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Research Article

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Sucrase-α-Dextrinase in the Rat

Postinsertional Conversion to Inactive Molecular Species by a Carbohydrate-free Diet

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Abstract

Absence of dietary carbohydrate decreases both activities of intestinal brush border sucrase-α-dextrinase. We examined the molecular mechanism causing this decrease. Adult rats were fed chow (70% CHO) or matched carbohydrate-free (CHO-free) diet for 7 d. Sucrase activity decreased by 50% in whole homogenates and brush borders. Enzyme kinetics revealed no change in sucrose affinity (CHO-free K_m = 18 mM, chow K_m = 21 mM), but fewer active sites (CHO-free V_{max} = 2,720, chow V_{max} = 5,000 μmol/min per g protein). Intraintestinal pulse-labeling of [^{35}S]methionine in vivo revealed no differences in incorporation into sucrase. Immunoreactive sucrase protein, assayed by ELISA and rocket immunoelectrophoresis, increased twofold per milliunit of sucrase enzymatic activity in CHO-free jejunum. Total immunosucrase (S_t), the sum of active and inactive enzyme ($S_t = S_a + S_i$), was unchanged with carbohydrate withdrawal, but > 50% of the sucrase protein became inactive. SDS-PAGE of sucrase immunoprecipitates revealed alteration of α, β, and γ subunits in CHO-free animals: (*a*) α and β subunits migrated farther (mass change − 2 kD); and (*b*) the α subunit became diffuse or was a doublet and was less abundant than the β subunit. Rather than representing loss of sucrase protein, the decline in sucrase activity is achieved with structural subunit changes, probably involving postinsertional processing. (*J. Clin. Invest.* 1993. 91:2785–2790.) Key words: sucrase-isomaltase • immunoprotein • inactive enzyme • brush border • subunit structure

Introduction

Sucrase-α-dextrinase (S-D)[1] is a hybrid glycoprotein digestive hydrolase initially synthesized as a 230–260-kD macromolecule in the endoplasmic reticulum (ER) of the small intestinal enterocyte. After acquiring *N*- and *O*-linked carbohydrate chains accounting for ~ 25% of its mass in the ER and Golgi (1), it is transported to the brush border where it is cleaved by luminal pancreatic proteases to two subunits, each having a specific catalytic site, which remain noncovalently associated and attached at the luminal surface of the brush border via a small hydrophobic anchor at the original NH_2 terminus. Although this digestive hydrolase is crucial for interfacial digestion of dietary sucrose and the α-dextrin products of starch, little is known of the molecular details of its regulation by these nutrients. When dietary carbohydrate is partially or completely withdrawn, sucrase hydrolytic activity is reduced by ≥ 50% (2, 3, 4). Ingestion of a diet rich in carbohydrate is followed by enhancement of activity within days in animals (4) and humans (2). The acute adaptation of intestinal enzymes to changes in diet may be partly explained by a reduction in synthetic rate resulting in a smaller S-D brush border pool, but the mechanism of catalytic reduction has not been clearly elucidated (5, 6).

We have examined the structure and function of S-D in intact rats that had achieved a new steady state after being fed a carbohydrate-free diet for 7 d. Despite an appreciable decrease in catalytic activity of both oligosaccharidase sites, neither total S-D protein nor the rate of in vivo synthesis was changed. However, a definite structural change in the mature subunits was identified. Thus, despite the loss of hydrolytic activity of its two components, S-D protein levels appear to be maintained in the brush border membrane, though as an altered structure.

