Human Intestinal Sucrase-Isomaltase

IDENTIFICATION OF FREE SUCRASE AND ISOMALTASE AND CLEAVAGE OF THE HYBRID INTO ACTIVE DISTINCT SUBUNITS*

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SUMMARY

Sucrase-isomaltase complex and its functional subunits have been identified in homogenates of human small intestinal mucosa by use of Sephadex G-200 (superfine) chromatography aided by affinity of the isomaltase moiety for the dextran gel. The isomaltase subunit binds strongly to the gel at 4°, and is eluted only after 2 column volumes; earlier recovery as a sharp peak can be achieved by raising column temperature to 37° after elution of other proteins.

Bio-Gel P-300 chromatography, density gradient, and equilibrium centrifugation demonstrated that the sucrase subunit (Stokes radius = 45 Å, frictional ratio = 1.32, s20,w = 6.9, MW = 130,000) and the isomaltase subunit (Stokes radius = 43 Å, frictional ratio = 1.30, s20,w = 6.6, MW = 120,000) are similar but unequal in size. The sucrase-isomaltase complex (Stokes radius = 70 Å, frictional ratio = 1.61, s20,w = 0.8, MW = 280,000), appears to be an elongated hybrid molecule that is less symmetrical than either of its subunits. Apparent Km and pH activity curves were indistinguishable for each enzyme whether present in the hybrid or in the free state. The sucrase-isomaltase complex, accounting for approximately 90% of native intestinal sucrase and isomaltase activities, was isolated and cleaved by 0.01 M β-mercaptoethanol/6 M urea treatment into active sucrase and isomaltase subunits having biochemical characteristics identical with those of the free native moieties. Sodium dodecyl sulfate acrylamide gel electrophoresis of the complex yielded a monospecific precipitating antibody that reacts with the hybrid and the sucrase subunit, but had minimal affinity for the isomaltase subunit, providing further evidence that the sucrase-isomaltase molecule is a hybrid consisting of two distinct α-glucosidases.

Immunization of rabbits with pure sucrase-isomaltase complex yielded a monospecific precipitating antibody that reacted with the hybrid and the sucrase subunit, but had minimal affinity for the isomaltase subunit, providing further evidence that the sucrase-isomaltase molecule is a hybrid consisting of two distinct α-glucosidases.

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Sucrase and isomaltase activities have been found in the brush border membranes of the small intestine of rabbit (1), rat (2), and man (3, 4). These membrane-bound activities can be recovered in soluble form after treatment with papain, and purification appears to yield a single protein, sucrase-isomaltase, with two independently acting enzyme sites. This digestive enzyme is analogous to the α-glucosidase in liver and muscle that has both glucosidase and transferase activities (5). Studies of human intestine after papain solubilization have revealed not only sucrase-isomaltase, but also an enzyme of smaller molecular size having only sucrase activity that is believed to be a preparative artifact (2, 6). However, isomaltase free of sucrase activity has been recently identified in a single human intestinal preparation (7), and Cogoli et al. (8) have shown that alkaline treatment of rabbit intestinal sucrase-isomaltase produces an active isomaltase subunit, a high molecular weight aggregate possibly containing inactive sucrase and a trace amount of an active sucrase subunit.

The present study concerns the interrelationship of the hybrid sucrase-isomaltase and its active sucrase and isomaltase subunits in human small intestine. The experiments suggest that the native hybrid is formed by union of two nonidentical subunits that act independently on their respective substrates.

EXPERIMENTAL PROCEDURE

Chemicals and Reagents—Tris, d-mannitol, 2-mercaptoethanol, urea, glucose oxidase, crystalline papain, horseradish peroxidase (salt-free), and N,N,N',N'-tetramethylenediamine (TEMED) were from Sigma. Acrylamide, BIS (N,N'-methylenbisacrylamide), agarose, and Bio-Gel P-300 were from Bio-Rad, and Sephadex (G-200) from Pharmacia. 3,3'-Dimethoxybenzidine (o-dianisidine) and riboflavin were products of Eastman. Isomaltose was a gift of Dr. Allene Jeanes, U.S.D.A. Northern Regional Research Laboratory, Peoria, Ill. All other chemicals were Baker Analyzed Reagents. The disaccharides showed no detectable impurity by paper chromatography.

Preparation of Intestinal Mucosa—Human small intestine obtained at autopsy 1 to 16 hours after death from patients without gastrointestinal disease was cut longitudinally and scraped with a glass microscope slide to remove the mucosa which then was wrapped tightly in Parafilm (American Can Company) and stored at −20° to prevent loss of oligosaccharidase activities (9). One to thirty days later, the mucosa was weighed, rapidly thawed, and allowed to autolyze for 1 hour at 37°. Whereas papain has been customarily used as a means of solubilization for brush border membrane enzymes (10), there is some doubt whether such treatment allows study of native membrane enzymes, and autolysis proved to release a high yield of oligosaccharidases including the active free sucrase and isomaltase moieties. Each gram of autolyzed tissue was homogenized with 0.75 ml of 0.01 M sodium-potassium...
phosphate (NaH2PO4-K2HPO4) buffer, pH 6.0, usually containing 0.01 M 2-mercaptoethanol to reduce the tendency of intestinal peptidases to bind to the disaccharidases. The homogenate was then centrifuged at 100,000 \times g for 1 hour, and the supernatant containing 70 to 90% of the total intestinal sucrase and isomaltase was recovered. Although jejunal tissue was routinely selected, no differences were found whether upper or lower small intestine was used.

