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Sucrase-α-Dextrinase in the Spontaneously Diabetic Biobreed Wistar Rat: Altered Intracellular Carbohydrate Processing

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Abstract Sucrase-α-dextrinase (S-D), a glycoprotein hydrolase in the border surface of the enterocyte, is altered in congenitally diabetic BioBreed Wistar (BB_d) rats. Its intracellular assembly was examined in the current studies. Following pulse-chase experiments, S–D was specifically immuno-isolated from ER-Golgi and brush border membranes, and examined by SDS-PAGE and autoradiography. While synthesis and co-translational glycosylation of an immature species, P_i, in the ER proceeded normally, post-translational maturation of the N-linked carbohydrate chains was altered in the BB_d rat. The mature species, P_m, was 10 kDa larger in BB_d than in normal rats, and ~25% of its N-linked chains remained immature. The difference in mass was attributed to an appreciably larger mass of the O-linked chains of P_m in BB_d rats. In vivo kinetics of intracellular assembly displayed a delay in the appearance of P_m in Golgi (Wistar, 15 min; BB_d, 30–60 min). These experiments, revealing structural alterations in congenital diabetes suggest an important role for intracellular glycosylation in the orderly assembly and processing of brush border S-D in the enterocyte. J. Cell. Biochem. 81:252–261, 2001. © 2001 Wiley-Liss, Inc.

Key words: sucrase; dextrinase; intracellular assembly; kinetics; N-linked carbohydrates

Sucrase-α-dextrinase[1] (S-D), an intestinal brush border glycoprotein hydrolase necessary for the digestion of nutrient oligosaccharides, is initially synthesized as a single chain precursor, pro-(S-D) or P, in association with the endoplasmic reticulum (ER), transferred stoichiometrically to the Golgi apparutus where N- and O-linked carbohydrate chains are modified, and eventually translocated to the brush border surface [Cézard et al., 1979; Danielsen, 1982; Naim et al., 1988]. Anchored in the brush border membrane by a short hydrophobic segment of its N-terminus [Hunziker et al., 1986], P is then cleaved to non-covalently associated dextrinase (D) and sucrase (S) subunits by luminal pancreatic proteases [Quaroni et al., 1975; Hauri et al., 1979; Naim et al., 1988].

Abbreviations used: S-D, sucrase-α-dextrinase; P, pro(S-D) precursor; P_i, high-mannose glycosylated immature species of P; P_m, mature species of P; Endo H, Endo-β-*N*-acetylglucosaminidase H; PNGase F, Endoglycosidase F/N-glycosidase F; (e) subscript, Endo H-treated P species; (pf) subscript, PNGase F-treated P species; ER, endoplasmic reticulum; ERG, ER-Golgi; BBM, brush border membranes; Wist, normal Wistar rat; BB, BioBreed Wistar rat; BB_c, non-diabetic BB rat; BB_d, diabetic BB rat; $t_{1/2}$, half-life.

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[1]Sucrase-α-dextrinase, commonly known as sucrase-isomaltase, is usually assayed with sucrose and isomaltose as substrates. Because the α-1,4, 1,6 dextrin products of α-amylase action on starch rather than isomaltose they are the physiologic substrates in the intestinal lumen, we have used the name sucrase-α-dextrinase.

Catalytic activities of sucrase have been reported to be elevated in streptozotocin (STZ)- or alloxan-induced diabetes [Chaudhary and Olsen, 1973; Madara et al., 1982; Nakabou et al., 1985], and this has been correlated with low concentrations of immunoreactive insulin rather than with the toxicity of the diabetes-inducing drugs [Olsen and Korsmo, 1977]. The increase in total enzymatic activity has been correlated with an elevated specific catalytic activity in early stages and to an increase in intestinal mass in later stages of diabetes [Yamada et al., 1980; Goda et al., 1983]. Yet, no structural abnormality of S-D has been observed in chemically induced diabetes [Najjar et al., 1991].

The diabetic BioBreed rat (BB_d), selectively bred since 1974 [Wright et al., 1983], displays hyperglycemia, insulitis, polydipsia, polyuria and absolute dependence on insulin for survival, as well as many other common clinical and histopathological characteristics of human insulin-dependent diabetes mellitus. Hence it is an excellent animal model to study the molecular structure of S-D in diabetes mellitus.

