Biochimica et Biophysica Acta, 661 (1981) 346–349
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 61399

RAT INTESTINAL MICROVILLUS MEMBRANE SUCRASE-ISOMALTASE IS A SINGLE HIGH MOLECULAR WEIGHT PROTEIN AND FULLY ACTIVE ENZYME IN THE ABSENCE OF LUMINAL FACTORS

ROBERT K. MONTGOMERY, MARIANA A. SYBICKI, ANN G. FORCIER and RICHARD J. GRAND

Division of Gastroenterology and Nutrition, Department of Medicine, The Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115 (U.S.A.)

(Received February 2nd, 1981)
(Revised manuscript received June 15th, 1981)

Key words: Sucrase; Isomaltase; Disaccharidase; (Rat intestine)

Sucrase-isomaltase immunoprecipitated from brush border of an intestinal transplant lacking pancreatic proteases was found to be a single, high molecular weight protein. Elastase digestion converted this protein into two subunits which co-migrated on electrophoresis with those normally found on the microvillus membrane. The high molecular weight form had full sucrase and isomaltase activities.

Sucrase-isomaltase is a digestive enzyme located on the luminal surface membrane of the epithelial cells of the small intestine. It is largely exposed to the luminal environment, attached to the cell membrane by only a relatively small 'anchor' sequence [1]. In this position it is readily affected by luminal pancreatic proteases, which have been implicated both in the turnover of this and other epithelial cell surface proteins [2] and recently, by indirect evidence, in the final conversion of sucrase-isomaltase to the form normally found in the cell membrane [3]. Using a previously described intestinal transplant technique [4,5] we have identified by immunoprecipitation a single high molecular weight form of sucrase-isomaltase isolated from the membranes of mature transplants. These data give direct evidence of the role of pancreatic proteases in sucrase-isomaltase maturation.

In normal rat epithelial cell microvillus membranes, sucrase-isomaltase consists of two large subunits (sucrose α-D-glucohydrolase, EC 3.2.1.48, and dextrin 6-α-D-glucanohydrolase, EC 3.2.1.10, respectively) making up a functional dimer [6], which can be readily separated into two subunits by heating with SDS under reducing conditions [3]. It has been suggested that sucrase-isomaltase may be initially synthesized as a single polypeptide, then later processed to the dimeric form [8]. Hauri et al. [3] immunoprecipitated from the Golgi fraction of normal rat intestinal cells a form of sucrase-isomaltase consisting of a single large protein, which could not be separated into subunits by reduction, but could be separated by treatment with elastase, one of the pancreatic proteases, into subunits which co-migrated with those derived from microvillus membrane sucrase-isomaltase. This evidence suggests that the final step in the processing of sucrase-isomaltase may occur at the cell surface, through the activity of pancreatic proteases.

We have developed and characterized a fetal intestinal transplant model in which the transplanted intestine matures and develops the normal complement of brush border enzymes, including sucrase-isomaltase [5]. Since no pancreatic proteases are present in these transplants, this model provides a unique opportunity to test the hypothesis that the pancreatic proteases play a significant role in the final processing of the sucrase-isomaltase molecule.

Accordingly, we have purified brush borders from mature intestinal transplants and subjected them to structural and functional analysis [9]. First, specific

