SOME CHARACTERISTICS OF EARLY APPEARING ISOMALTASE IN INTESTINAL MUCOSA OF SUCKLING RAT

Kazuhiko YAMADA, Sachiko MORIUCHI, and Norimasa HOSOYA

Department of Nutrition, School of Health Sciences, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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Summary Characteristics of early appearing free isomaltase in the soluble fraction were investigated in rat intestinal mucosa. Soluble isomaltase and membrane-bound sucrase-isomaltase complex were prepared from 15-day-old rat intestine. Immunochemical properties, optimal pH and heat sensitivity of soluble isomaltase were compared with those of membrane-bound isomaltase.

Optimal pH of free isomaltase in the soluble fraction was lower than that of membrane-bound isomaltase. Soluble and membrane-bound isomaltase showed different sensitivities for temperature. Furthermore, membrane-bound isomaltase in 15-day-old suckling rat intestine gave a single line with antiserum. However, soluble isomaltase gave no precipitin line. From these results, it could be concluded that soluble isomaltase is not derived from the isomaltase moiety of membrane-bound sucrase-isomaltase complex as a result of mechanical fragility and rather it would be lysosomal in origin.

Keywords soluble isomaltase, sucrase-isomaltase complex, suckling rat, intestinal mucosa, immunochemical properties

It has been well established that isomaltase exists as a complex with sucrase in the intestinal microvillous membrane (1-3). Recently, we reported that a significant amount of free isomaltase was detected in the soluble fraction of suckling rat intestine, in addition to isomaltase which was integral to the microvillous membrane with sucrase as a sucrase-isomaltase complex (4). However, it was not clear whether the early appearing free isomaltase was derived from the isomaltase moiety of the membrane-bound sucrase-isomaltase complex.

Therefore, in order to gain further understanding of the early appearing soluble isomaltase, the immunochemical properties and some characteristics of the...
soluble isomaltase were compared with those of membrane-bound isomaltase using 15-day-old rat intestine.

METHODS

1. Animals. Intestinal mucosa was obtained from 15-day-old rats of Wistar strain. The suckling rats remained with their mothers until the experiment. Water and laboratory chow (Oriental Yeast Co.) were given ad libitum. Rats were killed by decapitation between 1,200 and 1,500 hr.

2. Separation of soluble and membrane-bound disaccharidases. Intestinal mucosa obtained from 15-day-old rats was homogenized with 4 volumes of 10 mM potassium phosphate buffer (pH 7.0). To separate soluble enzymes from insoluble membrane-bound enzymes, the homogenate was centrifuged at 105,000 × g for 1 hr. The supernatant was lyophilized, resuspended in water and dialyzed against water for 2 days (soluble disaccharidases).

For the isolation of membrane-bound enzymes, preparation of the brush border membrane was carried out according to the method of Kessler et al. (5). The 105,000 × g precipitate was suspended in 30 volumes of ice-cold 50 mM mannitol in 2 mM Tris-HCl buffer (pH 7.1), and homogenized in a Waring blender at maximum speed for 2 min. Solid calcium chloride was added to the homogenate to give a final concentration of 10 mM. After standing in the cold for 20 min, the suspension was centrifuged at 3,000 × g for 15 min. The supernatant was centrifuged at 27,000 × g for 30 min. The pellet was then resuspended in 10 ml of 50 mM mannitol in 10 mM Tris-HCl buffer (pH 7.1) and centrifuged once more at 27,000 × g for 30 min. This pellet was resuspended in 2 ml of 10 mM potassium phosphate buffer (pH 7.0) (brush border membrane suspension). The brush border membrane suspension (ca. 14 mg protein/ml) in 10 mM potassium phosphate buffer (pH 7.0) was incubated with 0.3 mg papain and 0.75 mg cysteine-HCl for 90 min at 37°C. After papain treatment, the incubation mixture was centrifuged at 105,000 × g for 1 hr. The supernatant was used as membrane-bound disaccharidases.