**Enzyme Assays**—All assays were performed using 0.01 M sodium-potassium phosphate buffer (Buffer A), pH 6.0, unless otherwise noted. Sucrose was used at 28 mm and isomaltose at 5.5 mm in the reaction mixture. One-tenth milliliter of enzyme solution, diluted to ensure linear reaction with time, was incubated with 0.1 ml of buffered substrate for 20 to 60 min at 37°C. Longer incubation times were occasionally employed to identify minimal activity, but results were not considered to be quantitative. The reaction was stopped by transferring the tubes to a boiling water bath for 2 min and the glucose produced by the reaction was specifically assayed by a Tri-glucose oxidase reagent (11). Enzyme-disaccharide blanks boiled at zero time showed no significant glucose. Activity was expressed in international units (i.u.) = micromoles of substrate hydrolyzed per min. For kinetic studies, six different substrate concentrations up to 5 times the apparent K_m were used at the pH optimum. Michaelis constants were determined by the method of Lineweaver and Burk (12). In the pH activity studies, no enhancement or inhibition of enzyme activity was found for any of the buffers used.

Lactase, alcohol dehydrogenase, catalase, alkaline phosphatase, and leucyl-β-naphthylamidase were used as marked enzymes, and antibody to them was always included as a control. Activity was measured by standard methods (11, 13-16).

**Gel Filtration and Affinity Chromatography**—Bio-Gel P-300 was handled according to the manufacturer's instructions (17), except that the gel columns were poured at room temperature, and later allowed to equilibrate at the running temperature of 4°C to allow any trapped air bubbles to dissolve at the lower temperature.

Unsubstituted Sephadex G-200 possesses special advantages for the separation of these disaccharidases because of the affinity of the isomaltase for the α-1,6-glucosidic linkages of the gel (1, 18). The superfine grade allowed both superior resolution in molecular sieving and improved binding of the sucrase-isomaltase complex and the isomaltase moiety, as compared to the coarser grade.

Pressures were maintained at a maximum of 10 cm of water in order to maintain flow rates of 1 ml/cm²/hour, and to prevent the gel particles from being forced through the 0.5 mm mesh nylon screen at the column outlet.

**Density Gradient Centrifugation**—Continuous 5-ml linear gradients of 5 to 20% d-mannitol in 0.01 M Buffer A, pH 6.0, were made at 4°C and used within 1 hour after preparation. Samples of 0.01 to 0.1 ml were layered on the gradient, and centrifugation carried out at 100,000 \times g for 72 hours in a SW 65Ti swinging bucket rotor or for 97 hours at 4°C in an SW 65Ti rotor and collected as described above. The enzyme sedimented to equilibrium by 72 hours, and the density at that point was determined from the refractive index at 25°C (19) with interpolation to 4°C from the International Critical Tables (20).

**Polyacrylamide Gel Electrophoresis**—A subsystem of the multiphasic buffer System 53A of Jorvan et al. (21-24) was used for acrylamide gel electrophoresis. A separation gel of 7.5% or 10% total acrylamide was used with 2% cross-linking; a stacking gel of 4% total acrylamide with 12% cross-linkage was co-polymerized with 0.5% agarose. The pH values for upper reservoir buffer, stacking gel buffer, and separation buffer were 9.11, 8.74, and 9.20, respectively. For isolation of the single enzyme band, gels were cut into 0.5-mm tranverse sections by a Mickle gel slicer (Brinkmann Instruments), and the active enzyme in each slice was allowed to diffuse overnight at 4°C into 0.5 ml of 0.2 M Buffer A, pH 6.0. Control gels from the same run were stained for protein with Coomassie blue.

Sodium dodecyl-sulfate-gel electrophoresis was carried out according to Weber and Osborn (25) in gels of 5.3% total acrylamide with 2.67% cross-linking.

**Immunoaffinity with Sucrase-Isomaltase Complex**—Pure enzyme isolated from polyacrylamide gel electrophoresis was brought up to 1 ml (100 to 200 μg of protein) with 0.02 M Buffer A and homogenized with 4 ml of complete Freund's adjuvant. The material was then injected in equally divided volumes subcutaneously into each footpad, shoulder, and thigh of a New Zealand white rabbit. A few milliliters of blood were removed from an ear vein 30 days later for immunodiffusion analysis. A precipitating antibody was always found at this stage, and an additional 100 μg of pure sucrase-isomaltase in 1 ml of complete Freund's adjuvant were then injected intramuscularly into a thigh. Two weeks later, an identical booster dose was given. Blood was removed from an ear vein 2 weeks after the last injection, and the serum was made 50% saturated with (NH₄)₂SO₄. The precipitate produced was reconstituted with 0.02 M sodium phosphate/0.145 M NaCl, pH 7.0, to the original serum volume and dialyzed against the same buffer for 18 hours.