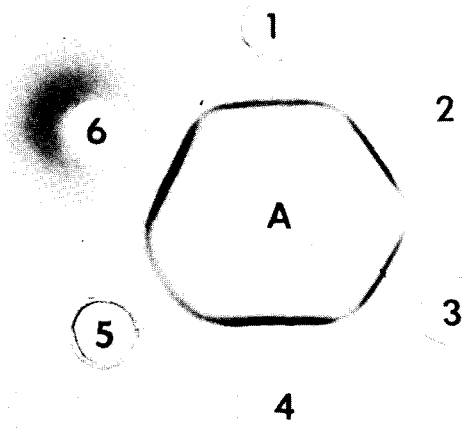

Fig. 1. Comparison of purified adult rat sucrase-isomaltase and solubilized transplant brush borders reacted against rabbit antibody to purified sucrase-isomaltase by Ouchterlony immunodiffusion. Sucrase-isomaltase was isolated from adult rat mucosa by the method of Cogoli et al. [15] using papain solubilization of the enzyme and column chromatography purification. Transplant brush borders were prepared by the method of Kessler et al. [16] and solubilized in 0.6% Lubrol. Aliquots of the purified sucrase-isomaltase were added to the odd numbered wells (1, 3, and 5) and aliquots of three different samples of solubilized transplant brush borders were added to the even numbered wells (2, 4, and 6). Rabbit antibody to purified adult rat sucrase-isomaltase was placed in the central well (A). After development of the precipitation lines, the gel was dried and stained with amido black.

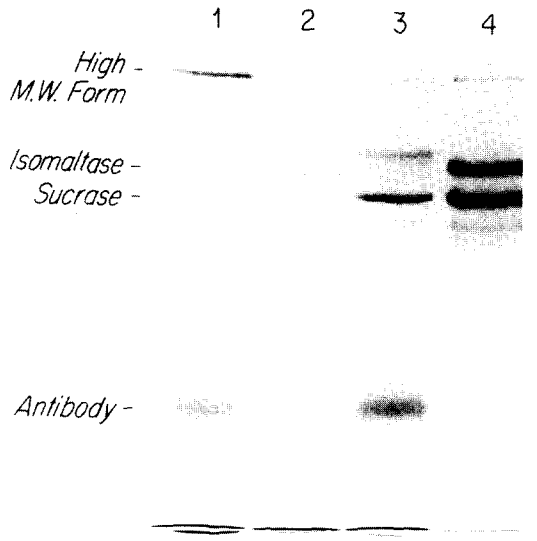

Fig. 2. SDS-polyacrylamide slab gel electrophoresis of immunoprecipitated transplant sucrase-isomaltase. Brush borders were isolated [16] from transplanted intestine and solubilized with 0.6% Lubrol PX. Sucrase-isomaltase was immunoprecipitated, the immunoprecipitates solubilized by heating to 100°C for 5 min in 1% SDS/5% mercaptoethanol, and run on 7.5% polyacrylamide slab gels prepared according to Neville [17]. Lanes 1 and 2 show immunoprecipitates prepared with equal amounts of antibody in each case. Lane 1 shows that from untreated solubilized brush borders. Lane 2 shows that from an equal amount of the same preparation treated with purified pancreatic elastase as described by Hauri et al. [3], then immunoprecipitated. Lanes 3 and 4 show two different samples of normal adult brush border sucrase isomaltase run on the same gel as markers for the transplant enzyme. Lane 3 shows that immunoprecipitated from Lubrol-solubilized normal adult brush border by the identical procedure used for the transplant sample shown in Lane 1. Lane 4 shows a sample of papain-solubilized and purified adult enzyme. The two smaller bands in Lanes 3 and 4 consistently appear in both types of preparation, but appear to be more enzymatically labile than the isomaltase and sucrase subunits and may be degraded by elastase treatment of solubilized brush borders. Their role is unknown, although Cezard et al. [11] also observed two small subunits in their brush border sucrase-isomaltase preparations.

rabbit antibody to rat sucrase-isomaltase was used to immunoprecipitate the enzyme from Lubrol-PX solubilized brush borders. This antibody was demonstrated to be mono-specific for normal brush border sucrase-isomaltase by immunodiffusion and immunoelectrophoresis [10]. Fig. 1 shows an example of an immunodiffusion test in which the reactions of this antibody with purified normal adult rat sucrase-isomaltase and with solubilized transplant brush border are compared. This figure demonstrates the identity of the two reactions.

