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IMMUNOCHEMICAL STUDIES ON THE SUBUNITS OF RABBIT-INTESTINAL SUCRASE-ISOMALTASE COMPLEX

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Summary

Purified sucrase-isomaltase complex (sucrose α-glucohydrolase, EC 3.2.1.48 — dextrin 6-α-glucanohydrolase, EC 3.2.1.10) solubilized by papain from rabbit intestine was dissociated by citraconylation into its subunits, sucrase and isomaltase, which were then isolated in a form active immunologically as well as enzymatically by affinity chromatography on Sephadex G-200 and gel-filtration on Bio-gel P-300. Antibodies against the purified complex inhibited isomaltase but not sucrase and formed precipitation lines, crossing each other, with isolated sucrase and isomaltase, showing that the two enzymes differ in antigenicity from each other. By absorbing the antibodies with isolated sucrase and isomaltase, antibodies specific for isomaltase and sucrase, respectively, were obtained. Like the original antibodies, both of the specific antibodies quantitatively agglutinated microvillous vesicles. Sucrase was inhibited by neither of the antibodies. In contrast, isomaltase was greatly inhibited by the isomaltase-specific antibodies, but not by the sucrase-specific ones.

Introduction

Intestinal sucrase (sucrose α-glucohydrolase, EC 3.2.1.48) has been considered to be tightly associated with isomaltase (dextrin 6-α-glucanohydrolase, EC 3.2.1.10) to form a bienzyme complex on the microvillous membrane [1]. By immuno-electron microscopy using non-labeled antibodies against purified sucrase-isomaltase complex, we have demonstrated that the complex protrudes about 150 A from the membrane surface (Nishi and Takesue, ref. 2 and manuscript in

Abbreviations: P-SI complex, papain-solubilized sucrase-isomaltase complex; anti-SI IgG, immunoglobulin G against P-SI complex; anti-S and anti-I IgGs, anti-SI IgG absorbed with isolated isomaltase and sucrase, respectively.

preparation). However, the enzyme which is directly bound to the membrane has not yet been determined.

Enzymatically active sucrase and isomaltase moieties can be obtained from a purified complex [3—6]. Isolated sucrase and isomaltase have been found to be very similar to each other in catalytic and chemical properties [7]. However, their comparison in antigenicity has not been carried out, though the effect of antibodies on sucrase has been examined in several laboratories [6,8—11]. If antibodies specific for sucrase or isomaltase can be obtained, then these enzymes could be specifically located by the immuno-electron microscopy using the specific antibodies in the same way as for the location of the whole complex [2].

We have succeeded in obtaining antibodies specific for sucrase and for isomaltase, and have studied their effects on the enzyme activities and agglutination of microvillous vesicles.

Materials and Methods

Materials

Microvillous vesicles [11] and papain-solubilized sucrase-isomaltase complex (P-SI complex) [12] were prepared from rabbit intestines. Treatment of microvillous vesicles by 0.1% trypsin was carried out as described previously [13]. The immunoglobulin G fraction (anti-SI IgG) was obtained by ammonium sulfate fractionation from antisera raised in a goat against P-SI complex [8].

Isolation of sucrase and isomaltase from P-SI complex

P-SI complex was dissociated into its subunits by citraconylation [4]. Citraconic anhydride dissolved in 10 vols. of acetone was added in small aliquots (10—20 μl at a time) to a P-SI complex solution (5 mg protein/ml in 0.2 M sodium phosphate (pH 8.2)) which was being stirred at room temperature. The pH of the mixture was maintained between 8.0 and 8.4 by the addition of 1 N NaOH. The total amount of citraconic anhydride added was 1 μl/5 mg protein. At the end of the final addition, the mixture was stirred for an additional 20 min. Under these conditions the sucrase and isomaltase activities were inactivated by about 75 and 35%, respectively. The citraconylated complex was subjected to affinity chromatography on a Sephadex G-200 column (3 × 50 cm) with 0.01 M sodium phosphate (pH 7.0) as elution buffer at 2°C. The fractions having high sucrase activity around a elution volume of 160 ml were pooled for further purification of sucrase. Most of the isomaltase activity was eluted by raising the elution temperature to 30°C, as in the case of the parent complex [12], and the fractions around the peak of activity were pooled. The pooled eluates were separately concentrated to 3 ml by vacuum dialysis and chromatographed on a Bio-gel P-300 column (2 × 95 cm) with the same buffer at 4°C. The fractions containing high sucrase or isomaltase activity were pooled, concentrated, and deacylated according to Braun et al. [4], to yield an isolated sucrase or isomaltase preparation.