**RESULTS**

**Identification of Enzymes Having Sucrase or Isomaltase Activity**

Sephadex Gel Filtration Affinity Chromatography—As shown in Fig. 1, gel filtration of intestinal preparations on Sephadex G-200 (superfine) revealed a major peak containing both sucrase and isomaltase activities accounting for about 90% of total intestinal activity for these two enzymes. But there were also other distinct peaks of α-glucosidase activity with specificity for either sucrose or isomaltose. A small leading peak possessing activity only against isomaltose was eluted close to the void volume, and was in the same position on rechromatography. However, this enzyme was inconstant, and was identified in only 20% of the different tissues tested. Activity for sucrase alone followed this

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1 This method was generously provided by Dr. Leonad A. Herzenberg.
peak and was designated S. It was found in every intestinal tissue that we studied. A trailing peak of isomaltase activity appeared in approximately 50% of the 35 tissues that were studied, and was eluted at nearly 2 times the total column volume, apparently reflecting an avid affinity of this enzyme for the α-1,6 links of the dextran gel. Glucose, presumably produced by the hydrolysis of these 1,6 linkages, was eluted toward the end of the main sucrase-isomaltase peak, making routine measurement of disaccharidase blanks in each fraction critical for identification of true enzyme activity. Rearranging the Sephadex column temperature to 37° after elution of the main sucrase-isomaltase peak produced prompt elution of the trailing isomaltase peak, and was used as a method of identification of this enzyme (Fig. 1). Rechromatography of the enzyme showing activity only against sucrose (90 ml of effluent, Fig. 1) and of the trailing enzyme active against isomaltose (250 ml, if heated or 400 ml at 4°, Fig. 1) demonstrated consistent behavior for these two enzymes.

Bio-Gel P-300 Gel Filtration and Density Gradient Centrifugation—In contrast to Sephadex, Bio-Gel has no affinity for these α-glucosidases, and each enzyme can be expected to be eluted according to its molecular (Stokes) radius (26, 27). Fig. 2 is a composite representation of separate experiments, showing that sucrase-isomaltase complex is much larger than either sucrase or isomaltase, and that the smaller enzymes are similar to each other in size.

Although the discrepancy between the two gel filtration techniques seemed most likely to be explained by the affinity of the isomaltase active site for Sephadex, density gradient ultracentrifugation was selected as an additional method for studying the enzymes, particularly since it allows determination of the sedimentation coefficient (28). As expected, velocity centrifugation in 5 to 20% d-mannitol at 300,000 × g in an SW65Ti rotor for 6 hours (see under “Experimental Procedure”) of the sucrase-isomaltase (S-I), free sucrase (S), and free isomaltase (I) peaks isolated from Sephadex G-200 chromatography revealed a sedimentation pattern similar to the elution profile found for Bio-Gel chromatography. The apparent difference in size between small sucrase and small isomaltase demonstrated by both techniques was verified by a 10-hour ultracentrifugation study, in which small sucrase sediments more rapidly than small isomaltase (Fig. 3).

**Fig. 2. Bio-Gel P-300 (100 to 200 mesh) gel filtration of sucrase-isomaltase (S-I), sucrase (S), and isomaltase (I) isolated from Sephadex G-200 (superfine) chromatography (cf. Fig. 1). After the column (2.5 × 45 cm) had been allowed to equilibrate with 0.01 M Buffer A, pH 6.2, at 4° for 60 days (26), each peak and marker protein was run separately and eluted at 5.0 ml/hour. Arrows above peaks indicate positions of thyroglobulin, alcohol dehydrogenase, and albumin. O—O, sucrase; O—O, isomaltase.**

Purification of Sucrase-Isomaltase Complex

Human small intestinal mucosa was prepared, homogenized, and solubilized as described under “Experimental Procedure.” Autolysis at 37° for 1 hour released maximal amounts of sucrase- isomaltase, and the active sucrase and isomaltase moieties as monitored by density gradient centrifugation. No additional activity was solubilized, nor was there any change in the ratios of activities or enzyme species found even after 3 hours of incubation. Autolysis for longer periods produced symmetrical losses of activity for the hybrid and for free sucrase and isomaltase.

Specific activities of the original homogenate prior to solubilization were 51 i.u./g of protein for sucrase and 34 i.u./g of protein for isomaltase. Purification of the hybrid enzyme was accomplished by a series of procedures, as summarized in Table I.

**Step 1: Autolysis—After autolysis of 10 g (950 mg of protein) of homogenate for 1 hour at 37°, the 100,000 × g-hour supernatant contained 400 mg of protein and 70 to 90% of the sucrase and isomaltase activities at slightly higher specific activity than in the original homogenate. This was used as the starting material (Table I).**

**Step 2: 50 to 85° (NH₄)₂SO₄ Precipitation—The supernatant from Step 1 was made 50% saturated with (NH₄)₂SO₄ by addition of the solid salt at 4°, and the precipitate containing no sucrase or isomaltase activity was discarded. The 80% supernatant was then brought to 85% saturation with (NH₄)₂SO₄, and the precipitate washed twice in 85% saturated (NH₄)₂SO₄. It was then taken up in 5 ml of 0.01 M Buffer A/0.15 m NaCl buffer, pH 6.0, and dialyzed for 24 hours against 1 liter of the same buffer with three buffer changes.**

