isomaltase mRNA was a single polypeptide of M_r = 190,000. Similar experiments using antiserum against glucoamylase revealed a single polypeptide of M_r = 145,000.

These results are consistent with the single chain precursor hypothesis for sucrase-isomaltase. However, the glucoamylase product (145 kDa) is too small to be processed to a heterodimer of M_r = 230,000. Moreover, the mature subunits (M_r = 135,000 and 125,000) probably are derived from the 145 kDa precursor by proteolytic trimming and must include and share most of the precursor protein.

Sucrase-isomaltase (SI) and glucoamylase are abundant large glycoproteins of the intestinal brush border. SI consists of two nonidentical subunits (130 and 110 kDa) one of which hydrolyzes sucrase, and one isomaltase (1). This protein is synthesized as a single chain precursor, which is subsequently hydrolyzed in the intestinal lumen by pancreatic enzymes to its component subunits (1,2). Glucoamylase, on the other hand, is comprised of two subunits (135 and 125 kDa) with identical catalytic properties (3). It is not clear if these two subunits differ only by the presence of the anchor peptide found only on the larger subunit (3), nor is it clear if they are made as a large single chain precursor, as is SI. Recent evidence suggests that the two subunits are very similar, but not identical (4).

The sizes of the primary products of translation of SI and GA mRNAs are not known. Although both proteins are glycosylated,

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SI mRNA is reported to stimulate production of a 240 kDa peptide, larger than the mature glycosylated form (230 kDa) (5). No reports regarding the size of the glucoamylase primary product have appeared. To clarify this initial step in the biosynthesis of these proteins, we have isolated the products of their mRNAs in a cell-free translation system.

MATERIAL AND METHODS

Total RNA was isolated from proximal intestine of fasted rats by the guanidine-HCl method as described previously (6). *In vitro* translations were performed in a nuclease-treated rabbit reticulocyte lysate system (Promega Biotec, Madison, WI), with a final potassium and magnesium concentration of 154 mM and 1 mM respectively. Sixty μCi of ^{35}S-methionine was used for each 50 μl translation reaction. Benzamidine (1 mM) and phenylmethylsulfonylfluoride (0.1 mM) were added to reduce proteolysis. Immunoprecipitates were obtained by adding 5 μl of specific antiserum and staph A cells (BRL, Bethesda, MD) as described previously (7). The products were separated on reduced 5% SDS gels after boiling in 2% SDS (7), and the dye front run off the gel to allow greater separation of the large proteins. Labeled protein bands were visualized by autofluorography. Molecular weight standards used were myosin, β-galactosidase, phosphorylase, and bovine serum albumin.

Pancreatic duct-ligated animals were prepared as described previously (8) and injected intraperitoneally with 1 mCi of [^{3}H]leucine and 100 μCi of [^{35}S] methionine (New England Nuclear, Boston, MA). Brush borders were isolated, extracted with 2% Triton, and the supernatant used for immunoprecipitation as described previously (9), except that Staph A cells were used to isolate the immune complex. Papain-solubilized SI and glucoamylase were purified as described (10,11) to specific activities of 236 U/mg and 127 U/mg respectively. Purified SI contained 2 peptides of 120 and 110 kDa; and glucoamylase a single peptide of 115 kDa. Antiserum raised in New Zealand white rabbits precipitated only a single protein on Ouchterlony immunodiffusion plates which stained for the enzyme activity, using bromochloro indolyl α-glucoside as substrate.

RESULTS

Immunoprecipitation with SI antiserum of products obtained from cell-free translation of rat intestinal RNA yielded a single polypeptide with a M_r of 190 kDa (Fig. 1B). This antiserum also identified the mature, single-chain pro SI (M_r = 210 kDa) from intestinal brush border preparations prepared from animals with ligated pancreatic ducts (Fig. 1A). The complete product of