3. Sephadex G-200 column chromatography. Disaccharidases (1.5 ml) were applied to a Sephadex G-200 column (1.5 × 30 cm) which was equilibrated with 10 mM potassium phosphate buffer (pH 7.0) and eluted with the same buffer in the cold room. The flow rate was 4.5 ml/hr. Three-milliliter fractions were collected and aliquots were used for the enzyme assay.

4. Preparation of antiserum against sucrase-isomaltase complex. Sucrase-isomaltase complex of rat intestine was purified by the method of Kolinska and Kraml (3) with some modification (6). Briefly, the 105,000 × g supernatant after papain treatment was precipitated with ammonium sulfate between 45% and 65% saturation. The precipitate was dissolved in 10 mM potassium phosphate buffer (pH 7.0) and was applied to Sephadex G-200 column chromatography. The fractions
containing sucrase-isomaltase complex were further purified by DEAE-Sephadex A-25 column chromatography.

The purified sucrase-isomaltase complex (0.8 mg) was mixed with Freund's complete adjuvant in a 1:1 volume ratio and injected subcutaneously into a rabbit to raise antiserum. The injection (0.15 mg protein) was repeated 3 weeks later; 1 week thereafter the rabbit was bled from an ear vein. Serum supplemented with 0.1% sodium azide was stored at -20°C until use.

5. Quantitative precipitation of isolated sucrase by antibody against sucrase-isomaltase complex. Sucrase-isomaltase complex was dissociated into its subunits by citraconylation according to the method of Braun et al. (7). The fractions containing high sucrase activity without isomaltase activity on Sephadex G-200 column chromatography were pooled, concentrated and deacylated to yield an isolated sucrase.

Antiserum against sucrase-isomaltase complex (0.5 mg protein) was incubated with various amounts of isolated sucrase in 0.15 ml of sodium phosphate buffered saline (pH 7.4) at 37°C for 60 min and then 4°C for 20 hr. After incubation, the mixture was centrifuged at 1,500 × g for 30 min. Aliquots of the mixture and supernatant were used for enzyme assay.

6. Double immunodiffusion. Double immunodiffusion in agar gel was carried out as described by Ouchterlony (8). The thickness of the gel was uniformly set at 2 mm on the immunoplate by pouring the warm 1.2% fluid agar in barbital buffer (pH 8.6) containing 0.9% sodium chloride, 0.1% sodium azide and 0.1% Tween 80. After solidification, wells of 2-mm diameter were punched. The wells were filled with antibody against sucrase-isomaltase complex, antibody against sucrase-isomaltase complex without anti-sucrase activity or sample antigen.

7. Heat inactivation. Heat inactivation was studied by the method of Dahlqvist (9). Enzyme solution in 10 mM potassium phosphate buffer (pH 7.0) was inactivated at 45, 50, 60 and 75°C in a thin-walled glass tube. The tube was shaken continuously and the desired temperature was reached within 1 min. Samples for enzyme assay were taken at intervals of 10 min.

8. Assay procedure. Disaccharidase activities were determined by the Tris glucose oxidase of Dahlqvist (10). Sucrose and maltose were used in a final concentration of 28 mM, and isomaltose was 2.8 mM. All sugars were dissolved in 0.1 M sodium maleate buffer (pH 6.0). Protein concentration was determined by the method of Lowry et al. using bovine serum albumin as a standard (11).

9. Chemical. Papain and glucose oxidase were obtained from Worthington Biochemicals Co. Tris was obtained from Sigma Chemical Co. Citraconic anhydride, sucrose and maltose were from Wako Pure Chemical Industries Ltd. Isomaltose was kindly provided by Hayashibara Co., Ltd.
RESULTS

1. Sephadex G-200 column chromatography of soluble and membrane-bound disaccharidases

In our previous study (4), free isomaltase, which had not formed a complex with sucrase, was demonstrated in the intestinal mucosa of suckling rat. In order to determine if the free isomaltase is bound or not to brush border membranes, the intestinal disaccharidases of 15-day-old rats were fractionated to soluble and brush border membrane-bound ones.