**Step 3: Sephadex G-800 (Superfine) Affinity Filtration Chromatography—The final preparation from Step 2 was layered under the 0.01 M Buffer A, pH 6.0, in a column (5.0 × 90 cm) of the dextran gel with the precautions taken in preparing and running the column as outlined under “Experimental Procedure.”**

**Step 4: Bio-Gel P-300 Filtration Chromatography—Fractions from the sucrase isomaltase peak from Step 3 were combined and the volume reduced to 3 ml by vacuum dialysis against 0.01 M Buffer A/0.15 M NaCl. The final sample was applied to the column (2.5 × 45 cm) of Bio-Gel P-300 (prepared as described under “Experimental Procedure”) in the same manner as described in Step 3.**

**Step 5: Polyacrylamide Gel Electrophoresis—The sucrase-isomaltase peak from Step 4 was concentrated as described for Step 4, except that dialysis was against the stacking gel buffer to be used for acrylamide electrophoresis. The sample was then made 40% v/v with glycerol and 50 μl containing 10 to 20 μg of protein were applied to cylindrical gels (0.6 × 10 cm). Acrylamide electrophoresis was carried out on System 2518 for 3 hours at 2 ma/gel, as described under “Experimental Procedure.” The single protein band identified at this stage contained both sucrase and isomaltase activities in the usual 1.5:1 ratio. The pure hybrid was recovered from the appropriate segment of 36 to 48 gels as described under “Experimental Procedure,” and was used in subsequent characterization experiments as well as for immunization of rabbits and for cleavage into active subunits.

**Kinetics and pH Optima—Kinetic and pH activity experiments were performed using sucrase and isomaltase as substrates as described under “Experimental Procedure.” The Michaelis constants with sucrase as substrate were indistinguishable for the sucrase-isomaltase complex (Kₘ(app) = 20.2 ± 1.3 mM, 95% confidence limits) and for partially purified free sucrase (Kₘ(app) = 19.6 ± 1.9 mM). Similarly, with isomaltose as substrate, the same Kₘ(app) was found for the sucrase-isomaltase complex (4.30
peaks recovered from Sephadex G-200 (superfine) (cf. Fig. 1). The enzyme peaks were combined with alcohol dehydrogenase, layered over a linear gradient of 5 to 20% d-mannitol/0.01 M Buffer A, pH 6.0, and centrifuged at 300,000 X g in an SW 65Ti rotor for 10 hours. ●—●, sucrase; ○—○, isomaltase. A.D., position of alcohol dehydrogenase.

Fig. 4 (right) pH activity curves for pure sucrase-isomaltase (S-I) (Step 5, Table I) and partially purified sucrase (S) and isomaltase (I) isolated from Sephadex G-200 chromatography and density gradient experiments. Reaction mixtures containing 28 mM sucrose or 5.5 mM isomaltose in 0.2 M buffers (citrate-phosphate, pH 4.20 to 6.60; sodium-potassium phosphate, pH 6.20 to 7.75; borate, pH 7.60 to 8.89). pH values were measured in the reaction mixture. ●—●, sucrase for S-I; △—△, sucrase for S; ○—○, isomaltase for S-I; ▲—▲, isomaltase for I.

Table I

| Step | Sucrase | Isomaltase | Recovery | Purification factor |
|------|---------|------------|----------|---------------------|
| 1. Autolyzed 100,000 X g supernatant | 0.087 | 0.008 | 100 | 1 |
| 2. 50 to 85% (NH₄)₂SO₄ ppt. | 0.28 | 0.18 | 90 | 3 |
| 3. Sephadex G-200 (superfine) | 9.0 | 6.3 | 75 | 103 |
| 4. Bio-Gel P-300 | 12 | 7.8 | 70 | 134 |
| 5. Acrylamide gel electrophoresis | 47 | 31 | 40 | 540 |

* Activities given are V_max.

Enzyme activity became labile at the final purification step so that albumin 50 μg/ml reaction mixture was required for maximal activity. This reversible loss of activity was as high as 70% with buffer alone.

± 1 mM) and for free isomaltase (4.36 ± 0.5 mM). V_max values for the pure hybrid are given in Table I. Although maximal rates for free sucrase and isomaltase might be expected to be even greater than those found for the complex, the small amounts of free sucrase and isomaltase in human intestine made reliable measurement of protein content difficult. However, maximum velocities for the free sucrase and isomaltase moieties could be approximated to be 85 to 160% of those found for the S-I complex.

The entire pH activity curve for each enzyme was identical whether the enzyme was free or in the hybrid form (Fig. 4).

Molecular Parameters—The Stokes radius of each enzyme was determined according to the methods of Acker (26) using a Bio-Gel P-300 column calibrated with marker proteins (Fig. 2), where the volume of the gel (V_P) was assumed to be 5% of the inclusion volume (V_i) of the column.

Table II

| Enzyme | V_P (%) | Stokes radius | Molecular weight (apparent) | Axial ratio |
|--------|---------|---------------|----------------------------|------------|
| S-I    | 8.0     | 0.8           | 328,000                    | 8.0        |
| S      | 4.0     | 0.9           | 130,000                    | 4.0        |
| I      | 4.0     | 0.9           | 120,000                    | 4.0        |

* Protein concentration in samples used for velocity centrifugation was low (0.5 to 3 μg/ml); no extrapolation was made to zero concentration.