Methods

Chemicals and reagents. Horseradish peroxidase, glucose oxidase, Tris, L-methionine, DTT, 2-mercaptoethanol, *p*-nitrophenyl phosphate, *p*-nitrophenyl-α-D-mannopyranoside, Nonidet 40, and leucyl-β-naphthylamide and 2,2′-Azinobis(3-ethylbenzthiazonlinesulfonic acid) (ABTS) were obtained from Sigma Immunochemicals (St. Louis, MO). [^{35}S]L-methionine was obtained from Amersham (Corp., Arlington Heights, IL). Goat anti-rabbit horseradish peroxidase was from Tago, Inc. (Burlingame, CA). Acrylamide, ammonium persulfate, *N*′-*N*′-methylene bisacrylamide, and *N*′,*N*′, *N*′,*N*′-tetramethylethylenediamine were from Bio-Rad Laboratories (Richmond, CA). Isomaltose was a gift from Dr. Allene Jeanes, United States Department of Agriculture Laboratory (Peoria, IL). Lactose, cellobiose, sucrose, and glucose were from Pfanstiehl. Diets were prepared by U. S. Biochemical Corp. (Cleveland, OH). All other chemicals were analyzed reagents (J. T. Baker Chemical Co., Phillipsburg, NJ).

Enzyme assays. Leucyl-β-naphthylamide hydrolase, sucrase, isomaltase, cellobiase, and lactase activities were assayed as previously described (7). Protein was determined by the Bio-Rad assay (8); BSA was the standard protein.

Diet and animals. After arrival, adult male Wistar rats (250–350 g) were placed on rat chow for 7 d. Weights were monitored, and the diet was changed to either carbohydrate-free or, in control animals, continued on rat chow (70% CHO) for the next 7 d. The carbohydrate-free diet was made isocaloric with standard rat chow by replacing the carbo-

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1. *Abbreviations used in this paper:* CHO-free, carbohydrate-free diet; ER, endoplasmic reticulum; S-D, sucrase-α-dextrinase.

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hydrate with corn oil. Protein, vitamins, and minerals devoid of carbohydrate were matched to the content in rat chow. The carbohydrate-free group consumed similar amounts of total calories and maintained similar weight accumulation as the control animals during the experiments (±3% variation).

Labeling of small intestine in the intact rat. Rats, allowed only water for 16 h before the beginning of the experiments, were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally. The abdomen was opened, a 20-cm loop of proximal jejunum was catheterized at its proximal and distal ends (9) and gently flushed with warmed 0.9% NaCl to remove residual debris. After preperfusion with the saline solution at 0.5 ml/min for 1 h, the loop was labeled for 5 min with 1 mCi of [^{35}S]methionine (1,200 Ci/mmol) in 1.5 ml of 145 mM NaCl. The isotope was then flushed out with unlabeled 1 mM methionine and 145 mM NaCl, and the loop was perfused at the same rate with the warmed methionine-NaCl solution. After a variable chase period, rats were killed and the labeled jejunal segment was removed and washed with cold 145 mM NaCl and 1 mM DTT to remove adherent mucus.

Intestinal organelle fractionation. The jejunal mucosa was obtained by gentle scraping with glass microslides and subjected to a series of graded homogenization and centrifugation steps, including a final 20–60% sorbitol density gradient to separate subcellular organelles (7). Basolateral membranes were identified by sodium potassium ATPase, as determined by modification of the methods of Fujita et al. (10), and Fiske and Subbarow (11). ER-G membranes were identified by mannosidase II activity as previously described (12). Brush border membranes were identified by leucyl-β-naphthylamidase activity. ER-Golgi membranes were purified an average of 10-fold over the initial 100,000 $g \times 1$ h particulate preparation with an average yield of 0.05 (where 1.0 is the total available in the original particulate). Brush border membranes were purified ~ 15-fold with a yield of ~ 0.20.

Quantitation of radioactivity and SDS electrophoresis analysis. Radioactivity in ER-G and brush border fractions was determined by liquid scintillation counting of an aliquot of detergent-solubilized membrane fractions. S-D was isolated by specific immunoprecipitation using a monospecific, polyclonal antibody; specificity has been established previously by the presence of only the authentic α, β, and γ subunits on SDS gels (13), verified by NH_2-terminal amino acid sequencing of the immuno-isolated subunits (14). SDS-electrophoresis was carried out as described previously in Ahnen et al. (15) with a few modifications. The separating gel was 6.5% total acrylamide and 2% cross-linking. Bands were cut from dried gels and the radioactivity was quantified per enzyme unit. Total radioactivity for the particular membrane organelle, was determined as previously detailed (7).