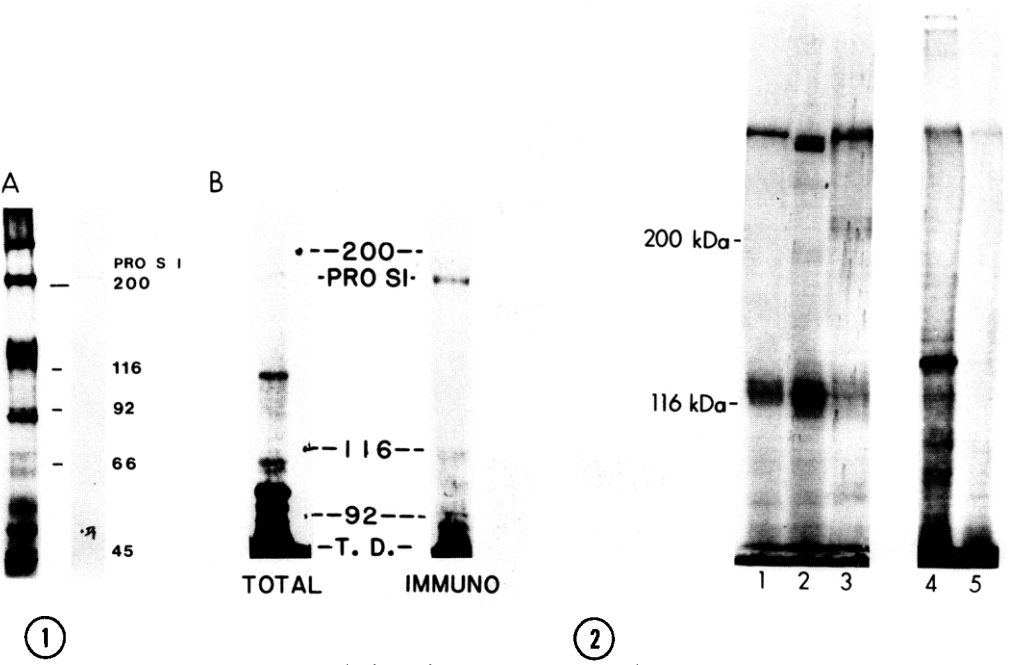

Fig. 1 - Immunoprecipitation of sucrase-isomaltase. A. In vivo - The brush border from dual labelled animals with ligated pancreatic ducts was isolated. The total brush border protein was solubilized with 2% SDS and subjected to electrophoresis on 5% denaturing acrylamide gels followed by fluorography (left lane). A fluorogram of immunoprecipated SI from Triton solubilized brush borders is shown in the right lane. B. In vitro, the fluorogram of similar experiments using the products of cell-free translation in a reticulocyte lysate are shown. Left lane, total; right lane, SI immunoprecipitate.

Fig. 2 - Fluorographs of glucoamylase immunoprecipitates. Lanes 1, 2. In vivo - immunoprecipitated glucoamylase from sham-operated and bile duct -ligated animals were analyzed as described in Figure 1A. Lane 1, control; lane 2, bile duct -ligated, lane 3, total brush borders. Lanes 4, 5 - in vitro. Lane 4, immunoprecipitation of glucoamylase from reticulocyte lysate translation. Lane 5, same as lane 4 after addition of 50 µg of papain-purified glucoamylase. The band at $M_r \equiv 250$ kDa was a constant finding, and migrates similarly to the previously reported translation product of SI.

translation of SI mRNA was thus significantly smaller than the proSI found in brush border membranes.

On the other hand, the complete product of translation of glucoamylase mRNA, as identified by glucoamylase antiserum, was smaller (M_r = 145 kDa), as shown in lane 4, Fig. 2. This pro-maltase was thus larger by M_r = 20-30,000 than the form of

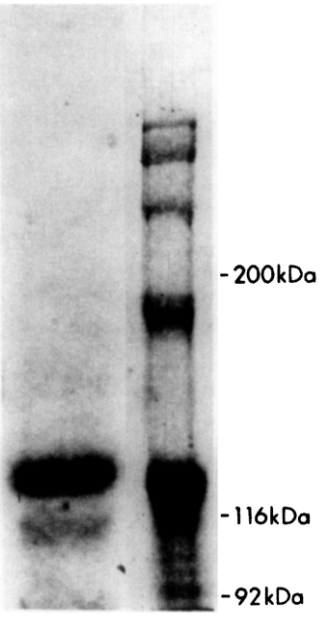

Fig. 3 - Aggregation of glucoamylase. Papain-solubilized glucoamylase from two preparations were electrophoresed in 5% denaturing acrylamide gels. Left lane, preparation 1, 10 μg protein, stained with silver. Right lane, same preparation after storage at -20°C for 2 months and freeze-thawing five times, 20 μg protein, stained with Coomassie Blue.