Equilibrium ultracentrifugation in a CsCl gradient (10) yielded a density of 1.331 g/cm³ for the hydrated sucrase-isomaltase complex, indicating a solvated partial specific volume of 0.751 cm³/μg. Hence, the true anhydrous partial specific volume (V̄) must be very close to the typical V̄ of 0.725 found for many proteins and the sedimentation coefficients of the sucrase-isomaltase complex, sucrase and isomaltase could be calculated according to the method of Martin and Ames (28), assuming ρ_0 of 7.0 for alcohol dehydrogenase, 11.3 for catalase, and 0.5 for 125I-labeled rabbit γ-globulin.

Since ρ_0, Stokes radius, and the partial specific volume were known, the molecular weight could then be calculated from a combination of the Stokes-Einstein and Svedberg equations (26, 27):

\[ M = \frac{6\pi\eta N a}{1 - \frac{V}{\rho}} \]

where N = Avogadro’s number, η = viscosity of water at 20°C, a = Stokes radius, s = sedimentation coefficient at 20°C (s_0,0), \( \bar{V} \) = partial specific volume, ρ = density of water at 20°C.

The frictional ratio f/f_0 provides a measure of molecular symmetry and hydration and can be determined when the molecular weight is known:

\[ f = a \left( \frac{3M\bar{V}}{4\pi N} \right)^{-1/3} \]

Assuming that the hybrid enzyme and the active free forms of sucrase and isomaltase are equally hydrated at approximately 0.3 g of water/g of anhydrous protein, then the axial ratios of these enzymes can be estimated (29).

Table II compares the various molecular parameters for the three enzymes having sucrase or isomaltase activity. Although the S and I moieties are similar in shape and size, the free sucrase is larger than its isomaltase counterpart. When the molecular weights and axial ratios of the three α-glucosidases are compared (Table II), it is clear that S and I may constitute the active subunits of the native hybrid. The studies described below verify this interpretation.

Immunological Characterization with Monospecific Antibody—The 0 to 90% (NH₄)₂SO₄ fraction of serum obtained from rabbits immunized (see under “Experimental Procedure”) with pure sucrase isomaltase complex was used for immunoprecipitation studies of the enzymes. Immunodiffusion of the antiserum against crude and pure sucrase-isomaltase complex revealed a single line of precipitation (Fig. 5), providing further evidence that the enzyme is homogeneous. The antiserum did not precipitate other
Serum (A). Samples were allowed to diffuse at room temperature within 16 hours and remained unchanged for 2 days.

in 1% agarose/0.075 M barbital, pH 8.6. The precipitin line formed was determined by adding varying amounts of enzyme until crude intestinal autolyzed supernatant (C) against rabbit anti-

for 30 min and 4° for 24 hours. Maximal capacity of the antibody pure sucrase-isomaltase was incubated with each enzyme at 37°

tate S-I.

Percent is given relative to capacity of the antiserum to precipi-

from nonimmunized rabbits did not precipitate any enzyme. Residual enzyme could be detected in the supernatant. Serum

revealed that 0.01 ml of the rabbit antiserum precipitated 40 milliunits of sucrase-isomaltase and an even greater amount of free sucrase. In contrast, the antiserum showed minimal affinity for free isomaltase. This suggests that the major antigenic determinants of the hybrid molecule are confined to the sucrase moiety.

**Table III**

Quantitative Immunoprecipitation with Anti-S-I Serum

| Enzyme | Milliunits precipitated | % maximal |
|--------|------------------------|-----------|
| S-I    | 40                     | 100       |
| S     | 62                     | 155       |
| I      | 1.1                    | 4         |

brush border enzymes such as lactase, alkaline phosphatase, and leucyl-β-naphthylamidase, as estimated by absence of these activities in the immunoprecipitate and by the total recovery of each activity in postprecipitin supernatant when crude autolyzed intestine was reacted with antiserum.

As shown in Table III, quantitative immunoprecipitation revealed that 0.01 ml of the rabbit antiserum precipitated 40 milliunits of sucrase-isomaltase and an even greater amount of free sucrase. In contrast, the antiserum showed minimal affinity for free isomaltase. This suggests that the major antigenic determinants of the hybrid molecule are confined to the sucrase moiety.

**Clearance of Sucrase-Isomaltase Complex into Active Subunits**

A highly active preparation of sucrase-isomaltase complex (sucrase 38 i.u./mg of protein, isomaltase 23 i.u./mg of protein) free of any sucrase or isomaltase subunits was dialyzed for 24 hours against 6 M urea/0.01 M mercaptoethanol in 0.02 M Buffer A, by dialysis for 24 hours, and then to buffered 0.01 M mercaptoethanol alone for an additional 24 hours. The control sample was exposed to buffer alone for 48 hours. Centrifugation was then carried out in tubes (3½ inch X 3½ inch) of 5 to 20% manitol at 200,000 X g for 16 hours in the SW 41Ti rotor and in the control run (left). The treated sample was cleaved into discrete subunits that sedimented at different rates (right). Recovery of total activity was 92% of the control for sucrase and 87% for isomaltase. After urea-mercaptoethanol treatment, and 5 to 10% of the sucrase-isomaltase hybrid was cleaved into active sucrase and isomaltase subunits. These active subunits had identical biochemical characteristics ($K_m$, pH optimum, behavior on Sephadex filtration affinity columns) to those of the native free sucrase and isomaltase found in small intestinal preparations.

