Demonstration of Sucrase-Isomaltase Complex in Chick Intestine

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Summary It was investigated whether sucrase and isomaltase form an enzyme-enzyme complex in chick intestine or not, and some properties of the disaccharidases were compared with those of other species.

1) Chick intestinal sucrase and isomaltase were shown to exist in the form of an enzyme-enzyme complex from the results of polyacrylamide disc gel electrophoresis, DEAE Sephadex A-25 ion exchange column chromatography and citraconylation, although the chick intestinal sucrase and isomaltase were not retained on a Sephadex G-200 column. 

2) The molecular weights of sucrase-isomaltase complex, maltase I, maltase II, maltase III and isomaltase dissociated from sucrase-isomaltase complex were estimated to be 250,000, 250,000, 160,000, 225,000 and 80,000, respectively, by polyacrylamide disc gel electrophoresis. 

3) The optimum pH for all the enzymes was 6.0.

4) \(K_m\) values of sucrase, isomaltase, maltase I and maltase III were 10.0, 3.5, 1.0 and 4.6 mM, respectively. \(V_{max}\) values for sucrase, isomaltase, maltase I and maltase III were 217.4, 281.4, 147.1 and 454.5 µmol substrate hydrolyzed/mg protein/hr, respectively.

Key Words sucrase-isomaltase complex, chick intestine, citraconylation

It is well known that various membrane digestive enzymes including disaccharidases exist in the microvilli of small intestinal mucosa and are involved in the terminal digestion and absorption of nutrients (1, 2). Among these enzymes, sucrase [EC 3.2.1.48] and isomaltase [EC 3.2.1.10] have been isolated as an enzyme-enzyme complex from rabbits (3), humans (4) and rats (5), and these have been characterized. However, it is not well established that chick intestinal sucrase and isomaltase also exist as an enzyme-enzyme complex in the microvilli. Therefore, the present study was conducted to investigate whether sucrase and isomaltase form an enzyme-

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enzyme complex in chick intestine or not.

MATERIALS AND METHODS

Homogenate of intestinal mucosa. Male 4-week-old White Leghorn chicks were killed by decapitation between 10:00 and 12:00 hr. The jejunum and the ileum were immediately taken out, slit open and rinsed with ice cold saline. The mucosa was scraped off and was homogenated with 4 volumes of 10 mM sodium potassium phosphate buffer (pH 7.0) to give a 20% homogenate. The homogenate was centrifuged at 1,000 × g for 5 min at 4°C. The resulting supernatant was stored at −20°C until use.

Solubilization of disaccharidases by papain treatment. Ten ml of the supernatant were incubated with 2.4 mg of papain and 7.5 mg of cysteine-HCl at 37°C for 60 min (6). After the incubation, the reaction mixture was dialyzed against 5 liters of 10 mM sodium potassium phosphate buffer (pH 7.0) for 18 hr at 4°C. The dialysate was centrifuged at 100,000 × g for 90 min at 4°C. The supernatant was used as “papain solubilized disaccharidases.”

Sephadex G-200 column chromatography. Papain solubilized disaccharidases were applied onto a Sephadex G-200 column (2.5 × 67 cm) which was equilibrated with 10 mM sodium phosphate buffer (pH 7.0) and the enzymes adsorbed were eluted with the same buffer in the cold room (6). Flow rate was 7.7 ml/hr. Three ml fractions were collected and their aliquots were used for the enzyme assay.

DEAE Sephadex A-25 ion exchange column chromatography. The fraction having sucrase and isomaltase activities from Sephadex G-200 column was applied onto a DEAE Sephadex A-25 column (1.5 × 20 cm) which was equilibrated with 10 mM sodium potassium phosphate buffer (pH 7.0). The elution was carried out using sodium potassium phosphate buffer (pH 7.0) with a linear gradient of 10 to 500 mM in the cold room.