Absorption of Anti-SI IgG with isolated subunits

For absorption by isolated sucrase, 95 mg protein of anti-SI IgG was incu-

bated with a little excess isolated sucrase (15.4 mg protein) in a total volume of 20 ml in sodium phosphate buffered isotonic saline at 37°C for 60 min and then at 4°C for 20 h, after which the mixture was centrifuged at 10000 × *g* for 30 min. The supernatant, having about 3% of the initial sucrase activity, was passed through a column (2 × 5 cm) of Sepharose 4B coupled with anti-SI IgG prepared as described previously [14]. The sucrase-free eluate was used as anti-I IgG.

For absorption by isolated isomaltase, 108 mg protein of anti-SI IgG was incubated with isolated isomaltase (13 mg protein) in a total volume of 20 ml in the saline and processed as above; the supernatant had about 6% of the isomaltase added. The isomaltase-free eluate from the Sepharose column was used as anti-S IgG.

Reaction of enzymes with antibodies in test tubes

The reaction of different enzyme preparations with antibodies in test tubes and the presentation of the results obtained were carried out as described previously [14]. The detailed experimental conditions are described in the legends to figures. As the isomaltase activity was inhibited by antibodies, the activity in supernatants obtained after agglutinated antigens were precipitated was corrected for the inhibition in the same way as in the case of leucine β-naphthylamidase which is also inhibited by antibodies against the amidase [14].

Assays and analysis

The sucrase activity was determined as described previously [12]. The isomaltase activity was assayed using palatinose (6-*O*-α-D-glucopyranosido-D-fructofuranose) as the substrate [4]; the enzyme was incubated with 33 mM palatinose in the total volume of 0.2 ml in 0.05 M sodium phosphate (pH 6.5) at 37°C for 60—120 min. The glucose produced was determined by the Tris/glucose oxidase-peroxidase reagent [15]. Activity was expressed in international units, i.e. micromoles of substrate hydrolyzed per min. Protein was determined by the method of Lowry et al. [16] using bovine serum albumin as the standard.

Sodium dodecyl sulfate disc electrophoresis was carried out according to the method of Weber et al. [17] with 5% gel system and 0.2 M sodium phosphate (pH 8.0) containing 0.2% sodium dodecyl sulfate as the gel buffer. Protein samples were boiled for 2 min in 0.01 M sodium phosphate (pH 8.0) containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol. With sodium dodecyl sulfate disc electrophoresis in the presence of urea, the buffers used were made 8 M with respect to urea. For molecular weight determination by dodecyl sulfate disc electrophoresis, β-galactosidase from *E. coli*, rabbit muscle phosphorylase *a*, bovine serum albumin, bovine liver catalase and ovalbumin were used as molecular weight markers [17].

Double immunodiffusion was performed in 0.5% agar in 0.05 M sodium phosphate (pH 7.0) with 0.03 ml of reactant in each well [11]. Diffusion was allowed to continue for at least 24 h at 4°C and at the end of diffusion the plates were photographed or stained with Amido Black.

Results

Isolation of sucrase and isomaltase from P-SI complex

Sucrase and isomaltase isolated in this work were comparable to those isolated by Braun et al. [4] in specific activity for their respective substrates (Table I). Isolated isomaltase had also a low but significant activity towards sucrose, probably attributable to a contaminating undissociated complex as mentioned later. In sodium dodecyl sulfate disc electrophoresis (Fig. 1A—C) the P-SI complex, isolated sucrase and isomaltase migrated as a single protein, sometimes accompanied by a weak slow-moving band. The minimum molecular weights of the P-SI complex, isolated sucrase and isomaltase were estimated to be 130000, 133000 and 122000, respectively, being approximately the same as those reported by Braun et al. [4]. In the presence of 8 M urea (Fig. 1D) the P-SI complex gave two closely located bands as in the case of human intestinal sucrase-isomaltase complex [6], one probably corresponding to the sucrase moiety and the other to the isomaltase one.