Immunoprecipitation. Aliquots of detergent-solubilized membrane fractions and monospecific, polyclonal anti–S-D antibody at twice the estimated antibody binding capacity for chow fed rat sucrase were incubated for 20 min at 22°C and then rotated in the same tube for 18 h at 4°C to allow maximal binding. Prewashed inactivated staphylococcus aureus (50–200 μl) was then added to the mixture in a shaking water bath for 1 h at 37°C, and the immune pellets were recovered after microfuge centrifugation for 5 min. Supernatants were assayed for any residual unbound sucrase activity. In parallel control experiments, preimmune rabbit globulin was used instead of anti–S-D antibody.

Rocket immunoelectrophoresis. Rocket immunoelectrophoresis was performed as described by Axelsen and Bock (16). S-D was solubilized with 0.5% NP-40 from normal, chow-fed adult rat brush border membranes that were isolated as previously described (6, 15). This S-D preparation served to define the standard immunoassay curve. Rocket-like immunoprecipitin lines formed and area dimensions were correlated to the amount of antigen.

Solid-phase immunoassay of sucrase-α-dextrinase. Immunoassay of S-D immobilized on nitrocellulose according to the method of Palfree and Elliott (17) was used with a few modifications. Membrane samples were solubilized with 50 pulses of sonication with a cell sonicator (Branson Ultrasonics Corp., Danbury, CT) in a detergent buffer (0.5% NP-40, 0.15 M NaCl, and 0.01 M sodium-potassium phosphate, pH 7.5). This released ~ 90% of the sucrase activity into the 100,000 *g*-h supernatant. Samples were diluted to lower the detergent concentration below 0.01% of NP-40, applied to scored sheets of nitrocellulose (prewashed with water and dried), and allowed to dry on a sheet of Parafilm. Repeated applications were sometimes necessary when large amounts of antigen were being assayed. Residual binding sites on the nitrocellulose were then blocked by exposure to 2% FCS for 1 h. The sheet was washed three times for 15 min on a rocking platform with 200 ml of 0.15 M NaCl, and 0.05 M Tris, pH 7.0 (Tris-saline), picked up with Teflon-coated tweezers, and submerged sequentially (four times) into plastic dishes containing 200 ml of Tris-saline, and then incubated with 100 ml of a 1:1,000 dilution of monospecific, polyclonal anti–S-D in 2% FCS for 3 h. The nitrocellulose was then washed three times with Tris-saline and exposed to goat anti-rabbit IgG bound to horseradish peroxidase at 1:3,000 dilution for 2 h at 22°C. After three washes with Tris-saline, 1 × 1–cm squares were cut and transferred to 12 × 75–mm test tubes and the colorimetric reaction was performed with ABTS as the peroxidase substrate as described by Porstmann et al. (18). The reaction was stopped with azide, and, after removal of the nitrocellulose square, the absorbance of the substrate solution was measured at 418 nm. Each immunoblot assay was standardized against purified brush border sucrase as previously described by Cezard et al. (6) for normal, chow-fed adult rats. This assay allowed quantitation of sucrase protein associated with sucrase activity in the approximate range of 0–1 mU, and was reproducible (±5% coefficient of variation) from assay to assay.

Results

Effect of carbohydrate-free diet on sucrase-α-dextrinase activities in brush border. As shown in Table I, the sucrase specific activity in whole homogenate preparations from animals ingesting no carbohydrate for 7 d was only one third of that for animals consuming chow (70% CHO). Sucrase specific activity in brush border was also reduced comparably. In contrast, sucrase-specific activity in the purified intracellular ER-Golgi membranes was not significantly different in the carbohydrate-free and control groups. When calculated from recovery of marker enzymes, sucrase activity in the ER-Golgi membrane pool was not changed by carbohydrate withdrawal. α-Dextrinase activity (isomaltose as the specific substrate) was measured in some animals, and the sucrase to isomaltase activity ratio was consistently ~ 1.2 for both the control and carbohydrate-free groups, in agreement with previous findings (6). Hence, there was a parallel dramatic decrease in activity of both the sucrase and α-dextrinase activities in response to carbohydrate withdrawal.

