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CONCANAVALIN A BINDING OF SOLUBLE NEUTRAL MALTASE-GLUCOAMYLASE IN SUCKLING RAT INTESTINE

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SUMMARY

The distal intestine of suckling rats contains an anomalously high proportion of its brush border membrane-typical enzymes in soluble, or easily released form. As a sensitive probe of carbohydrate structure, we compared the binding of four forms of maltase-glucoamylase (I, membrane-bound, proteolytically solubilized, adult rat; II, membrane-bound, detergent solubilized, adult rat; III, membrane-bound, proteolytically solubilized, suckling rat; IV, soluble, suckling rat) to Concanavalin A sepharose, eluted by gradient α-methylmannoside. The three membrane-bound enzymes were readily eluted at low concentrations of α-methylmannoside in an identical manner, whereas the soluble enzyme contained a component which was more tightly bound. These results suggest that a portion of the soluble maltase-glucoamylase in the suckling rat intestine has a carbohydrate structure which differs from that of the membrane-bound enzyme. This difference may prevent or at least weaken attachment of the enzyme to the brush border membrane.

INTRODUCTION

A number of glycoprotein enzymes attached to the outer surface of the luminal microvillus plasma membrane of the small intestine are present in the rodent small intestine during the suckling period, but markedly increase in activity at the time of weaning (19-21 days) or earlier, if induced by cortisone or thyroxine (1-5). Before this abrupt increase in activity an anomalously high proportion of the activity of many of these enzymes is present in a soluble form easily separated from membrane fractions by centrifugation (6,7). In the most extensively characterized example, maltase-glucoamylase (EC 3.2.1.3), the soluble enzyme is identical to its membrane-bound counterpart in size, electrophoretic mobility, substrate and inhibitor affinities, pH and heat sensitivity (6) and antigenicity (8). In contrast to membrane-bound enzymes which are predominantly found in the mid-intestine after weaning or hormonal induction (2,9) the soluble enzymes are found chiefly in the distal intestine (7,9).

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In this report we describe differences in the binding of the soluble and membrane-bound forms of maltase-glucoamylase to Con A,[1] thereby raising the possibility that the binding of the enzyme to the plasma membrane may depend on carbohydrate structure.

MATERIALS AND METHODS

Membrane-bound maltase-glucoamylase: Pure maltase-glucoamylase was prepared from adult rat intestinal membranes after solubilization with papain (8) or triton X-100 (10). Both preparations were purified to the extent of appearing as a single band in sodium dodecyl sulfate polyacrylamide gel electrophoresis. The specific activity was approximately 60 u/mg protein in both cases and the purification 300x. In suckling rats the intestine was homogenized in 5 mM EDTA-NaOH pH 7.0 and a membrane-bound maltase-glucoamylase was separated from soluble maltase-glucoamylase by centrifugation at 100,000 g for 60 minutes as described previously (4-6). Membrane bound maltase-glucoamylase was released by incubation with papain (8). The neutral maltase peak obtained by chromatography on sepharose 4B was used for subsequent experiments.

Soluble maltase-glucoamylase: The 100,000 g homogenate supernatant was concentrated by lyophilization to 10 ml and placed on a sepharose 4B column. The column was eluted by gravity with 0.01 M KH_2PO_4-K_2HPO_4 buffer pH 7.0 as described previously (9). The neutral maltase peak was used in subsequent experiments. There was no evidence of acid maltase activity (6) in this peak.

Con A columns: Con A sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was diluted in 5 volumes 0.1 M KH_2PO_4-K_2HPO_4 buffer pH 7.0 containing 0.15 M NaCl, 0.1 M $MgCl_2$, 0.1 M $MnCl_2$ and 0.02% NaN_3 (buffer A), mixed and loaded by gravity into an I.D. 0.7 cm column to a height of 17 cm. The column was washed with 100 ml buffer A overnight. Enzyme samples 1.0 to 5.0 ml, in 0.1 M KH_2PO_4-K_2HPO_4 buffer pH 7.0 were loaded onto the column. After washing with 40 ml buffer A the columns were eluted with a linear gradient of 0.0 to 0.5 M α-MM[2] in 50 ml buffer A, and washed with an additional 25 ml of 0.5 M α-MM in buffer A.