Quantitative precipitation of isolated sucrase and isomaltase by anti-SI IgG

Fig. 2 shows the effect of anti-SI IgG on the enzyme activities of purified P-SI complex. As reported previously [8,11], anti-SI IgG slightly inhibited the sucrase activity of the complex, though the IgG completely precipitated the enzyme. In contrast, anti-SI IgG greatly inhibited the isomaltase activity; a greater amount of the IgG used would have caused greater inhibition than shown in Fig. 2 (see Fig. 6).

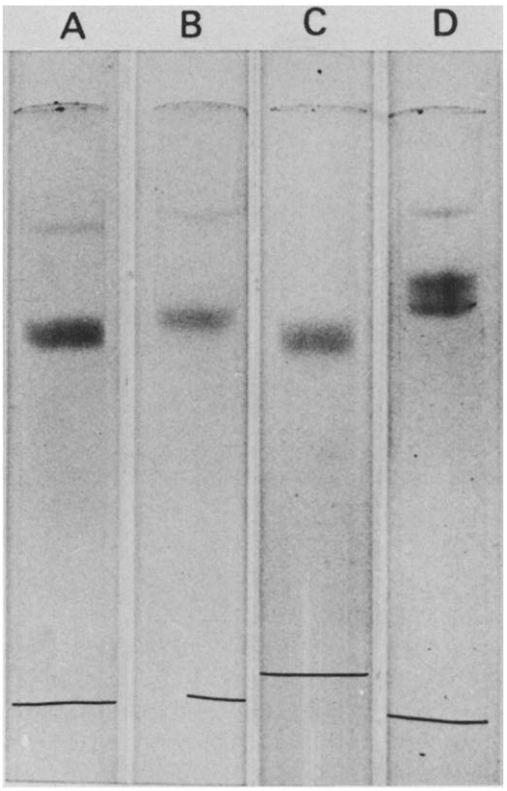
After the complex was dissociated into its subunits by citraconylation, the enzyme activities were still affected by anti-SI IgG in the same manner as before their dissociation (Fig. 3). The sucrase activity of isolated sucrase was slightly inhibited by the IgG, but the isomaltase activity of isolated isomaltase was greatly inhibited. Isolated sucrase and isomaltase were completely precipitated by anti-SI IgG in the range of antibody excess, indicating that anti-SI IgG contains antibodies to sucrase as well as to isomaltase. Therefore, a possibility can be excluded that the small inhibitory effect of anti-SI IgG on the sucrase activity might be due to the presumed absence of anti-sucrase antibodies in the anti-SI IgG used. About 100 and 75 μg protein of isolated sucrase

TABLE I

ISOLATION OF SUCRASE AND ISOMALTASE FROM P-SI COMPLEX

Purified P-SI complex (50 mg protein) was dissociated by citraconylation into its subunits, sucrase and isomaltase, which were isolated by affinity chromatography on Sephadex G-200, followed by gel-filtration on Bio-gel P-300, as described in the text.

Enzyme preparations	Sucrase activity		Isomaltase activity	
	Specific activity (units/mg protein)	Recovery (%)	Specific activity (units/mg protein)	Recovery (%)
P-SI complex	8.8	100	1.06	100
Isolated sucrase	11.5	23.5	0.0	0.0
Isolated isomaltase	1.13	1.7	1.49	18.5



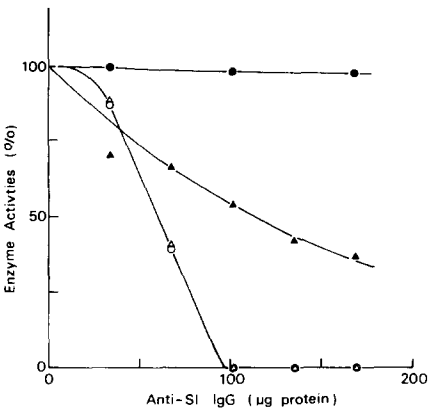


Fig. 1. Sodium dodecyl sulfate-disc electrophoresis of P-SI complex and its isolated subunits in the absence and presence of 8 M urea. Protein samples (5 μg protein in each) were electrophoresed for 4 h at 8 mA per gel in the gel and buffer system described in the text, and stained with Coomassie Brilliant Blue. A—C, without urea; D, with 8 M urea. A and D, P-SI complex; B, isolated sucrase; C, isolated isomaltase.