Kinetic parameters of brush border S-D. Classical enzyme kinetics were done on brush border samples for sucrase (Fig. 1). Average V_{max} for the chow-fed group was 5,000 μmol/min per g protein; the K_m was 21 mM for sucrose. In contrast, the

*Table I. Effect of Diet on Sucrase Activity**

Diet	Whole homogenate	Brush border	ER-Golgi	Total ER-G
	mU/mg protein	*mU/mg protein*	*mU/mg protein*	*mU*
Lab chow	280±50	2,500±674	70±9	2,100±610
CHO-free	94±12‡	830±94§	50±6	1,600±270

* $n = 29$ for each group; mean±SEM; normal were placed on a 70% carbohydrate or CHO-free diet for 7 d, and the intestine processed as detailed in Methods.
‡ $P < 0.001$.
§ $P < 0.02$.

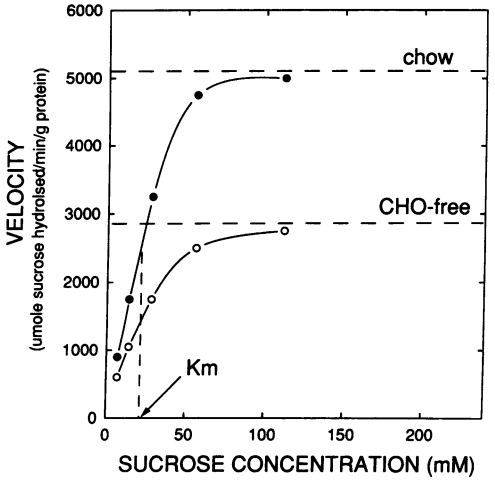

Figure 1. Comparative kinetics of sucrose hydrolysis by brush border membranes. After regular chow or CHO-free diet for 7 d, kinetic values, determined from Eadie-Hofstee plots are shown (V_{max}, *intersection of dashed line at ordinate*). K_m at abcissa was not significantly different for the two groups.

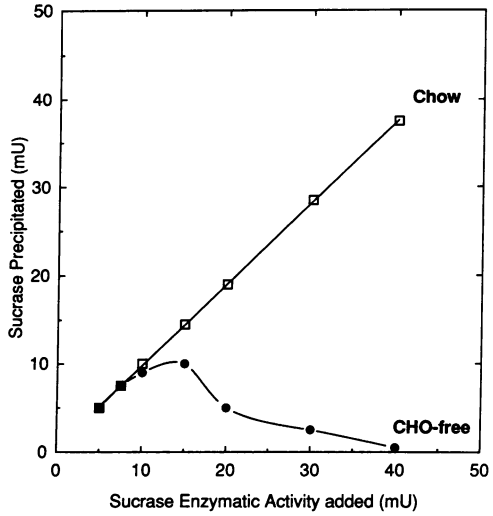

Figure 2. Immunoprecipitation of sucrase activity after regular chow or carbohydrate-free (CHO-free) diets. The monospecific, polyclonal anti-S-D antibody (50 μl; capacity 50 mU sucrase from normal rats) was added to 50 mU of sucrase activity in NP-40-solubilized brush border samples at 22°C for 20 min, and at 4°C overnight. Prewashed staph A cells (25 μl of 10% suspension) were then added at 22°C and the mixture agitated for 1 h. Samples were centrifuged at 10,000 *g* for 10 min, and any residual sucrase in the supernatant was subtracted from the original sucrase in the reaction to determine the quantity precipitated.

average V_{max} for the carbohydrate-free group was 2,720 μmol/min per g protein; the K_m was 18 mM for sucrose. Thus, while the K_m did not change significantly, the V_{max} of the carbohydrate-free group was reduced to ~ 50% of control. This implies a loss in the number of active catalytic sites, either by a reduction in the number of S-D glycoprotein molecules or by conversion of the S-D to a catalytically inactive glycoprotein species. Further studies were designed to distinguish between these possibilities.

Effect of carbohydrate-free diet on sucrase-α-dextrinase synthesis rates. Synthesis rates were compared by examining the incorporation of [^{35}S] methionine in vivo into newly synthesized S-D in purified ER-Golgi membranes (Table II). A maximal incorporation into S-D was achieved in these intracellular membrane preparations at 60 min and declined appreciably for both rat groups by 3 h. No significant difference was seen at the different time points.