The soluble fraction contained 30% of the total intestinal maltase activity and 13% of the isomaltase activity. However, no sucrase activity was found in the soluble fraction. Furthermore, chromatographic behavior on a Sephadex G-200 column of soluble fraction revealed that isomaltase did not exist as a complex with sucrase. Namely, most isomaltase activity was detected with maltase activity at

![Graph showing elution patterns on Sephadex G-200 column chromatography of soluble and membrane-bound disaccharidases in 15-day-old rat intestine. Membrane-bound disaccharidases were solubilized by papain. Sample solutions (1.5 ml) were applied to Sephadex G-200 column (1.5 x 30 cm) equilibrated with 10 mM potassium phosphate buffer and eluted by the same buffer. Three-ml fractions were collected and aliquots were used for the enzyme assay. a, Soluble disaccharidases; b, Membrane-bound disaccharidases.](image-url)
void column of the column and was not retained on the column (Fig. 1).

On the other hand, chromatographic profile on Sephadex G-200 column of the membrane fraction showed that isomaltase activity was eluted with sucrase activity in good agreement with our previous results (4).

The molecular weight of isomaltase eluted at void volume was estimated by polyacrylamide gel disc electrophoresis (12) to be about 100,000 daltons and that of membrane-bound isomaltase, which was in complex with sucrase and retained on Sephadex, to be about 200,000 (results not shown).

These results show that in the suckling period of the rat, a soluble isomaltase exists in the intestine which does not complex with sucrase.

2. Effects of pH and temperature on soluble and membrane-bound isomaltase

To characterize this free isomaltase further, the influence of pH and heat on the soluble disaccharidase activities were compared with those of membrane-bound ones. Membrane-bound sucrase and isomaltase were optimally active at pH 6.0 and had little activity at pH 4.0. Maltase possessed some activity at pH 4.0, but maximum activity occurred at pH 6.0. However, the optimal pH of soluble isomaltase and maltase were lower than membrane-bound enzymes. Furthermore, at pH 3.0 and 4.0, significant activities of both enzymes remained (Fig. 2).

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Fig. 2. pH activity curve of soluble and membrane-bound disaccharidases of 15-day-old rat intestine. The enzyme activities were expressed as a percentage of the maximum activity. Buffers used: pH 2.0–3.5, 0.05 M glycine-HCl; pH 3.5–5.5, 0.05 M sodium acetate-acetic acid; pH 5.5–7.0, 0.05 M sodium maleate.

a, Soluble disaccharidases; b, Membrane-bound disaccharidases. --●-- sucrase, --○-- isomaltase, --×-- maltase.
It is well known that intestinal disaccharidases have different temperature sensitivities (9). Therefore, heat sensitivity of membrane-bound and soluble disaccharidases was studied at 45, 50, 60 and 75°C. The inactivation of soluble isomaltase was about 50% after 50°C treatment for 60 min. Membrane-bound isomaltase activity was inactivated to 20% of total activity at 50°C, and then rapidly inactivated at 60°C. Maltase activities of the soluble and membrane-bound fraction behaved similarly to their respective isomaltases, though they were slightly heat stable. The heat stability of membrane-bound sucrase was more labile than other enzymes (Fig. 3).