Fig. 2. Specific inhibition of the isomaltase activity of P-SI complex by anti-SI IgG. Purified P-SI complex (22 μg protein) was incubated with various amounts of anti-SI IgG in phosphate-buffered isotonic saline in a total volume of 0.25 ml at 37°C for 30 min and then at 4°C for 20 h, after which aliquots were withdrawn from the mixtures for assay of the total activities and the remainders centrifuged at 1000 × *g* for 30 min. The supernatants were assayed for the enzyme activities not agglutinated. The data are given as activities relative to the activities without IgG; the total activity is given as the measured value, but the activity in the supernatants is corrected for the inhibition by antibodies. ●, total sucrase activity; ○, sucrase activity in supernatant; ▲, total isomaltase activity; △, isomaltase activity in supernatant.

and isomaltase, respectively, seemed equivalent to 10 μl (0.667 mg protein) of anti-SI IgG (Fig. 3).

Double immunodiffusion of isolated sucrase and isomaltase against anti-SI IgG

Isolated sucrase and isomaltase, together with P-SI complex, were diffused against anti-SI IgG in agar gel (Fig. 4). As reported previously [8], P-SI complex gave a single line. Isolated sucrase and isomaltase formed lines of non-identity to each other, each of which fused with the line of the complex accompanied by a spur. Citraconylation seems not to inhibit these interaction. This and the above-mentioned results indicated that sucrase and isomaltase carry antigenic determinants different from each other and that anti-SI IgG

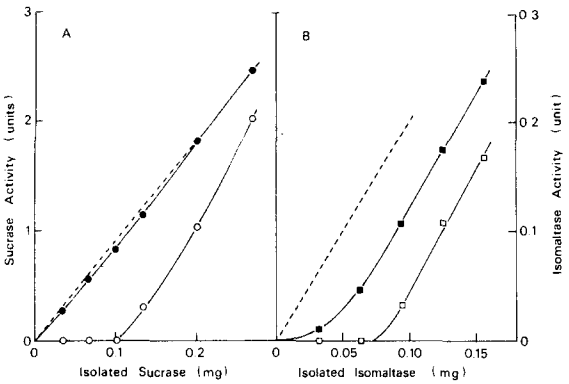


Fig. 3. Quantitative precipitation of isolated sucrase and isomaltase by anti-SI IgG. 10 μl (0.667 mg protein) of anti-SI IgG was incubated with various amounts of isolated sucrase (A) or isomaltase (B) in 0.5 ml of phosphate-buffered saline and processed as in Fig. 2. A: ●, total sucrase activity; ○, sucrase activity in supernatant. B: ■, total isomaltase activity; □, isomaltase activity in supernatant. Dashed lines show the total activities without antibodies.

contains antibodies directed toward these different antigenic sites, in addition to antibodies towards possible common antigenic sites.

Enzymatically active sucrase and isomaltase have been reported to be obtained from P-SI complex by extensive trypsin treatment [5] and mild alkaline pH treatment [3], respectively. The P-SI complex exposed to either of these treatments was also examined in a double immunodiffusion test. No precipitation line could be detected between anti-SI IgG and the P-SI complex

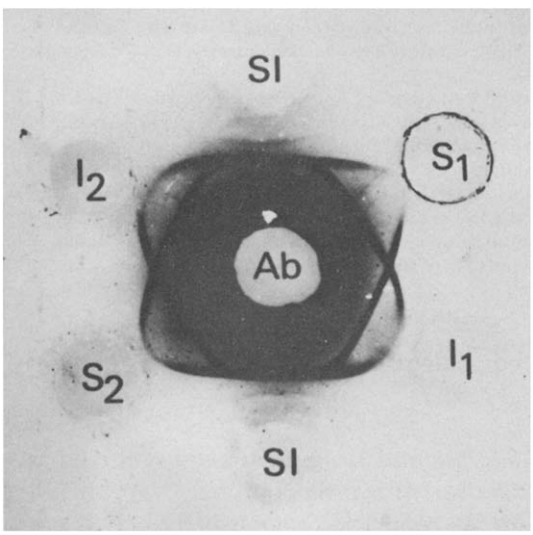


Fig. 4. Double immunodiffusion in agar gel of isolated sucrase and isomaltase against anti-SI IgG. Different enzyme samples were diffused against anti-SI IgG in 0.5% agar gel in 0.05 M sodium phosphate (pH 7.0). The agar plate with samples (about 10 μg protein per well) was incubated at 4°C for 4 days. Then it was washed repeatedly with isotonic saline and finally with water, dried, and stained with Amido black. Ab, anti-SI IgG; SI, P-SI complex, S_1; isolated sucrase before decitraconylation; S_2, isolated sucrase; I_1, isolated isomaltase before decitraconylation; I_2, isolated isomaltase.

