EFFECT OF VARIOUS SUGARS ON THE INDUCTION OF
CHICK EMBRYONIC INTESTINAL DISACCHARIDASES
IN THE ORGAN CULTURE SYSTEM

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Summary Disaccharidases activities in 20-day-old chick embryonic intestine were induced by the addition of sucrose, maltose, fructose and glucose to the culture medium. However, maltitol, which cannot be digested by intestinal enzymes, showed no effect on the induction of disaccharidase activity.

Kinetic study of the enzymes demonstrated that the maximum velocity ($V_{\text{max}}$) and the Michaelis constant ($K_m$) of sucrose induced disaccharidases activities of the explants showed changes similar to those observed in the chick of same developmental stage in vivo. Namely, $V_{\text{max}}$ values of sucrase and maltase were increased. $K_m$ values of sucrase did not change, but that of maltase showed a significant decrease during development.

Organ culture of intact intestinal explants represents many interesting possibilities such as the study of immediate effects of several drugs, the role of different nutrients or the regulation of the intestinal metabolism. We have studied the induction mechanism of intestinal disaccharidases of chick embryo using the organ culture system in which the effects of possible inducers on the activities of intestinal disaccharidases, i.e., maltase [EC 3.2.1.20] and sucrase [EC 3.2.1.48], were observed (1). In a previous study (1), it was clearly shown that hydrocortisone was required for the induction of disaccharidases in 15- and 17-day-old embryos and sucrose was for 20-day-old embryos. Moreover, the results using antibiotics suggested that the site of action of two factors is different.

Experiments on intestinal disaccharidases reported here were selected in an attempt to define the effect of several sugars on the induction of disaccharidases activities of the jejunum of 20-day-old chick embryos in organ culture and to compare it with that of in vivo induction.
MATERIALS AND METHODS

1. Chick embryo. One-day-old White Leghorn fertilized eggs were obtained from the Kazusa Hatchery (Chiba, Japan), and incubated at 37.5°C with 60% relative humidity. After hatching, chicks were raised in temperature controlled cages. Jejuna were harvested at the 20th day of incubation and at 2-days after hatching. Intestines were slit open with a razor blade, rinsed with ice cold redistilled water. Aliquots of the homogenate were used for enzyme assay.

2. Organ culture procedure. The jejunum was excised from the 20-day-old chick embryo and slit open longitudinally. Other methods were the same as those described previously (1). Eagle’s minimum essential medium set (a commercial product of Daigo Eiyo Kagaku Co., Osaka, Japan) was used as the culture medium. As test substances, sucrose, maltose, maltitol, fructose and glucose were used. The test substances were dissolved in redistilled water and added to culture medium at 5.56 mM. The final concentration of water from these sources was 0.1%. Each test substance was added through a Millipore filter. The intestines on the grids were cultured for 48 hr in a humidified incubator continuously supplied with 5% CO₂ in air. After forty-eight hours, the segments were rinsed well with iced saline in order to avoid contamination with glucose contained in the culture medium on the enzyme assay system. The tissues were homogenized with redistilled water and their aliquots were used for enzyme assay. Glucose content of tissue homogenate was negligible.

3. Assay procedures. Maltase and sucrase activities were determined by the method of DAHLQVIST (2). Substrate concentration was 28 mM maltose or sucrose in 50 mM sodium maleate buffer (pH 6.0) except where noted. Enzyme activity was expressed as μmoles substrate hydrolyzed per hour per mg of protein, or, expressed as the ratio of treated to control activity (relative activity).

Protein was determined by the procedure of LOWRY et al. (3). Bovine serum albumin was used to prepare a standard curve.

4. Chemicals. Maltose was purchased from Wako Pure Chemical Industries, Ltd. and sucrose, glucose and fructose were from Kokusan Chemical works, Ltd.. Maltitol was kindly provided by the Nikken Chemical Co., Ltd. and Hayashibara Co., Ltd.. Tris was obtained from Sigma Chemical Co., while glucose oxidase came from the Worthington Biochemical Company.

RESULTS

1. Effect of several sugars on the induction of disaccharidases activities in organ culture

A previous study demonstrated that hydrocortisone was required for the induction of disaccharidases in 15- and 17-day embryos. However, there was little induction of the enzymes in 20-day-old embryos with hydrocortisone. However, these enzymes activities of 20-day-old embryos were significantly stimulated by the addition of sucrose as a dietary factor. Maximal stimulation of disaccharidases
activities with sucrose was obtained at the sucrose concentration of 5.56 mM. Therefore, in order to assess the effect of several sugars on the induction of disaccharidases activities of 20-day-old chick embryonic intestine, we examined it using maltose, maltitol, fructose and glucose, respectively. The concentration of those sugars added in the culture medium was 5.56 mM each which was the same concentration that caused the maximal response showed in the sucrose dose response study.

As shown in Fig. 1, the disaccharidase activity of the explants obtained from 20-day-old embryos which was cultured in the absence of these substances, showed a little increase but did not increase to the same extent as observed in a post-hatched 2-day-old chick in vivo. The addition of maltose, fructose or glucose in culture medium as well as sucrose increased maltase activities to almost same extent as that observed in vivo (Fig. 1). The same tendencies were also obtained in the sucrase activity (Table 1). However, maltitol, which is a synthetic sugar alcohol of maltose, has no effect on the induction of disaccharidases (Fig. 1 and Table 1).