Assays: Maltase activity and protein were determined as described previously (6). Maltase activity in eluates was expressed as the optical density reading at 420 mu, after assay of the glucose produced in 15 min with the Tris-glucose-oxidase reagent. α-MM was measured with the phenol-sulfuric acid reagent as described by Dubois et al (11) using appropriate standards of α-MM.

RESULTS

The elution of pure membrane-bound adult maltase-glucoamylase from the Con A sepharose column was monitored as maltase activity (fig.1). At least 98% of the total activity was bound to the column. Maltase eluted as a single peak at the start of the α-MM gradient. Elution began with concentrations of 5-10 mM α-MM gradient. Elution began with

[1] Con A = Concanavalin A

[2] α-MM = α-methylmannoside

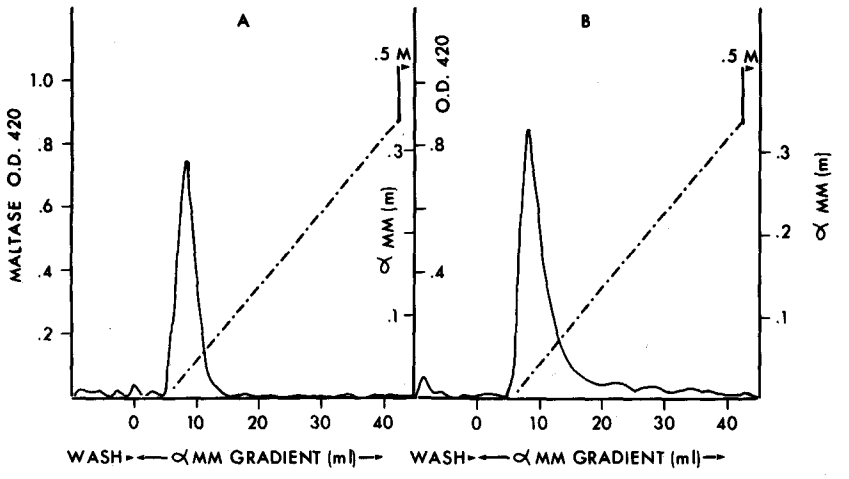

Figure 1: Con A sepharose column chromatography of (A) papain solubilized membrane-bound maltase, and (B) detergent-solubilized membrane-bound maltase from adult rat intestine.
Maltase _____ , α-MM _._._._

concentrations of 5-10 mM α-MM, reached its peak at approximately 20-40 mM α-MM and was essentially complete at 75 mM α-MM. The elution pattern of both the papain (1A) and detergent solubilized (1B) enzymes was very similar, indicating that the possible cleavage of proteolytically susceptible fragments of the membrane maltase-glucoamylase had no significant effect on Con A binding.

Figure 2a demonstrates the elution profile of a maltase-glucoamylase obtained from the plasma membrane of the distal one-half of the small intestine of suckling rats age 17 days. As in figure 1 the bulk of the enzyme activity was retained by Con A sepharose and once again a sharp elution profile was obtained, beginning at 5 mM α-MM and reaching its peak at approximately 20 mM α-MM. All of the enzyme was eluted by 100 mM α-MM.

Figure 2b shows the elution profile of the soluble neutral maltase-glucoamylase obtained from the distal one-half of the small intestine of suckling rats age 17 days. There was an early, rapidly eluted, peak similar to that of the membrane bound maltase at 25 mM α-MM. However,