Sodium dodecyl sulfate-acrylamide gel electrophoresis (25) of the pure sucrase-isomaltase that had been treated with urea and mercaptoethanol as described above revealed a 2-mm band with decreased staining in the center that migrated slightly ahead of β-galactosidase. This suggested that sodium dodecyl sulfate treatment also produces scission of the hybrid into its subunits of slightly over 100,000. In order to increase the sensitivity for detection of the subunits, the sucrase-isomaltase complex was labeled with $^{125}$I by the chloramine-T method (30), and by lactoperoxidase-catalyzed coupling (31). Chloramine-T produced a higher specific radioactivity and did not destroy the enzyme activities. Acrylamide gel electrophoresis was used to ensure that all radioactivity was confined to the hybrid enzyme. The $^{125}$I-sucrase-isomaltase was then examined by sodium dodecyl sulfate-acrylamide gel electrophoresis and gels were sliced at 0.5-mm intervals for determination of radioactivity. As shown in Fig. 7, two radioactive protein peaks were produced by sodium dodecyl sulfate treatment. In a separate gel in the same electrophoresis experiment, the $^{125}$I-labeled free sucrase moiety migrated to the identical position of the slower moving peak, thereby locating the sucrase subunit. The more rapidly moving peak, must, therefore, constitute the isomaltase subunit. This interpretation is supported by estimation of molecular weights with the standard proteins, β-galactosidase, catalase, and ovalbumin. When the mobility was plotted as a log function of molecular weight (25), values for the sucrase sub-
**Discussion**

The hybrid enzyme has been isolated from human small intestine without additional treatment (Fig. 1). Sucrase activity was found to be approximately 235,000 by ultracentrifugation analysis (8, 18), but values as low as 120,000 and 110,000 for the subunits were also obtained (33). The comparable pure rabbit isomaltase has been reported to have a molecular weight of 220,000 (33). The combined molecular weights of free S and I are reasonably close to the weight of the S-I complex. Further, the S-I complex is a relatively elongated molecule whose axial ratio is approximately twice that of either S or I. Even though determination of axial ratios gives only an approximate indication of molecular shape (29), the 2-fold difference in these ratios for the S-I hybrid as compared to the free S and I moieties suggests that the complex is formed by union of S and I at their long axes.

Small intestines solubilized by allowing autolysis to occur at pH 9.6 at 37° for 30 min (8). However, this technique destroyed more than 90% of the sucrase activity, so that it was necessary to use papain treatment to release the enzymes from the brush border surface membrane. This solubilization technique releases various membrane proteins at different rates, and might therefore be used to release the free isomaltase subunit. Second, the isomaltase subunit binds so avidly to the Sephadex gel at 4° that it is eluted long after any proteins that migrate through the column by permeation alone (Fig. 1). Finally, the free isomaltase is accompanied by high concentrations of glucose that it has hydrolyzed from the Sephadex gel matrix. This produces high blanks in the disaccharidase assay, making identification of the isomaltase activity difficult. We were able to identify the free isomaltase subunit in only half of human small intestines solubilized by allowing autolysis to occur at 37°. This, along with other data discussed above, suggests that the isomaltase moiety becomes labile when released from the native sucrase-isomaltase complex. Whether sucrase and isomaltase exist separately as well as in the hybrid molecule within the intestinal surface membrane is impossible to decipher from our experiments, but, given the finding that 90% of the two activities are contained in the hybrid molecule, it seems likely that the predominant native species is the sucrase-isomaltase complex.

Since kinetic and pH activity experiments revealed identical characteristics whether the α-glucosidases were present in the complex or as smaller separate proteins (Fig. 4), it seemed reasonable that they may constitute the enzymatically active subunits of the hybrid enzyme. Comparison of the size and shape of the hybrid to the free sucrase and isomaltase moieties also suggests such a relationship for the enzymes. As shown in Table II, the combined molecular weights of free S and I are reasonably close to the weight of the S-I complex. Further, the S-I complex is a relatively elongated molecule whose axial ratio is approximately twice that of either S or I. Even though determination of axial ratios gives only an approximate indication of molecular shape (29), the 2-fold difference in these ratios for the S-I hybrid as compared to the free S and I moieties suggests that the complex is formed by union of S and I at their long axes.