Quantitation of S-D protein by immunoassay. Immunoprecipitation of S-D was performed on NP-40 solubilized brush border samples. The amount of sucrase activity precipitated was calculated by subtracting the activity remaining in the post-precipitation supernatant from the total in the sample, as shown in Fig. 2. Anti-S-D antibody precipitated much more catalytically active sucrase from chow-fed (70% carbohydrate) brush border membranes than from the carbohydrate-deprived animals. This suggested that the carbohydrate-free samples contained some catalytically inactive S-D protein that competes with the active S-D for binding to the antibody, resulting in more residual sucrase activity in the postprecipitation supernatant. To examine this possibility, we designed two different quantitative immunoassay systems.

Rocket immunoelectrophoresis was performed on detergent solubilized brush border samples (Fig. 3). Immunoreactivity per mU of brush border sucrase activity for the CHO-deprived rats was increased two times over the CHO-fed controls, as estimated along the linear portions of the curve. For example, addition of 4 mU of sucrase activity for CHO-free animals

*Table II. Synthesis Rate of S-D**

	10^{-3} × Total sucrase radioactivity in ER-G			
	30′	60′	120′	180′
Chow-fed	712±27	861±68	610±33	450±56
CHO-free	778±42	945±91	591±62	394±45

* n = 4 animals for each period; mean DPM in immunoprecipitated S-D±SEM after a 5-min intraintestinal pulse with [^{35}S]methionine and chase with 1 mM methionine for 30–180 min. Total specific radioactivity represents adjustment for losses caused by the purification process, determined by monitoring of membrane marker proteins; for details, see Methods.

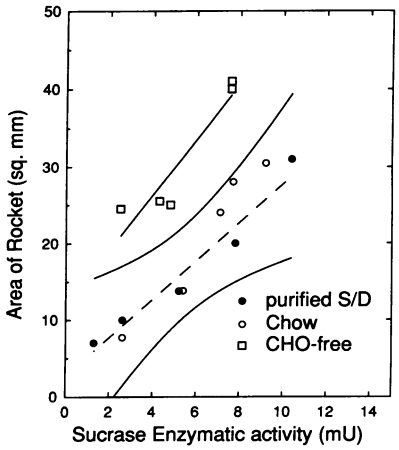

Figure 3. Quantitation of immunoreactive sucrase by rocket immunoelectrophoresis. Detergent solubilized brush border samples were placed in wells cut in agarose impregnated with monospecific, polyclonal anti-S-D antibody. Area under the precipitin line was measured and compared to enzymatic activity. Purified S-D standard (20 IU/mg protein) served as the standard.

produced a rocket area (25 mm^2) comparable to that achieved by 9 mU of sucrase in the normal controls. This confirms the concept that there is an appreciable amount of sucrase protein present in the carbohydrate-free samples that cannot be identified by its catalytic activity.

Although rocket immunoelectrophoresis is quantitative, it is less sensitive than other immunoassay techniques. Thus, a sensitive and specific quantitative solid phase immunoassay was performed on both ER-Golgi and brush border membrane samples that were immobilized on nitrocellulose (see Methods). Again, immunoreactivity, expressed as a function of brush border sucrase activity added, was about two times greater for CHO-free rats than for normal rats (Fig. 4), confirming the results from the rocket immunoelectrophoresis experiments. Separate experiments using monoclonal anti-S-D antibody instead of the polyclonal antibody were also carried out and the curves essentially duplicated those generated by the polyclonal antibody (not shown). The immunoassay can be used to estimate the total S-D glycoprotein, and the following equation can be applied: $S_t = S_a + S_i$, where S_t is the total activity measured by immunoassay, S_a is catalytically active sucrase, and S_i is the inactive sucrase, the unknown to be determined. As shown in Table III, applying this equation and presuming that S-D in the normal chow fed rats is completely active (i.e., $S_i = 0$ and therefore $S_t = S_a$), we calculate that the total S-D protein (S_t) is maintained in carbohydrate deprived animals, but that a large amount (60%) is inactive (S_i).