Based on immunological and SDS-acrylamide electrophoresis studies, we have noted structural alterations of the S and D subunits in the BB_d rat as compared to healthy control animals. α-subunit,[2] the slower migrating 140 kDa subunit on SDS acrylamide electrophoresis is either smaller (−5 kDa) or indistinguishable from normal, and the 125-kDa β subunit is uniformly larger (+5 kDa) than that in normal Wistar rats [Najjar et al., 1991]. These structural changes, observed in both the newly-synthesized and steady state brush border membrane pool of S-D regardless of the severity of diabetes in the BB_d rat, are independent of hyperglycemia and are restored to normal when animals are treated with insulin for 2–3 weeks [Najjar et al., 1991]. The basis of the structural alteration in the S-D glycoprotein and its recovery remains unknown.

[2]When our previous paper was published [Najjar et al., 1991], the larger α-subunit was believed to carry the dextrinase active site and the β-subunit to possess the sucrase site; therefore they were designated as the D-subunit and the S-subunit respectively. But subsequent detailed analysis of rat S-D subunit assignment by differential denaturation–renaturation has revealed that sucrase is confined to the α-subunit and dextrinase to the β-subunit Zhu et al. [1991].

In the present study, we examined the intracellular synthesis and trafficking of the single-chain precursor of S-D in intact BB_d rats. We showed that the structural changes of this glycoprotein hydrolase occur during its intracellular assembly, involving the post-translational maturation of its carbohydrate chains. We also showed that the intracellular assembly of the newly synthesized S-D precursor appears to undergo altered kinetics of trafficking within intracellular membrane compartments.

MATERIALS AND METHODS

Materials

The source of most chemicals and reagents are given, when they are listed in the subsections below. All other chemicals were Baker analyzed reagents.

Experimental Animals

Male BioBreed diabetic (BB_d), non-diabetic control (BB_c) and normal Wistar rats were obtained from the Animal Resources Division, Health Protection Branch, Health and Welfare, Ottawa, Canada. The BB_d animals, derived originally from a colony of Wistar rats that developed spontaneous diabetes [Chappel and Chappel, 1983], were 60–90 days of age, weighed 250–350 g and had developed diabetes 4–8 days prior to the date of the experiments. These rats were injected subcutaneously at 4:00 p.m. daily with 2–4 units of NPH, U-100 (Lilly) as previously described [Najjar et al., 1991]. At the time of the experiments, the BB_d rats had hyperglycemia without ketoacidosis (serum glucose; ≥ 300 mg/dl; serum ketones 0.1–0.5 mM). BB_c, the siblings of BB_d rats that failed to develop diabetes even after 120–180 days of age, weighed 300–450 g. Normal Wistar rats were age- and weight-matched with BB_d rats, after preliminary experiments revealed that normal Wistar rats showed no structural change in S-D from 60 to 180 days of age [Najjar et al., 1991]. All animals were housed in the same room in cages equipped with air filters (Lab Care Caging System, Research Equipment Co. Ltd-Byran, TX) and were fed laboratory rat chow ab libitum. Rats were given 1 mCi of [^{35}S]methionine intraluminally at 7:00 a.m. after an overnight fast (only water was provided). During the experiment, whole blood glucose was assayed by Glucose Analyzer (Boehringer Mannheim Co., Indianapolis, IN),

and urinary and blood ketone and glucose were checked by the Ketositix and Dipstick methods (Roche Diagnostics), respectively. Serum was kept at −20°C for later determination of D-(−)-β-hydroxybutyrate [Williamson and Mellanby, 1984], a ketoacidosis marker, and glucose (Beckman Glucose Analyzer-2).

Intraluminal Labeling of Proximal Jejunum

Jejunal loops (20 cm length begining at the ligament of Trietz) were isolated in anesthetized rats as described previously [Ahnen et al., 1982]. The jejunal segment was preperfused with 0.9% NaCl (37°C) at 0.5 ml/min for 30–60 min, pulsed with the radiolabeled L-[^{35}S]methionine trace (1 mCi; 1200 Ci/mmole; Amersham Pharmacia Biotech) in 3 ml of 0.9% NaCl for 5 min, and chased with 1 mM of unlabeled L-methionine in 0.9% NaCl for 15–180 min. The jejunal segment was then removed, flushed with 0.9% NaCl containing 1 mM dithiothreitol, cut longitudinally and the mucosa scrapped off and processed for organelle fractionation as described below.