Polyacrylamide disc gel electrophoresis. Polyacrylamide disc gel electrophoresis of papain solubilized disaccharidases was performed according to the method of Davis (7.5% in acrylamide, pH 9.4) (7). After the electrophoresis, the gels were either stained with 1% amido black in 7% acetic acid for 60 min and then destained in 7% acetic acid or were frozen immediately and sliced into 2 mm pieces, which were soaked in 0.35 ml redistilled water and used for enzyme assay. Molecular weight analysis of disaccharidases on polyacrylamide disc gel electrophoresis was carried out according to the method of Hedrick and Smith (8). For the molecular weight standardization of gel, ovalbumin, albumin, γ-globulin and apoferritin were used.

Separation of sucrase and isomaltase by citraconylation. Sucrase and isomaltase fraction from DEAE Sephadex A-25 ion exchange column (0.99 mg protein/ml) was treated with 10% citraconic anhydride in acetone (290 μl) according to the method of Braun et al. (9). After citraconylation, the enzymes were chromatographed on a Sephadex G-200 column (2.5 × 67 cm) with 10 mM sodium potassium phosphate buffer.
phosphate buffer (pH 7.0) in the cold room. The flow rate was 7.7 ml/hr.

*Assay procedure.* Disaccharidase activities were determined by the method of Dahlqvist (10). Substrate concentration was 28 mM in sucrase and maltase and 2.8 mM isomaltase. Enzyme activities were expressed as μmol substrate hydrolyzed per ml-elute per hr or per mg protein per hr. Protein concentration was determined by the method of Lowry et al. using bovine serum albumin as standard (11).

*Chemicals.* Papain and glucose oxidase were obtained from Worthington Biochemical Co. Acrylamide was purchased from Tokyo Kasei, Ltd. Standard marker proteins used for the determination of molecular weight were the products of Schwarz/Mann. Glucose and sucrose were obtained from Kokusan Chemical Works, Ltd. Tris(hydroxymethyl)aminomethane was from Sigma Chemical Co. Citraconic anhydride was obtained from Wako Pure Chemical Industries, Ltd. Isomaltose and maltose were kindly provided by Hayashibara Co., Ltd.

**RESULTS**

*Demonstration of sucrase and isomaltase complex in chick intestinal mucosa*

It is known that the sucrase-isomaltase complex of rabbits (3), humans (4) and rats (5) is retained by Sephadex G-200 owing to a substrate-enzyme interaction

![Graph showing the chromatography of papain solubilized disaccharidases from chick intestinal mucosa.](image)

*Fig. 1.* Sephadex G-200 column chromatography of papain solubilized disaccharidases from chick intestinal mucosa. Eight ml of papain solubilized disaccharidases specimen prepared from 4-week-old chick intestine (7.3 mg of protein) were applied to a Sephadex G-200 column (2.5 × 67 cm) equilibrated with 10 mM sodium potassium phosphate buffer (pH 7.0) and eluted by the same buffer. Three ml fractions were collected and aliquots were used for the enzyme assay.
mainly via its isomaltase site. Therefore, in order to know whether the chick intestinal sucrase and isomaltase exist as a complex, papain solubilized disaccharidases from chick intestinal mucosa were subjected to a Sephadex G-200 column chromatography.

As shown in Fig. 1, the activities of maltase were separated into 3 fractions, named maltases I, II and III according to the elution order. Maltase I was eluted at the void volume of the column, maltase III was retained by Sephadex G-200 and eluted at the total volume of the column and a small amount of maltase activity was eluted as maltase II between maltases I and III. However, sucrase and isomaltase activities were eluted at the void volume of the column together with maltase I and their chromatographic behaviors on the Sephadex G-200 column differed from those of the enzymes of the other animals.

Polyacrylamide disc gel electrophoretic pattern of papain solubilized disaccharidases from chick intestinal mucosa showed 10 different protein bands. Sucrese

Fig. 2. Disaccharidase activities after electrophoresis of 4-week-old chick intestinal papain solubilized specimen on 7.5% polyacrylamide gel at pH 9.4. The ordinate shows the enzymatic activities determined on 2 mm slices of gel and expressed as absorbance at 500 nm. The abscissa shows the distance along the gel.