treated by trypsin under the conditions as described by Quaroni et al. [5], suggesting that this treatment destroyed antigenic determinants of the complex. The P-SI complex treated by mild alkaline pH formed two weak lines against anti-SI IgG, possibly related with the dissociation of the complex into sucrase and isomaltase. However, this treatment has been reported to result in great inactivation of sucrase [3], though recently Cogoli and Semenza [18] mentioned the isolation of enzymatically active sucrase by this method without referring to the reason for the discrepancy. Unlike these two treatments, the method used in this work, citraconylation, was found to dissociate P-SI complex into immunologically as well as enzymatically active sucrase and isomaltase at the same time.

Preparation of antibodies specific for sucrase and for isomaltase

It was expected that antibodies specific for one of the two moieties would be obtained by absorbing anti-SI IgG with the other moiety. As described in Materials and Methods, anti-SI IgG was absorbed with isolated sucrase and isomaltase to yield anti-I and anti-S IgGs, respectively. The immunological specificity of the IgGs obtained was examined in a double immunodiffusion test (Fig. 5). Anti-S IgG formed a single line with isolated sucrase, which completely fused with that of the parent complex. It did not form any detectable line with isolated isomaltase, but the line of isolated sucrase seemed to be little curved by isolated isomaltase, possibly because of undissociated P-SI complex contaminating isolated isomaltase (see Table I). On the other hand, anti-I IgG formed a distinct line with isolated isomaltase, which completely fused with the line of the complex. It did not form any line with isolated sucrase. It should be noted that the line of P-SI complex given by anti-S IgG was much weaker than that by anti-I IgG on the basis of the protein amounts used. This is

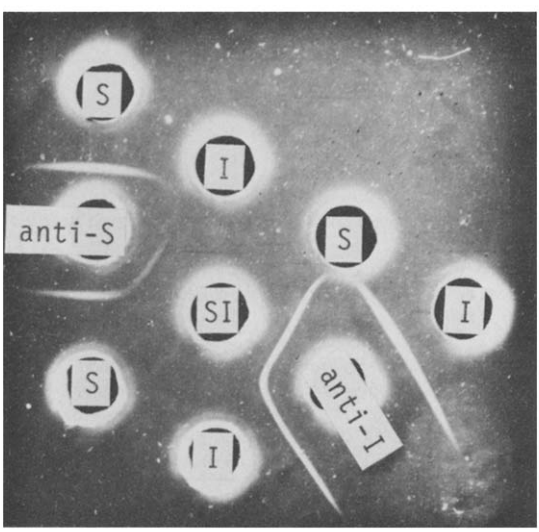


Fig. 5. Double immunodiffusion test of anti-S and anti-I IgGs. Different enzyme samples were diffused in agar gel against anti-S and anti-I IgGs at 4°C for 4 days. Anti-S, anti-S IgG; anti-I, anti-I IgG; SI, P-SI complex; S, isolated sucrase; I, isolated isomaltase.

in contrast to the human sucrase-isomaltase complex system, in which isolated isomaltase has much weaker affinity for antibodies than isolated sucrase [6]. However, these results indicate that anti-S and anti-I IgGs are specific for sucrase and isomaltase, respectively.

Effects of anti-S and anti-I IgGs on sucrase and isomaltase

The specificity of isolated IgGs was supported by the following experiments (Fig. 6). Like anti-SI IgG, anti-S IgG slightly inhibited the sucrase activity, whether this activity was exhibited by the membrane-bound or solubilized SI complex, or by isolated sucrase. It has also little effect on the isomaltase activity. Anti-S IgG quantitatively agglutinated microvillous vesicles, but its agglutinating activity was extraordinarily weak toward P-SI complex; even a prolonged incubation of 4 days at 4°C could result in only a little precipitation of the complex. This behavior of P-SI complex was consistent with the result in double immunodiffusion (Fig. 5). On the other hand, isolated sucrase was agglutinated by anti-S IgG, but its agglutinants were made soluble by the addition of excess anti-S IgG, in contrast to its agglutinants by anti-SI IgG which were completely precipitated in the region of antibody excess (Fig. 3A).