When the immunoprecitated transplant sucrase-isomaltase, solubilized by heating with SDS and mercaptoethanol, was electrophoresed on an SDS-polyacrylamide slab gel as shown in Fig. 2, Lane 1, only a single high molecular weight band appeared, which co-migrated with the small amount of high molecular weight form which remains in sucrase-isomaltase from adult intestine, solubilized in the same manner (Lane 3). In fact, when normal adult sucrase-isomaltase and immunoprecipitated transplant sucrase-isomaltase were added to the same lane and electro-

phoresed, the high molecular weight forms migrated as a single band in which the two were indistinguishable (data not shown). Since the putative precursor form isolated from the Golgi fraction by Hauri et al. [3], also co-migrated with the high molecular weight form from microvillus membranes, it appears that the high molecular weight form found in the transplants is very close, if not identical, in molecular weight to the Golgi form of the precursor. As shown in Lane 2 of Fig. 2, if the solubilized transplant brush borders are incubated with purified pancreatic elastase prior to the immunoprecipitation, electrophoresis of the resulting immunoprecipitate reveals two lower molecular weight bands, and little or none of the high molecular weight form remains. These two lower molecular weight bands co-migrate with the isomaltase and sucrase subunits derived from sucrase-isomaltase purified from normal adult microvillus membrane. When the precursor immunoprecipitated from Golgi fractions was split by elastase, Hauri et al. [3] found only two subunits, one of which co-migrated with the smaller subunit from microvillus membrane, while the larger migrated slightly slower than the large subunit from microvillus membrane. As shown in Fig. 2, Lanes 2, 3 and 4, we have found that when the larger subunit (isomaltase) from elastase-treated transplant brush borders (Lane 2) is compared to that from papain-purified and detergent-solubilized normal adult sucrase-isomaltase, it co-migrates with the papain-purified subunit (Lane 4) and migrates somewhat faster than the subunit from Lubrol-solubilized sucrase-isomaltase (Lane 3). The smaller subunit (sucrase) co-migrates in all three samples. In rabbit intestine, papain acts by cleaving the anchor sequence which is part of the isomaltase subunit and releasing the remainder of the enzyme into solution [1]. In the present study, removal of the anchor sequence could account for the difference in migration rate between the papain-solubilized and the detergent-solubilized sucrase-isomaltase (which is not exposed to enzymatic cleavage during preparation). Since the elastase-treated subunit co-migrates with the papain-treated one, and since elastase also releases sucrase-isomaltase from the normal brush border [2], elastase treatment probably also cleaves off the anchor sequences from the transplant isomaltase. Thus, the form of sucrase-isomaltase found in the transplants in the absence of pancreatic proteases matches the characteristics described for the precursor form found in the Golgi fraction isolated from normal cells, confirming that this precursor is transported intact to the cell surface. These data support the hypothesis that luminal pancreatic proteases normally present in the intestinal lumen play a key role in the conversion of sucrase-isomaltase to the form found on the microvillus membrane.

The second question we examined was whether or not this high molecular weight form of sucrase-isomaltase was enzymatically active. Enzyme activity of the Golgi fraction precursor form was not evaluated in the report of its discovery [3]. However, active sucrase is present in homogenates of transplanted fetal intestine, both in our transplants and in those grown by others in the kidney capsule [10,11]. This sucrase has been found by us to have a consistently lower specific activity than that of normal adult rats, even in the brush borders from our mature transplants (0.51 vs. 1.46 μmol glucose/min/mg protein), as did the other disaccharidases. The lowered activity could be either the result of incomplete activation of the enzyme since it is still in an immature form, or it could simply be due to decreased amounts of fully active enzyme protein relative to non-enzyme protein. In order to resolve this question, we compared enzyme kinetics of normal adult and transplant sucrase-isomaltase.