Comparative SDS electrophoresis of S-D from normal and CHO-free rats. As shown in Fig. 5, discrete and consistent changes in patterns for the α, β, and γ subunits of S-D in rats fed the CHO-free or the regular chow diet were observed by SDS electrophoresis. Although the 230-kD P precursor, the S-D species initially synthesized and inserted into the brush border membrane, migrated identically in control and CHO-free rats (Fig. 5, all lanes near top of gel), the 140-kD α subunit was less prominent and migrated farther and more diffusely in the CHO-free animals (Fig. 5, lanes *1, 3, 5,* and *7*) than in chow-fed controls (Fig. 5, lanes *2, 4,* and *6*), often separating as an α–α' doublet (Fig. 5, lanes *5* and *7*). The relative decrease in the quantity of the α subunit can be appreciated from the α/β ratios determined from transmitance scans of Fig. 5: chow-fed, 0.86; CHO-free, 0.29. The β subunit also migrated slightly farther in CHO-free (Fig. 5, lanes *1, 3, 5,* and *7*) than in control animals (Fig. 5, lanes *2, 4,* and *6*). The apparent mass changes for both the α and β subunits were ~ -2 kD. Also, one or more bands migrating in the region of the γ subunit (a species derived from processing of the α by removal of an $\sim$ 40-kD NH_2-terminal segment; see references 13 and 14), were more prominent in CHO-free samples. In some CHO-free animals, additional species migrating above or below than the γ unit could be identified (Fig. 5, lanes *5* and *7*). In the aggregate, these differences appear to reflect a more extensive postinsertional processing in response to withdrawal of dietary carbohydrate.

*Table III. S-D in Brush Border Membranes**

	Total sucrase	Active sucrase	Inactive sucrase
		mU/mg protein	
Chow-fed	280	280	0
CHO-free	235	94	141

* Calculated values from the equation $S_t = S_a + S_i$, where S_t is the total S-D by quantitative immunoassay, S_a is active sucrase, and S_i = inactive sucrase. S_i is assumed to be 0 for the normal chow-fed rat. Data for activity are from Table I. Total sucrase, expressed in equivalent units, was determined from the linear portion of the immunoassay curve (see Fig. 4).

Discussion

Mammalian small intestine responds rapidly to an increase in intraluminal carbohydrate by incrementally increasing the appropriate intestinal hydrolases. Short term starvation decreases total protein, DNA content, and especially the digestive oligosaccharidase levels. Feeding an isocaloric diet with a reduction in its carbohydrate content decreases the surface membrane α-glucosidases, and these are rapidly restored upon reintroduc-

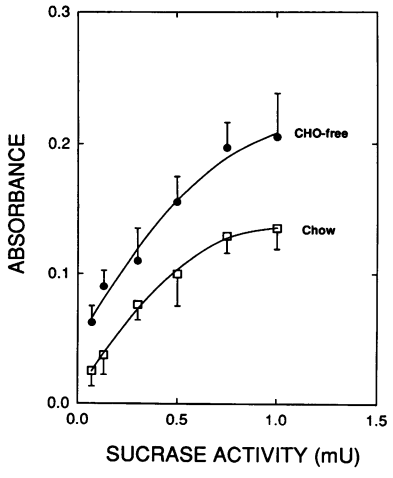

Figure 4. Quantitation of immobilized sucrase by dot-blot immunoassay. NP-40 solubilized brush border samples were applied to a washed, scored (1 cm × 1 cm–squares) sheet of nitrocellulose. Each sheet containing both chow-fed and CHO-free samples were blocked, washed, and then probed sequentially with primary and secondary antibodies. The individual samples were then separated for the final colorometric reaction. The chromagen released into the liquid phase was quantified at 418 nm. Each point represents the mean of four animals±2 SE.