Like anti-SI IgG, anti-I IgG also inhibited the isomaltase activity, and its inhibition curve was the same, whether the membrane-bound or solubilized

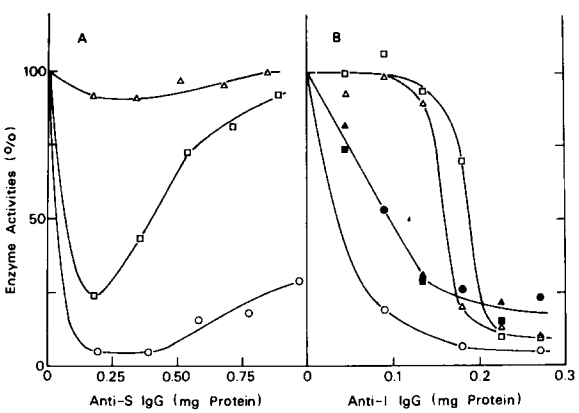


Fig. 6. Effects of anti-S and anti-I IgGs on the activities and agglutination of different enzyme preparations.

(A) Various amounts of anti-S IgG were incubated with microvillous vesicles (the amount equivalent to 18 μg protein of P-SI complex), P-SI complex (20 μg protein), or isolated sucrase (9.5 μg protein) in 0.5 ml of phosphate-buffered saline at 37°C for 30 min and then at 4°C for 20 h (microvillous vesicles) or 4 days (the two other samples), and processed as in Fig. 2. Only the data concerned with the sucrase activity in the supernatant are shown. ○, microvillous vesicles; △, P-SI complex; □, isolated sucrase.

(B) Various amounts of anti-I IgG were incubated with microvillous vesicles (the amount equivalent to 40 μg protein of P-SI complex), P-SI complex (20 μg protein) or isolated isomaltase (10 μg protein) in 0.5 ml of phosphate-buffered saline at 37°C for 30 min and then at 4°C for 20 h, and processed as in Fig. 2. Only the data concerned with the isomaltase activity are shown. ●, ○, microvillous vesicles; ▲, △, P-SI complex; ■, □, isolated isomaltase. Solid symbols, total activity; open symbols, activity in supernatant. The amount of antibody used is expressed in mg protein of IgG per 20 μg protein of P-SI complex or the amount of enzyme preparation equivalent to it: the amount of microvillous vesicles equivalent to 20 μg protein of P-SI complex was determined based on the specific activities of the purified P-SI complex and of the microvillous vesicles used, and 10 μg protein of isolated sucrase or isomaltase was assumed to be equivalent to 20 μg protein of P-SI complex.

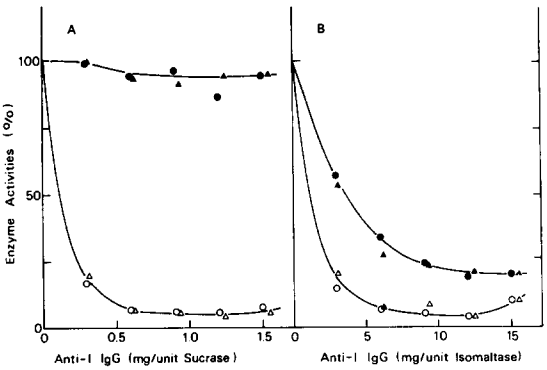


Fig. 7. Effect of trypsinization on the interaction of microvillous vesicles with antibodies. Microvillous vesicles (0.62 unit sucrase and 0.059 unit isomaltase) or trypsin-treated microvillous vesicles (0.59 unit sucrase and 0.058 unit isomaltase) were incubated with various amounts of anti-I IgG in 0.3 ml of phosphate-buffered saline under the same conditions and processed in the same way as in Fig. 2. (A) Sucrase activity; (B) isomaltase activity, ●, ○, microvillous vesicles; ▲, △, trypsin-treated microvillous vesicles. Solid symbols, total activity; open symbols, activity in supernatant. The amount of antibody used is expressed in mg protein per unit of the sucrase (A) or isomaltase (B) activity of the enzyme preparations used.