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Fig. 3. DEAE Sephadex A-25 ion exchange column chromatography of peak I fraction eluted from Sephadex G-200 column. Peak I fraction containing about 40 µg of protein from Sephadex G-200 column chromatography was applied to DEAE Sephadex A-25 column (1.5 × 15 cm) equilibrated with 10 mM sodium potassium phosphate buffer (pH 7.0) and eluted by changing the concentration of sodium potassium phosphate buffer from 10 to 500 mM. Four ml fractions were collected and their aliquots were used for the enzyme assay.

and isomaltase activities were comigrated and detected at band 1 and maltase activities were detected at bands 1 and 3 (Fig. 2). Furthermore, DEAE Sephadex A-25 ion exchange chromatography of sucrase-isomaltase fraction of Sephadex G-200 column chromatography showed that sucrase and isomaltase were eluted together at 120 mM sodium potassium phosphate buffer (pH 7.0) (Fig. 3).

Therefore, it could be assumed that sucrase and isomaltase formed a complex, though the behaviors of those enzymes on Sephadex G-200 column chromatography differed from those of the enzymes of the other animals as described above.

In order to confirm the presence of sucrase-isomaltase complex in chick intestinal mucosa, sucrase-isomaltase fraction of DEAE Sephadex A-25 ion exchange column chromatography was subjected to citraconylation. Before citraconylation, the sucrase-isomaltase fraction gave a single peak having sucrase, isomaltase and maltase activities (see Fig. 3), however, after citraconylation, sucrase-isomaltase fraction gave 2 peaks. The first peak had sucrase, isomaltase and maltase activities (Fig. 4). The activity ratio of sucrase to isomaltase in the first peak after citraconylation was 1.85 and higher than that before citraconylation. This suggested that sucrase dissociated from isomaltase would be eluted in the first peak together with the native sucrase-isomaltase complex. The second peak had an isomaltase activity. This result indicates a part of isomaltase dissociated from sucrase by citraconylation. These results clearly demonstrated the presence of sucrase-
Fig. 4. Preferential dissociation of isomaltase from sucrase and isomaltase fraction obtained by DEAE Sephadex A-25 ion exchange column chromatography. Sucrase and isomaltase fraction obtained by DEAE Sephadex A-25 ion exchange column chromatography was citraconylated as described under MATERIALS AND METHODS. About 750 μg of protein was applied onto the column.

isomaltase complex in chick intestinal mucosa.

Some properties of chick intestinal disaccharidases

The molecular weight of sucrase-isomaltase complex, maltase I, maltase II, maltase III and isomaltase dissociated from sucrase-isomaltase complex was 250,000, 250,000, 160,000 225,000 and 80,000, respectively (Fig. 5). The optimum pH for all the enzymes was 6.0. However, the isomaltase activity was decreased rapidly when pH exceeded 6.0, compared with sucrase and maltase (Fig. 6). Michaelis constants (Km) determined for sucrase and isomaltase were 10 and 3.5 mM, respectively. Km value for maltase I was slightly lower than that for maltase III. Vmax values for sucrase and isomaltase were 217.4 and 281.4 μmol substrate hydrolyzed/mg protein/hr, respectively and those for maltase I and maltase III were 147.1 and 454.5 μmol substrate hydrolyzed/mg protein/hr, respectively (Table 1).

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Fig. 5. The slope-molecular weight relation of chick intestinal disaccharidases and standard proteins. The enzymes fractionated by the Sephadex G-200 column were used. P I, P II and P III represent peak I, peak II and peak III in Fig. 1, respectively. The molecular weight of sucrase-isomaltase complex, maltase I, maltase II, maltase III and isomaltase dissociated from sucrase-isomaltase complex was estimated according to the method of Hedrik et al. a, ovalbumin (M.W. 45,000); b, albumin monomer (M.W. 67,000); c, γ-globulin (M.W. 160,000); d, apoferritin (M.W. 480,000).