The relative enzyme activity induced by several sugars, except maltitol, showed that that of sucrase was higher than maltase in all. In addition, the highest relative activity of maltase and sucrase (i.e. 2.16 and 3.12, respectively) was obtained in the presence of sucrose in the culture medium (Table 1).
Table 1. Effect of sugars on the induction of disaccharidases of 20-day-old chick embryonic intestine in organ culture. Jejunum explants from 20-day-old embryos were used and cultured for 2 days. Several sugars were added to the medium to a level of 5.56 mM. For further details see “MATERIALS AND METHODS.” Values are indicated as the ratio of treated and control disaccharidases activities and indicated as the mean ± S.E. of 4-5 cultures.

| Inducer   | Relative enzyme activity | Maltase | Sucrase |
|-----------|--------------------------|---------|---------|
| None      | 0.99 ± 0.02              | 1.03 ± 0.01 |
| Sucrose   | 2.16 ± 0.63              | 3.12 ± 0.28 |
| Maltose   | 1.97 ± 0.59              | 2.21 ± 0.58 |
| Maltitol  | 0.96 ± 0.24              | 0.99 ± 0.25 |
| Fructose  | 1.47 ± 0.24              | 2.05 ± 0.26 |
| Glucose   | 1.87 ± 0.14              | 2.19 ± 0.17 |

2. Properties of the chick intestinal disaccharidases in vivo and in organ culture

In the case of the sucrose-evoked disaccharidases activities in 20-day-old embryos, the relative enzyme activity was the highest in the values obtained from several sugars (Table 1). Then the properties of disaccharidases induced by sucrose in organ culture were analyzed and compared to that obtained during development in vivo. The disaccharidases activities were determined for maltose and sucrose at various substrate concentrations (7–56 mM). From these, the Michaelis constant ($K_m$)
and the maximal velocity ($V_{\text{max}}$) for sucrose and maltose were determined by the graphical method of Lineweaver-Burk plots (Figs. 2 and 3).

In the case of maltase, the $K_m$ value of the 20-day-old embryo and post-hatched 2-day-old chick was 15.6 mM and 5.5 mM, respectively. On the other hand, the $K_m$ value of the explants cultured in the absence of sucrose and of those cultured in the presence of sucrose was 8.0 mM and 4.9 mM, respectively. Thus the $K_m$ values of maltase showed a significant decrease after hatching, and these results were also observed in the explants cultured in the presence of sucrose in organ culture, although a little decrease of the values was observed in the explants cultured in the absence of sucrose. However, in the case of sucrase, there was no change of the $K_m$ values as shown in maltase (Table 2).

In addition, the $V_{\text{max}}$ values of maltase of the explants cultured in the presence of sucrose and of post-hatched 2-day-old chicks were increased to the same degree. However, that of the explants cultured in the absence of sucrose did not increase and showed rather a value near to that of the 20-day-old embryos. In the case of sucrase, the $V_{\text{max}}$ values indicated similar changes (Table 2).
DISCUSSIONS

The effect of several sugars on the induction of intestinal maltase and sucrase in 20-day-old chick embryos was studied using the organ culture system. Furthermore, the properties of the enzymes, which were induced by sucrose in the culture medium, and of those obtained during development in vivo were estimated by kinetics study.

Our previous studies indicated that disaccharidases activities in 20-day-old embryos are induced by sucrose in the culture system (1). Moreover, in the present study, it was found that maltose, glucose and fructose were also effective, but maltitol was ineffective for the induction of disaccharidases activities (Table 1). Although maltitol and maltose react at the single active center of maltase, the $K_m$ value for maltitol was very large and the $V_{\text{max}}$ value was very small, as compared to those values for maltose (4). Therefore, it has been suggested that only very little hydrolysis of this synthetic sugar alcohol would occur in the small intestine (4). Consequently, glucose in maltitol could not contribute to the induction of the enzyme activity. From these results, it is likely that the products of substrate hydrolyzed by the enzymes, which are thought to play an important role in terminal digestion and absorption of nutrients in the microvillar surface of the small intestine (5-7), would act on the induction of the enzymes.

From the results obtained by kinetic study (Figs. 2 and 3, Table 2), the $K_m$ and $V_{\text{max}}$ values of sucrase suggest that the increase in enzyme activity was due to an increase in the enzyme protein or in the active state enzyme. However, in maltase, the decrease in the $K_m$ value was shown in the enzyme obtained in the explants cultured in the presence of sucrose as well as in that of those in vivo (Figs. 2 and 3, Table 2). In the case of maltase of the explants cultured in the absence of sucrose, a slight change in the $K_m$ value was observed and a nearly middle value between that of 20-day-old embryos and of 2-day-old chicks of that of those in vivo. This change observed in the $K_m$ value of maltase would show the effect of remaining information which was in the individual at the time of cultivation. Therefore, it will be expected that the increase of maltase activity in this developmental stage of chick embryos is caused by the increase of the enzyme protein with the induction of maltase isozymes.

Concerning the intestinal disaccharidases of avian species, there is very little information available (8-10). Although, recently, SIDDONS reported that the disaccharidases activity of the small and large intestines of chicks was being widely studied (11, 12). SIDDONS also proposed that heat-inactivation studies showed that there were at least three different enzymes with maltase activity (12). Although the species of chicks used in our experiment was different from those in his study, the decrease of the $K_m$ values showed in the present study suggests the induction of maltase isozymes.

Consequently, although further studies will be required to confirm the induction mechanism of these enzymes by sugars, the present study suggests that accumulation of products of hydrolyzed substrate acts as a stabilizer of disaccharidases or as an activator of the enzymes, which results in an increase of the active state enzyme
protein or the induction of isozymes. These and other possibilities are presently being investigated.

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