complex, or the isolated isomaltase was examined. In each case the maximum inhibition was approximately 80% under the conditions used. In contrast to anti-S IgG, anti-I IgG quantitatively precipitated P-SI complex and isolated isomaltase as well. The sucrase activity of the P-SI complex was precipitated in parallel to its isomaltase activity. It is reasonable that microvillous vesicles were precipitated earlier than P-SI complex and isolated isomaltase, because the same number of crosslinkages probably formed larger agglutinants with the first than with the latter two.

Effect of trypsinization of microvillous vesicles on their agglutination

Trypsin cannot solubilize the sucrase-isomaltase complex, but it releases two-thirds of protein and one-third of hexosamine from the microvillous membrane [19]. Electron microscopic examination has shown that trypsinization of microvillous vesicles gives no effect on their membrane structure and interaction with anti-SI IgG [2]. In this work the effect of trypsinization was checked by quantitative precipitation in test tubes (Fig. 7). The agglutination curve and inhibition curve of enzyme activity for trypsinized microvillous vesicles were the same as those for the control vesicles, whether the effect was followed by assaying the sucrase or isomaltase activity. Furthermore, the results shown in Fig. 7 indicate that the sucrase and isomaltase activities are precipitated in parallel with each other.

Discussion

The present work demonstrates that the sucrase and isomaltase moieties of intestinal sucrase-isomaltase complex have antigenic determinants different from each other and that the sucrase activity of P-SI complex is precipitated in parallel with the isomaltase activity by isomaltase-specific anti-I IgG as well as by anti-SI IgG. These findings clearly confirm the view that intestinal sucrase-

isomaltase, at least in the purified state, is a complex of two distinct glucosidases.

It has been reported that the major antigenic determinants of human intestinal sucrase-isomaltase complex are confined to the sucrase moiety [6]. On the other hand, anti-SI IgG used in this work contains antibodies toward the sucrase moiety and those toward the isomaltase moiety in rougly equal amounts (Fig. 3), and antibodies specific for sucrase and for isomaltase can be effectively obtained by absorbing anti-SI IgG with isolated isomaltase and sucrase, respectively. Previous results with quantitative precipitation in test tubes [see Fig. 2 in ref. 11] indicate that about four molecules of anti-SI IgG bind to one molecule of P-SI complex at the equivalence zone; that is, eight or more antigenic determinants exist on the P-SI complex molecule. Since isolated sucrase can be precipitated, albeit incompletely, by anti-S IgG and since its agglutinants are easily solubilized by an excess of the antibody, there must be present at least, but probably little more than, two determinants on the sucrase moiety towards which anti-S IgG is directed. In the P-SI complex the associated isomaltase moiety probably inhibits the interaction of anti-S IgG with either of the two determinants on the sucrase moiety, resulting in a little precipitation of P-SI complex by anti-S IgG. As for vesicle agglutination by anti-S IgG, two vesicles are held together by the links formed by a certain number of divalent antibodies located close in a limited area, each of which crosslinks an antigenic site of the sucrase moiety on one vesicle to the one on the other vesicle. Therefore it is not absolutely necessary for the two determinants of the sucrase moiety to be fully saturated with anti-S IgG in order that microvillous vesicles may be agglutinated by anti-S IgG. This is most probably the reason why anti-S IgG, which cannot quantitatively agglutinate P-SI complex, can induce the agglutination of microvillous vesicles.

It has been reported that sucrase and isomaltase of intestinal sucrase-isomaltase complex are almost identical maltases differing in the detailed structure of the aglycone binding site [7]. Since anti-I IgG inhibits specifically and greatly the isomaltase activity, the antigenic determinant(s) toward which anti-I IgG is directed is probably located at or near this site.

The sucrase- and the isomaltase-specific antibodies have been utilized to localize the sucrase and isomaltase moieties of sucrase-isomaltase complex on the microvillous membrane by immuno-electron microscopy. The results obtained indicate clearly that the sucrase moiety is bound to the membrane through the isomaltase moiety which is directly bound to the membrane continuum, the whole complex protruding about 150 Å from the membrane surface [20].

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