Fig. 6. pH activity curve of chick intestinal disaccharidases. Peak I obtained by Sephadex G-200 column chromatography was used. Buffers: 0.05 M glycine-HCl, pH 2.0–3.5; 0.05 M Na-acetate, pH 3.5–5.5; 0.05 M Na-maleate, pH 5.0–7.0; 0.025 M HCl-diethylbarbituric acid, pH 7.0–9.0. ●, sucrase; ○, isomaltase; △, maltase.

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Table 1. Michaelis constant ($K_m$) and maximum velocity ($V_{max}$) of chick intestinal disaccharidases.

The enzymes fractionated by the Sephadex G-200 column were used. P I and P III represent peak I and III in Fig. 1, respectively. The $K_m$ and $V_{max}$ values were calculated by the graphical method of Lineweaver and Burk. Substrate concentration was varied from 1.75 to 28 mM.

| Maltase | Sucrase | Isomaltase |
|---------|---------|------------|
| P I     | P III   | P I        | P I        |
| $K_m$ a | 1.0     | 4.6        | 10.0       | 3.5         |
| $V_{max}$ b | 147.1 | 454.5      | 217.4      | 281.4       |

$^a K_m$: mM. $^b V_{max}$: μmol substrate hydrolyzed/mg protein/hr.

DISCUSSION

In the present study, it was clearly shown that chick intestinal sucrase and isomaltase existed as an enzyme-enzyme complex in rabbits (3), humans (4) and rats (5), although the behaviors on Sephadex G-200 column chromatography and some properties of chick intestinal disaccharidases differed from those of other animals.

Semenza et al. (3, 9, 14) showed the properties of sucrase-isomaltase complex as follows: a sucrase-isomaltase complex is retained by Sephadex G-200 owing to a substrate-enzyme like interaction via its isomaltase site. The purified sucrase-isomaltase complex had different catalytic sites for sucrase and isomaltase activities and sucrose and isomaltose were hydrolyzed at different sites; however, electrophoresis or ultracentrifugation did not dissociate the complex into sucrase and isomaltase; as sucrase and isomaltase formed an enzyme-enzyme complex, if the deficiency of either sucrase or isomaltase was found, always both enzymes were deficient; moreover, they showed that sucrase-isomaltase complex was dissociated by citraconylation into sucrase and isomaltase without cleaving the covalent bond.

The fact that chick intestinal sucrase and isomaltase activities were not retained on Sephadex G-200 column is in good agreement with the results of Siddons (6). However, polyacrylamide disc gel electrophoresis of chick intestinal papain solubilized disaccharidases did not dissociate sucrase and isomaltase and they comigrated on the gels. DEAE Sephadex A-25 ion exchange column chromatography of sucrase and isomaltase fraction from Sephadex G-200 column of papain solubilized disaccharidases did not separate sucrase and isomaltase activities. Moreover, citraconylation product of sucrase and isomaltase fraction from DEAE Sephadex A-25 ion exchange column showed that isomaltase was separated from sucrase. Therefore, it will be concluded that chick intestinal sucrase and isomaltase exist in the form of an enzyme-enzyme complex which has also maltase activity as shown in other animals (3–5).

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The molecular weight of sucrase-isomaltase complex was estimated as 250,000 daltons. It has been reported that the molecular weight of sucrase-isomaltase complex was estimated as about 220,000 for rabbit (12, 13), 280,000 for human (4) and about 215,000 for rat (5). Thus, the size of the complex of chick is similar to that of rabbit, human and rat. The optimum pH for sucrase, isomaltase and maltase in the chick was 6.0 as reported for other animals (5, 13, 14).

The \( K_m \) value of chick isomaltase (3.5 mM) was similar to those of rabbit (5 mM) (14) and rat (4.5 mM) (5). Therefore, the reason why chick isomaltase was not retained by the Sephadex G-200 column remains as an interesting problem to be solved.

On the other hand, it is very interesting to note that significant amounts of maltase activities (maltase III), which does not have isomaltase activity, have an affinity to Sephadex. Properties and physiological significance of maltase III will be subjected to future studies.

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