To evaluate enzyme activity in the single polypeptide transplant form, we determined the kinetic parameters of both sucrose and isomaltose hydrolysis in transplant brush border preparations. Utilizing the Lineweaver-Burk double-reciprocal plot technique, we calculated K_m values from measurements of initial reaction rates at various substrate concentrations for both enzymes. The results are displayed in Table I

TABLE I

ENZYME KINETICS OF SUCRASE AND ISOMALTASE IN TRANSPLANT BRUSH BORDER PREPARATIONS

	K_m (mM)		
	Transplant	Control	Literature [18]
Sucrase	19.7 ± 0.5 (n = 21)	20.2 ± 1.0 (n = 8)	20.0
Isomaltase	6.6 ± 0.2 (n = 14)	–	6.1

and demonstrate that the K_m values for both the transplant enzyme activities are indistinguishable from control values, indicating full enzyme activity for both sucrase and isomaltase in the single polypeptide form.

These findings provide additional evidence in support of the hypothesis that sucrase-isomaltase is initially synthesized as a single polypeptide chain which is later cleaved to the microvillus membrane form [8], although one report [13] suggests that the enzyme subunits in the cytosol are assembled after independent synthesis to form the dimer found on the microvillus membrane. Results in agreement with ours, in which a single high molecular weight form of sucrase-isomaltase was found in pancreatic duct ligated pigs, have recently been reported [14], although these authors found that enzymatic digestion of their high molecular weight form could not produce the normal pattern of sucrase-isomaltase on electrophoresis. Our data indicate that luminal factors, probably pancreatic proteases, convert sucrase-isomaltase to the form normally found on the epithelial cell membrane, but that the high molecular weight, single polypeptide form is already a fully active enzyme before this conversion. Thus, conversion to the dimeric form appears to have no role in activation of sucrase-isomaltase.

This work was supported by U.S. Public Health Service Grants Am-14523 and HD-14498.

References

1 Brunner, J., Hauser, H., Braun, H., Wilson, K.J., Wacker, H., O'Neill, B. and Semenza, G. (1979) J. Biol. Chem. 254, 1 821–1 828

2 Alpers, D.H. and Tedesco, F.J. (1975) Biochim. Biophys. Acta 401, 28–40

3 Hauri, H., Quaroni, A. and Isselbacher, K.J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5 183–5 186

4 Leapman, S.B., Deutsch, A.A., Grand, R.J. and Folkman, J. (1974) Ann. Surg. 179, 109–114

5 Montgomery, R.K., Sybicki, M. and Grand, R.J. (1981) Dev. Biol., in the press

6 Moismann, H., Semenza, G. and Sund, H. (1973) Eur. J. Biochem. 36, 489–494

7 Conklin, K.A., Yamashiro, K.M. and Gray, G.M. (1975) J. Biol. Chem. 250, 5 735–5 741

8 Semenza, G. (1979) Ciba Found. Symp. 70, 133–145

9 Montgomery, R.K., Sybicki, M.A., Forcier, A.G. and Grand, R.I. (1981) Gastroenterology 78, 1 225

10 Williams, C.A. and Chase, M.W. (1971) in Methods in Immunology and Immunochemistry, Vol. 3, pp. 146–160, 234–273, Academic, New York

11 Ferguson, A., Gerskowitch, V.P. and Russell, R.I. (1973) Gastroenterology 64, 292–297

12 Kendall, K. Jumawan, J. and Koldovsky, O. (1979) Biol. Neonate 36, 206–214

13 Cezard, J., Conklin, K.A., Das, B.C. and Gray, G.M. (1979) J. Biol. Chem. 254, 8 969–8 975

14 Sjostrom, H., Noren, O., Christiansen, L., Wacker, H. and Semenza, G. (1980) J. Biol. Chem. 255, 11 332–11 338

15 Cogoli, A., Mosimann, H., Vock, C., Von Balthazar, A. and Semenza, G. (1972) Eur. J. Biochem. 30, 7–14

16 Kessler, M., Acuto, O., Storilli, C., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154

17 Neville, D.M. (1971) J. Biol. Chem. 246, 6 328–6 334

18 Messer, M. and Dahlqvist, A. (1966) Anal. Biochem. 14, 376–392