**Urea-mercaptoethanol treatment of the sucrase-isomaltase complex by dialysis to remove the urea consistently produced free sucrase and isomaltase moieties that differed slightly from each other in molecular weight (Fig. 6). Although the yield of active subunits was not high, it was several-fold greater than that produced by spontaneous cleavage observed in the control sample dialyzed against buffer alone and the recovery of total enzyme activity in hybrid and free form was nearly quantitative. In other experiments, we have observed up to 2% conversion of sucrase from the hybrid to the free form when pure sucrase-isomaltase is stored for 30 to 60 days at -20°. Interestingly enough, no free active isomaltase is produced under these conditions, suggesting again that the isomaltase subunit may be more labile after separation from its sucrase partner. Recently, an isomaltase subunit has been produced from rabbit sucrase-isomaltase at twice the specific activity of that in the native complex by exposure to pH 9.0 at 4° for 3 days followed by pH 9.6 at 37° for 30 min (8). However, this technique destroyed more than 90% of the sucrase activity, so that it was difficult to compare the biochemical and physical characteristics of the two subunits. Hence, we have not resorted to alkaline treatment of human sucrase-isomaltase.

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**Fig. 7. Sodium dodecyl sulfate-acrylamide gel electrophoresis**

(25) of the native hybrid (Fig. 6). Despite extensive studies of mammalian intestinal α-glucosidases, the free native isomaltase had not been previously identified except in a single human intestinal preparation (7). There appear to be several important reasons for this. First of all, papain treatment has routinely been used to release the enzymes from the brush border surface membrane. This solubilization technique releases various membrane proteins at different rates, and might denature the free isomaltase subunit. Second, the isomaltase subunit binds so avidly to the Sephadex gel at 4° that it is eluted long after any proteins that migrate through the column by permeation alone (Fig. 1). Finally, the free isomaltase is accompanied by high concentrations of glucose that it has hydrolyzed from the Sephadex gel matrix. This produces high blanks in the disaccharidase assay, making identification of the isomaltase activity difficult. We were able to identify the free isomaltase subunit in only half of human small intestines solubilized by allowing autolysis to occur at 37°. This, along with other data discussed above, suggests that the isomaltase moiety becomes labile when released from the native sucrase-isomaltase complex. Whether sucrase and isomaltase exist separately as well as in the hybrid molecule within the intestinal surface membrane is impossible to decipher from our experiments, but, given the finding that 90% of the two activities are contained in the hybrid molecule, it seems likely that the predominant native species is the sucrase-isomaltase complex.

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Since the bulk of the sucrase-isomaltase is recovered in the
complexed state after removal of urea-mercaptoethanol (Fig. 7), major stable intermolecular bonds between sucrase and isomaltase must exist to maintain the integrity of the molecule. Exposure to urea alone followed by dilution or dialysis produced no subunits. The requirement for mercaptoethanol in addition to urea in order to produce cleavage suggests that one or more disulfide bonds may play a role in the union of the S and I moieties, but further study of the structure of the native hybrid and its subunits depends upon isolation of far greater quantities of the proteins than have been recovered at present.

Sodium dodecyl sulfate-acrylamide gel electrophoresis also revealed the presence of sucrase and isomaltase subunits (Fig. 7) having molecular weights very close to those for the enzymatically active free subunits (Table II). Since the sum of the molecular weights of the sucrase and isomaltase subunits is 30,000 less than the apparent weight of the sucrase-isomaltase complex (Table II), a polypeptide of this size might serve as a linkage peptide between the two enzymes. However, sodium dodecyl sulfate electrophoresis of \(^{32}P\)-labeled sucrase-isomaltase revealed only the two subunits. A 30,000 molecular weight peptide was identified (Fig. 7), nor was there evidence of smaller subunits comprising sucrase or isomaltase. However, it is still possible that a junctional peptide, especially if it were located in the interior or hydrophobic region of the hybrid complex, might not be accessible to the radiodine in the labeling procedure. Regardless of whether a junctional peptide is a component of the native hybrid molecule, it appears that the major components are two distinct macromolecules that represent the monomeric forms of each \(\alpha\)-glucosidase.

In addition to the slight but distinct difference in molecular weight, the free S and I moieties show markedly different affinities for the rabbit anti-S-1 antibody. Whereas free sucrase binds even more avidly than the hybrid enzyme used as the immunogen, free isomaltase has very weak affinity (Table III).

The reason for the existence of the two distinct \(\alpha\)-glucosidases in a single molecule is unclear. It is difficult to believe that the partnership exists solely to afford stability for isomaltase. Yet, it is possible that a junctional peptide, especially if it were located in the intestinal lumen for the isomaltase moiety (34), and it is possible that the two distinct subunits act in concert at different linkages of the relatively large \(\alpha\)-dextrin molecule (average molecular weight, 1500), especially since sucrase is capable of hydrolyzing \(\alpha\)-1,4-glucosidic linkages (3). 

Sucrase-isomaltase deficiency (35) is a rare recessively inherited malady that produces an osmotic diarrhea when the appropriate sugars remain undigested in the intestinal lumen. Although absence of both enzymatic moieties has suggested a single mechanism of genetic control, the distinct differences in the sucrase and isomaltase subunits and the apparent lability of the isomaltase moiety when it is separated from sucrase imply that defective synthesis of sucrase may constitute the primary defect, while the isomaltase moiety is secondarily depressed because of the unavailability of its partner. Indeed, Eggemont and Hers (32) have noted the presence in sucrase-isomaltase-deficient intestine of some residual isomaltase that is distributed between 6.2 S and 12.6 S components upon ultracentrifugation. In light of our studies, the smaller component is likely to constitute the free isomaltase subunit (Table II), and the 12.6 S may represent an aggregate of the subunit either with itself or other glycoproteins (32).