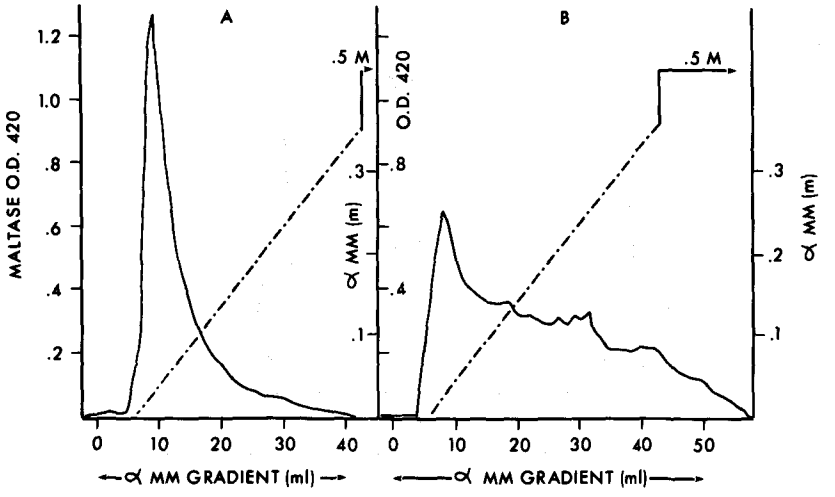

Figure 2: Con A sepharose column chromatography of (A) papain solubilized membrane-bound maltase from suckling rat intestine, and (B) soluble maltase from suckling rat intestine. Wash (not shown) contained neglible maltase.
Maltase ——— , α-MM —·—·—

a major portion, approximately 40% of the activity, was eluted at concentrations of α-MM greater than 100 mM. This material seemed to be extremely heterogeneous in its binding characteristics, appearing as a poorly defined broad peak over a range of 100 to 250 mM α-MM. The rate of elution of the peak depended on the concentration of α-MM. Reduction of the slope of the α-MM gradient and the use of larger eluting volumes failed to define discrete peaks of activity. Over 90% of the activity was bound, and subsequently eluted by 300 mM α-MM. Prolonged washing at 0.5 M α-MM failed to elute additional enzyme. Equilibration and elution with α-MM in 0.1% sodium-deoxycholate to reduce apolar interactions (12) had no effect on the elution profile.

DISCUSSION

These experiments provide the first conclusive evidence of a molecular difference between a soluble, and membrane-bound "membrane-typical" (9) enzyme in the suckling rat intestine. Membrane-derived maltases from adult and suckling intestine were bound by Con A in the

same manner. The difference in binding cannot be attributed to a proteolytic modification of the membrane derived maltase, since both the pure papain-solubilized and the pure detergent-solubilized enzyme were bound identically.

Binding of mannose containing oligosaccharides by Con A is greatly influenced by the nature of the sugar substitutions on the three core mannose residues (13,14). Enhanced binding by the soluble maltase-glucoamylase suggests that the post core sugar residues in the membrane-bound and soluble forms of the enzyme are substantially different. It is not clear how such differences might affect the association of the enzyme with the brush border plasma membrane. Considerable evidence has accumulated however, which indicates that the carbohydrate component of lysosomal enzymes is extremely important in determining their localization within the cell (15-21). The mode of attachment appears to represent an intelligent "homing" mechanism through which lysosomal enzymes seek, and are found by, their specific membranes. It appears that the lysosomal enzymes studied to date may move by either a soluble extracellular route dependent on endocytosis (15) for recapture, or a membrane-bound route (20,21) in their search for the specific receptors which characterize the lysosomal membrane. Our results raise the possibility that a similar carbohydrate-dependent homing mechanism may be involved in determining the placement of plasma membrane-bound enzymes.

Current evidence indicates that intestinal membrane-bound glycoproteins move from their site of synthesis within the RER to the plasma membrane by an extracytoplasmic route along the surface of smooth vesicular membranes (22). Aminopeptidase (23) and sucrase-isomaltase (24,25) are anchored to the plasma membrane by an N-terminal peptide segment which can be cleaved proteolytically. An apolar peptide which presumably serves an "anchor" function, has been isolated from membrane

maltase-glucoamylase (24). It is possible that the carbohydrate portion of the enzyme provides a recognition signal for the insertion of the apolar peptide tail at a specific anchoring site either in the RER or later during the evolution of the plasma membrane. If such is the case, the anomalously high quantity of soluble "membrane-typical" enzymes in the suckling rat intestine may be understood as a reflection of a delay in the development of an adequate carbohydrate recognition signal.

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