glucoamylase purified from papain-treated brush border membranes. Glucoamylase antiserum identified only one peptide with a M_r = 125 kDa, isolated from the papain-solubilized brush borders prepared from animals whose pancreatic duct had been previously ligated or from sham-operated animals (Fig. 2, lanes 1, 2). The purified glucoamylase used to raise antiserum was added to the translation mix simultaneously with the glucoamylase antiserum and completely inhibited immune precipitation of the 145 kDa protein (Fig. 2, lane 5). Identical results were obtained for SI addition and the 190 kDa protein(data not shown).

Glucoamylase purified from rat brush borders is usually reported to have a M_r in excess of 250 kDa, yet the cell-free translation product was only 145 kDa. In freshly-purified preparations of papain-solubilized glucoamylase, we obtained a single protein band of M_r = 125 kDa (Fig. 3, lane 1). After

storage and multiple freeze-thaw cycles, this preparation of glucoamylase contained multiple bands, consistent with the formation of multimers of the 125 kDa subunit (Fig. 3, lane 2).

DISCUSSION

The primary translation product of SI is the appropriate size for the mature pro-protein. It is known to be glycosylated, and the addition of carbohydrate (10-15% by weight) would convert the 190 kDa primary translation product to its mature 230 kDa size. These data are consistent with the single-chain SI model originally proposed by Semenza (2). However, previous attempts to demonstrate the size of the product of SI mRNA translation reportedly showed two bands of a M_r about 240,000 (5).

The situation regarding glucoamylase seems more complex. The two subunits reported (135 and 125 kDa) seem to differ largely by the presence of an anchor peptide. Purification of papain-solubilized enzyme leads to only one peptide of M_r = 115-125 kDa, although there is some evidence that minor differences, perhaps post-translationally produced, may be present between the subunits (4).

The finding of a single cell-free product of 145 kDa is interesting for two reasons. First, it suggests that the smaller 125 kDa subunit is derived from the larger one by post-translational proteolytic processing. In fact, the larger subunit (135 kDa) must also undergo post-translational proteolysis, especially considering that it, like the smaller subunit is a glycoprotein (3). The minor differences in these subunits seen on peptide mapping (4) or heat inactivation (12) could be explained by post-translational modifications. Second, the 145 kDa subunit is neither consistent with the two site, single-chain model for SI biosynthesis, nor with data in mammalian kidney and avian intestine for a similar pathway for

glucoamylase synthesis. A large single-chain (330 kDa) glucoamylase has been found in pig intestine with bypassed pancreatic enzymes (13), and in normal pigeon intestine (M_r = 260 kDa) (14). It is this form of the enzyme which appears in intracellular membranes during biogenesis, at which site it is split into two final subunits by proteases, especially elastase (15). Glucoamylase in the rat kidney has a large size also (M_r = 260 kDa), consistent with the lack of proteolytic lumenal enzymes in that tissue.

How can the finding of a single smaller cell free product apparently identical with glucoamylase be resolved with these data? The glucoamylase complex has been isolated comprised of 2 (16) or 3 (17) subunits. Sizes have varied from 260 kDa to 335 kDa, suggesting that oligomers can be formed. We have demonstrated that such aggregation occurs even when the enzyme is purified after papain solubilization (Fig. 3). Perhaps pancreatic proteases affect this aggregation, but do not clip a single chain into two separate chains. It is also possible that the rat intestinal enzymes differs from that in the pig and pigeon intestine.

The available data suggest at least 3 patterns for insertion of intestinal brush border proteins. (1) a single chain, 2 active site molecule with one anchor piece, cleaved post-translationally and intralumenally by pancreatic proteases (i.e. SI); 2) a single chain one active site molecule, post-translationally modified by intracellular proteases to two closely related subunits (e.g. glucoamylase); and 3) a single subunit containing one active site (e.g. membranous alkaline phosphatase (16). Further work will determine whether these are the only possible pathways for brush border insertion.

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