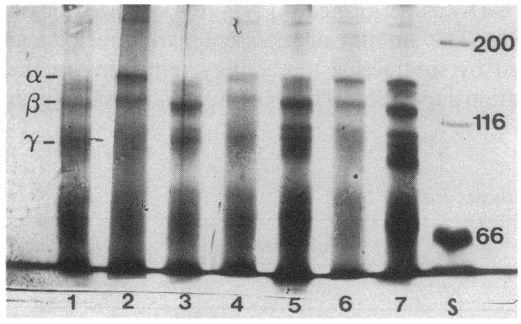

Figure 5. SDS-PAGE of S-D immunoprecipitates. Rats were fed regular chow or a CHO-free diet for 7 d. Intestinal brush borders were solubilized and S-D (25 mU) immunoprecipitated by monospecific, polyclonal anti-S-D (50 μl; capacity 1.5 mU/μl) as detailed in the Fig. 2 legend, and the postpreciptin supernatants were assayed for residual sucrase catalytic activity. Protein bands were silver stained. Three bands are indicated α, β, and γ (from top to bottom). Lanes *2, 4,* and *6,* chow-fed samples; lanes *1, 3, 5,* and *7,* CHO-free fed samples.

tion of dietary carbohydrate (2, 3, 4, 6, 19–22). This correlation of carbohydrate substrate intake with the appropriate saccharidase activities has been presumed to be regulated through decreased synthesis and consequent contraction of the oligosaccharidase pool. When other brush border hydrolases such as lactase and amino-oligopeptidase have been examined by immunoassay to assess the possibility of synthesis of catalytically inactive enzyme, the correlation of specific protein with catalytic activity have been very close, suggesting that there is no catalytically inactive enzyme protein, but rather a reduction of the enzyme protein pool caused by lower synthetic rates or enhanced degradation from the brush border (5, 23, 24). Despite the close correlation of dietary sucrose intake with sucrase activities, previous studies have examined primarily the catalytic activity alone, and have focused on the transient changes associated with acute dietary modification (6, 20, 25). Sucrase mRNA increases abruptly in response to acute reintroduction of dietary carbohydrate, compared to levels in cellulose-fed controls (25). Our studies were designed to explore the parameters of the new steady state acquired 7 d after the withdrawal of dietary carbohydrate with maintenance of caloric intake by isocaloric substitution of fat. Because the change in activity in response to diet (compare Table I) was manifested by a reduction in the V_{max} without a change in affinity (K_m) (Fig. 1), the reduced catalytic activity might be expected to be accounted for by changes in synthesis. Indeed, we were surprised to find no decrease in synthetic rates when a new lower level of sucrase and dextrinase activity was established by carbohydrate withdrawal for 7 d (Table II). Both the chow control and CHO-free diets were isonitrogenous and isocaloric conditions that have been documented previously to maintain a constant amino acid precursor pool (26).

These findings prompted us to examine other domains of the S-D glycoprotein by use of the polyvalent anti-S-D, which possesses several species of antibody that identify varied epitopes of the large S-D hybrid. An antiserum such as this serves to probe the overall S-D macromolecule rather than the enzyme active site, since a polyclonal anti-S-D can be expected to recognize several different regions of the glycoprotein molecule. Because we had observed essentially complete maintenance of sucrase catalytic activity in S-D immunoprecipitates (data not shown), we could presume there are no antibody species directed at the active site and that the antibodies recognize primarily other epitopes of the large S-D glycoprotein. Immunoassay by two different techniques, rather than revealing any reduction in immunoreactivity, identified a large pool of S-D glycoprotein in an inactive form (Figs. 2–4 and Table III).

The decline in catalytic activity associated with the maintenance of immunological activity implies that antigenic determinants are preserved when a new steady state is achieved after 7 d of carbohydrate withdrawal. A partial conformational change of the active site would be expected to decrease the affinity and consequently increase the required concentration (K_m). But the K_m was unchanged, while the V_{max} was reduced appreciably. The most likely explanation is a major conformational change in the area of the active site, conferring a total loss of specificity for sucrose for the majority of S-D molecules, but little or no change in the vast majority of other domains of the S-D glycoprotein. Dietary regulation by synthesis of an inactive enzyme pool has been shown for acetyl CoA carboxylase; this enzyme changes its subcellular distribution between the active cytosolic and inactive mitochondrial forms in response to a high carbohydrate diet (27). The inactive enzyme may represent a reservoir for activation during lipogenic conditions (28). In our studies, no such topographic redistribution of S-D occurred, the inactive enzyme remaining in the brush border membrane rather than being shuttled to intracellular sites (Table I).