3. Immunochemical studies of isomaltase against anti-sucrase-isomaltase complex

Antibody against rat intestinal sucrase-isomaltase complex was raised in rabbit (13). It is known that antibody against rabbit sucrase-isomaltase complex reacts immunochemically with the sucrase moiety as well as the isomaltase moiety (14). However, it is not clear that antibody against rat intestinal sucrase-isomaltase complex also has antigenicity for each moiety. Therefore, prior to examination of the antigenicity of soluble isomaltase against antiserum to the sucrase-isomaltase complex, it was observed that the antiserum to rat sucrase-isomaltase complex could react immunochemically with the sucrase and isomaltase moieties. For this purpose, sucrase-isomaltase complex was dissociated into subunits by citraconylation and the isolated sucrase was used to examine the
antigenicity against antiserum to sucrase-isomaltase complex in the range of antibody excess. As shown in Fig. 4, sucrase activity in the supernatants was abolished after incubation with anti-sucrase-isomaltase complex in the range of less than 5 μg of isolated sucrase. This suggests that the antibody reacts immunochemi-

![Fig. 4](image-url)

Fig. 4. Quantitative precipitation of isolated sucrase by anti-sucrase-isomaltase serum. Antiserum against sucrase-isomaltase complex (0.5 mg) was incubated with various amounts of isolated sucrase in 0.15 ml of phosphate buffered saline (pH 7.4) at 37°C for 60 min and then 4°C for 20 hr.

—Δ— total sucrase activity, —▲— sucrase activity in supernatant.

cally with the sucrase moiety. Therefore, using the antiserum, from which anti-sucrase activity was absorbed by a little excess of the isolated sucrase, the antigenicity of isomaltase against antiserum to the sucrase-isomaltase complex was examined. As shown in Fig. 5, the isolated sucrase did not give a precipitin line with the antibody without anti-sucrase activity, but sucrase-isomaltase complex did give a precipitin line. These results indicate that the antiserum against rat sucrase-isomaltase complex has antigenicity for the sucrase moiety as well as the isomaltase moiety.

Antigenicity of soluble isomaltase against antiserum to the sucrase-isomaltase complex was examined by Ouchterlony gel double diffusion technique. Soluble isomaltase was concentrated to give an activity per volume equal to the activity of the membrane-bound isomaltase preparation. Membrane-bound isomaltase in 15-day-old suckling and adult rat intestine gave a single line with antiserum (Fig. 6). However, soluble isomaltase gave no precipitin line.
Fig. 5. Micro-Ouchterlony double diffusion of sucrase-isomaltase complex and isolated sucrase against anti-sucrase-isomaltase antiserum without anti-sucrase activity. A, anti-sucrase-isomaltase antiserum without anti-sucrase activity; SI, sucrase-isomaltase complex; S, isolated sucrase.

Fig. 6. Micro-Ouchterlony double diffusion of membrane-bound sucrase-isomaltase complex and soluble isomaltase against antiserum specific for rat intestinal sucrase-isomaltase complex. A, antiserum; a, membrane-bound S-I complex solubilized by papain (adult); b, membrane-bound S-I complex solubilized by papain (15-day); c, purified S-I complex; d, concentrated soluble isomaltase (15-day); e, unconcentrated soluble isomaltase (15-day).

DISCUSSION

The activity of small intestinal brush border enzymes undergoes important
Changes during postnatal development. Previous studies in our laboratory have shown that isomaltase activity, which did not form a complex with sucrase, was detectable in the small intestine of a 15-day-old rat, though not in adult rats (4).

In the present paper, we report the differences between free isomaltase and sucrase-isomaltase complex in the suckling rat small intestine. The isomaltase activity found in the soluble fraction of intestinal homogenate was not present in the brush border membrane. It eluted at void volume on Sephadex G-200 column chromatography with an apparent molecular weight of 110,000. This isomaltase did not react with antibody raised against the brush border sucrase-isomaltase complex. These findings suggest that the soluble isomaltase in suckling rat small intestine differs from the isomaltase moiety of membrane-bound sucrase-isomaltase complex.

Recently, it was reported that, in the intestine of suckling rats, hydrolytic enzymes such as disaccharidase and alkaline phosphatase have been located in the soluble fraction (15). GALAND and FORSTNER demonstrated that soluble maltase represented a mixture of brush border maltase (optimal pH 6.0) and lysosomal maltase (optimal pH 3.0) (16).