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**REFERENCES**

1. **KOLINŠKA, J., and SEMENZA, G.** (1967) Biochim. Biophys. Acta 146, 181-195
2. **KOLINŠKA, J., and KRAML, J.** (1972) Biochim. Biophys. Acta 284, 235-247
3. **SEMENZA, G., AURICCHIO, S., and RUBINO, A.** (1965) Biochim. Biophys. Acta 86, 487-497
4. **CUMMINS, D. L., GITTELZMANN, R., LINDEMAN, J., and SEMENZA, G.** (1968) Biochim. Biophys. Acta 159, 396-403
5. **NELSON, T. E., KOLD, E., and LARNEF, J.** (1960) Biochemistry 8, 1419-1428
6. **DAHLQUIST, A., and TELENIUS, U.** (1969) Biochem. J. 111, 139-140
7. **ASP, N.-G., and DAHLQUIST, A.** (1973) FEBS Lett. 35, 303-305
8. **CIGNOLI, A., EDERLE, A., SIGURST, H., JOSS, C., ROBINSON, E., MOSSMANN, H., and SEMENZA, G.** (1973) Eur. J. Biochem. 33, 30-48
9. **WALTER, W. M., Jr., and GRAY, G. M.** (1968) Gastroenterology 54, 56-69
10. **AURICCHIO, S., DAHLQUIST, A., and SEMENZA, G.** (1969) Biochim. Biophys. Acta 73, 582-587
11. **GRAY, G. M., and SANTIAGO, N. A.** (1969) J. Clin. Invest. 48, 716-726
12. **LINEWEAVER, H., and BURH, D.** (1924) J. Am. Chem. Soc. 46, 658-666
13. **RACKER, E.** (1955) Methods Enzymol. 1, 500-503
14. **CHANCE, B., and MARSHALL, A. C.** (1955) Methods Enzymol. 2, 764-768
15. **LOWRY, O. H.** (1957) Methods Enzymol. 4, 371-372
16. **GOLDBERG, J. A., and HUTENBERG, A. M.** (1958) Cancer 2, 283-290
17. **Gel Chromatography. (1971) pp. 1-89, Bio-Rad Laboratories, Richmond, Calif.
18. **TAKESUE, Y. (1969)** J. Biochem. (Tokyo) 66, 545-552
19. **DUREHAM, J. P., and IVES, D. H.** (1970) J. Biol. Chem. 245, 2276-2284
20. **WASHBURN, E. W., Ed.** (1926) International Critical Tables, McGraw-Hill, New York
21. **RODBRAID, R., and OBERBACH. A.** (1971) Anal. Biochem. 40, 95-134
22. **JOVIN, T. M., DANTE, M. L., and CHRAMBACH, A.** (1970) Multiphasic Buffers System Catalogue, PB 196909, National Technical Information Service, Springfield, Va.
23. **JOVIN, T. M., DANTE, M. L., and CHRAMBACH, A.** (1970) Multiphasic Buffer Systems. Instructions for Use of the Systems Catalogue, PB 196909, National Technical Information Service, Springfield, Va.
24. **JOVIN, T. M., DANTE, M. L., and CHRAMBACH, A.** (1970) Multiphasic Buffer Systems. Systems Output Tape No. 3, PB 196907, National Technical Information Service, Springfield, Va.
25. **WEBER, K., and OSBORN, M.** (1969) J. Biol. Chem. 244, 4406-4412
26. **ACKERS, G. K.** (1964) Biochemistry 3, 723-730
27. **SIEGEL, L. M., and MONTY, K. J.** (1966) Biochim. Biophys. Acta 112, 340-362
28. **BARTN, E. G., and AMEIS, B. N.** (1961) J. Biol. Chem. 236, 1372-1379
29. **YOUNG, E. G.** (1963) in Comprehensive Biochemistry ("Proteins") (FLOWKIN, M., and STOTZ, E. M., eds) Vol. 7, pp. 45-55, Elsevier, Amsterdam
30. **GREENWOOD, F. C., HUNTER, W. M., and GLOYER, J. S.** (1963) Biochem. J. 89, 111-125
31. **BURT, S., NITETT, E. S., SHEEP, C. J., SIECHENKIN, S. I., and UHR, J.** (1971) J. Immunol. 106, 1133-1135
32. **EGGEMONT, E., and HERS, H. G.** (1969) Eur. J. Biochem. 9, 488-496
33. **YAMASHIRO, K. M., and GRAY, G. M.** (1967) Gastroenterology 58, 1056 (Abstract)
34. **GRAY, G. M.** (1967) Fed. Proc. 26, 1415-1419
35. **BURGESS, A. E., LEVY, B., MAHALANABIS, D., and TONGE, R. E.** (1964) Arch. Dis. Child. 39, 431-443
Human intestinal sucrase-isomaltase. Identification of free sucrase and isomaltase and cleavage of the hybrid into active distinct subunits.
K A Conklin, K M Yamashiro and G M Gray

*J. Biol. Chem.* 1975, 250:5735-5741.

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