Other possible mechanisms of nutritionally induced inactivation include covalent modification, primarily phosphorylation. For example, hepatic-branched chain α-ketoacid dehydrogenase becomes inactive and phosphorylated in rats fed a low protein diet and is reactivated by dephosphorylation (29). However, S-D has not been reported to be a phosphorylated glycoprotein.

While the biological reason for this preservation of the inactive S-D protein remains to be established, we initially suspected that the enzyme may be poised to reactivate when carbohydrate is reintroduced into the diet, as had been shown to be the case formethylenetetrahydrofolate reductase by adenosylmethionine (30). Once the structural change of subunits was discovered, however, it seemed unlikely that an efficient reactivation could be the result of simple allosteric regulation. In preliminary in vitro studies of the pure enzyme from carbohydrate deprived rats, we were unable to directly induce the recovery of catalytic sites by simply exposing brush border membranes from carbohydrate deprived rats to substrate (data not shown).

Analysis by SDS-electrophoresis revealed an alteration in the postinsertional processing of the brush border P protein precursor of the enzyme (Fig. 5). Recent studies in our laboratory by Shapiro et al. (13) had documented that the authentic α and β subunits are derived from P by a series of complex trimming steps catalyzed by luminal pancreatic trypsin. Now we find that animals deprived of dietary carbohydrate display an apparent enhancement of postinsertional processing of P to yield smaller α and β moieties (Fig. 5, odd-numbered lanes) as well as additional fragments that migrate to positions both above and below the authentic γ subunit (Fig. 5, lanes *5* and *7*). Notably, the γ unit is a physiological component of S-D in rats fed a regular chow diet which is produced by removal of a 40-kD segment from the NH_2 terminus of α (14). Whether the γ subunit retains a sucrase catalytic site is unknown. Thus the postinsertional processing of P appears to be more extensive in the absence of dietary carbohydrate, yielding some subunit species that are probably devoid of active sites. This is consistent with the reduction of V_{max}, but persistence of a normal K_m (Fig. 1), with the maintenance of S-D synthesis rates (cf Table I) and with stable quantity of S-D immunoprotein (Table III).

The molecular mechanisms of this additional processing of S-D subunits remains to be determined, but there is reason to believe that an extracellular process is involved. Because the P precursor is known to be glycosylated in the ER and Golgi before its insertion into the brush border, and its mass is unchanged in the CHO-free animals (Fig. 5, odd vs. even numbered lanes), altered glycosylation during intracellular processing cannot explain the changes that occur in response to carbohydrate deprivation. Since the final subunits of S-D are known to be derived from a single glycoprotein P precursor by action of luminal proteases, it seems likely that the relative quantities of secreted proteases and their subsequent action at the intestinal surface may be affected by the absence of carbohydrate in the luminal milieu. Perhaps the carbohydrate-rich glycocalyx

coat which defines the local microenvironment at the lumen-cell interface may also be altered in carbohydrate-free feeding, thereby permitting exposure of additional S-D domains to the bathing proteases. Other hydrolases, such as the amino-oligopeptidase, may be unaffected since they are relatively resistant to proteases. Whatever the events that enhance S-D processing to inactive species, they appear to produce definite structural effects on the enzyme and a resultant loss of functional activity.

Our experiments were not designed to examine the reacquisition of hydrolase activity after carbohydrate refeeding, but, considering the structural changes that appear to involve subunit trimming, it is doubtful that the reactivation will prove to be the result of rapid allosteric reversal. Instead, synthesis of new P precursor and a return to orderly restricted processing to the normal α and β components may be necessary, a modification that would require 2–3 d to replace the brush border S-D pool.

This appears to be the first example of regulation of a digestive enzyme by the postinsertional conversion to an inactive species at the brush border surface. We suspect that other examples of postinsertion conformational change in catalytic sites in response to changes in luminal dietary substrate withdrawal will be forthcoming.

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