Our study concerning the influence of pH on isomaltase activity suggests that soluble isomaltase would be lysosomal because of its activity at a lower pH than the sucrase-isomaltase complex. Furthermore, heat sensitivity studies of soluble isomaltase activity showed that it was slightly more labile than the membrane-bound isomaltase activity. Sucrase activity was inactivated rapidly at 50°C. However, it is interesting that in chick intestine, sucrase activity was more heat stable than maltase activity (17).

Our immunochemical studies excluded the possibility that soluble isomaltase was a contamination from membrane-bound sucrase-isomaltase complex as a result of mechanical fragility. Although the activity of soluble isomaltase is very small, we speculate that it may play a role in the digestion of macromolecules in the intestinal epithelial cells of the suckling rat.

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REFERENCES

1) KOLINSKA, J., and SEMENZA, G. (1967): Studies on intestinal sucrase and on intestinal sugar transport. V. Isolation and properties of sucrase-isomaltase from rabbit small intestine. Biochim. Biophys. Acta, 146, 181–195.

2) CONKLIN, K.A., YAMASHIRO, K.M., and GRAY, G.M. (1975): Human intestinal sucrase-isomaltase. Identification of free sucrase and isomaltase and cleavage of the hybrid into active distinct subunits. J. Biol. Chem., 250, 5735–5741.

3) KOLINSKA, J., and KRAML, J. (1972): Separation and characterization of sucrase-isomaltase and of glucoamylase of rat intestine. Biochim. Biophys. Acta, 284, 235–247.
4) Yamada, K., Moriuchi, S., and Hosoya, N. (1978): Developmental changes in the sucrase-isomaltase complex in rat intestinal mucosa. *J. Nutr. Sci. Vitaminol.*, 24, 177–184.

5) Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M., and Semenza, G. (1978): A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membrane. Their use to investigate some properties of d-glucose and choline transport system. *Biochim. Biophys. Acta*, 506, 136–154.

6) Takesue, T. (1969): Purification and properties of rabbit intestinal sucrase. *J. Biochem.*, 65, 545–552.

7) Braun, H., Cogoli, A., and Semenza, G. (1975): Dissociation of small-intestinal sucrase-isomaltase complex into enzymatically active subunits. *Eur. J. Biochem.*, 52, 475–480.

8) Ouchterlony, Ø. (1962): Diffusion-in-gel methods for immunological analysis. II., in *Progr. Allergy VI*, ed. by Kallos, P., and Watsman, D.H., Karger, Basel, pp. 30–154.

9) Dahlqvist, A. (1962): Specificity of the human intestinal disaccharidases and implications for hereditary disaccharide intolerance. *J. Clin. Invest.*, 41, 463–470.

10) Dahlqvist, A. (1964): Method for assay of intestinal disaccharidase. *Anal. Biochem.*, 7, 18–25.

11) Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.

12) Hedric, J.L., and Smith, A.L. (1968): Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. *Arch. Biochem. Biophys.*, 126, 155–164.

13) Sasaki, M., Yamada, K., Moriuchi, S., and Hosoya, N. (1979): Purification and characterization of rat intestinal sucrase-isomaltase complex. *Food and Nutrition*, 32, 201–208.

14) Takesue, Y., Tamura, R., and Nishi, Y. (1977): Immunochemical studies on the subunits of rabbit-intestinal sucrase-isomaltase complex. *Biochim. Biophys. Acta*, 483, 375–385.

15) Seethram, B., Yeh, K.Y., Moog, F., and Alpers, D.H. (1977): Development of intestinal brush border membrane proteins in the rat. *Biochim. Biophys. Acta*, 470, 424–436.

16) Galand, G., and Forstner, G.G. (1974): Soluble neutral and acid maltases in the suckling-rat intestine. The effect of cortisol and development. *Biochem. J.*, 144, 281–292.

17) Siddons, R.C. (1970): Heat inactivation and Sephadex chromatography of the small-intestine disaccharidases of the chick. *Biochem. J.*, 116, 71–78.