Intestinal Organelle Fractionation

ER-Golgi and brush border membrane fractions were prepared as previously described [Ahnen et al., 1982, 1983; Nguyen et al., 1987]. The scrapped mucosa was homogenized with ten vertical strokes of a loose-fitting pestle A Dounce homogenizer in 10 ml/g tissue of 5 mM histidine (adjusted to pH 7.4 with imidazole), 5 mM EDTA and a mixture of protease inhibitors of pepstatin A, chymostatin, antipain dihydrochloride, aprotinin and leupeptin (Sigma, St. Louis, MO), added to achieve a final concentration of 10 μg/ml each. The homogenate was subjected to a series of differential centrifugation steps and the final pellet was centrifuged to equilibrium on a 25–60% sorbitol gradient for 18 h. On this gradient, the ER and Golgi membranes co-migrated and were pooled together as ER-Golgi fractions. Specific enzymatic markers were used for ER-Golgi (α-mannosidase II) [Nguyen et al., 1987] and brush border (aminooligopeptidase) membranes [Ahnen et al., 1982]. Under these conditions, each fraction was purified 8–17 fold.

S-D Immunoprecipitation, Electrophoresis, and Autoradiography

ER-Golgi and brush border membranes were solubilized in Detergent Buffer (0.5% NP-40, 0.15 M NaCl, 0.01 M sodium potassium phosphate, pH 7.5). S-D was then assayed and specifically immunoprecipitated by polyclonal anti-S-D antibody. The immune pellet was then taken up in Solubilization Buffer (63 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 10 mM dithiothreitol, 1 mM EDTA), subjected to electrophoresis in 6% acrylamide gels, with the upper reservoir buffer including 0.05% SDS and the lower reservoir buffer lacking SDS. The gels were then fixed in 25% isopropanol, 10% acetic acid, stained with coomassie-blue, dried and exposed to Kodak XOMAT films at −70°C. The R_f, the distance from the origin that the glycoprotein migrated as a ratio to the distance from the origin to the front of the marking dye, was determined independently by three observers with agreement to ±0.020. The molecular mass was then estimated to the nearest 5 kDa by fitting on plots of log R_f versus molecular mass of standard proteins (Bio-Rad). In some experiments, the bands were excised from the dried gels (using the autoradiogram as template), solubilized in Solulyte–Lipofluor–water (1:10:0.2) for direct quantitation of radioactivity by scintillation counting. In other experiments, the autoradiogram was scanned on an imaging densitometer (Eagle Eye II-Stratagene, Inc), and the proteins quantitated on the Image NIH v1.60 Macintosh software program.

Treatment of S-D by Endoglycosidases

The S-D immunoprecipitates were boiled for 5 min in 150 mM sodium citrate, 2% SDS, pH 5.5, centrifuged at 15,000*g* for 5 min and treated with Endo-β-*N*-acetylglucosaminidase H (Endo H, Boehringer Mannheim Co.), as described previously [Choice et al., 1999]. Briefly, Endoglycosidase F/N-glycosidase F Endo H (10 mU) was added to the recovered supernatant, and the digestion mixture placed at 37°C for 1 h with a thin overlay of toluene. Control samples were treated identically except that incubation was in buffer without Endo H. After addition of albumin (15 μl of 10 mg/ml) to enhance recovery of the protein pellet, the Endo H-treated samples were precipitated with 10% trichloroacetic acid, washed with cold acetone (−20°C), and analyzed by SDS-PAGE.

Treatment of the S-D pellet by Endoglycosidase F/N-glycosidase F (Endo F) (PNGase F, Boerhinger Mannheim Co.) was performed as described previously [Choice et al., 1999]. Briefly, the S-D immune pellet (~20 mU of S

from ER-Golgi or ~200 mU from brush border) was washed three times with 62.5 mM Tris–HCl (pH 6.8), and heated at 100°C for 2 min in 100 μl of 2% SDS, 2% β-mercaptoethanol, 0.2 M sodium phosphate (pH 8.6). After centrifugation at 15,000*g* for 5 min, the supernatant was recovered in 800 μl of Buffer A (0.2 M sodium phosphate, pH 8.6, 1% NP-40, 0.5% β-mercaptoethanol) and combined with PNGase F, 5 units in 100 μl of Buffer A. The mixture was covered with a thin layer of toluene and left at 37°C for 19 h. The reaction was stopped by placing the mixture on ice. After addition of albumin (15 μl of 10 mg/ml) as a carrier, and reaction with trichloroacetic acid (final concentration 5%), the mixture was placed on ice for 1 h. The final protein pellet was washed with cold acetone and subjected to SDS-electrophoresis and autoradiography as described above.

RESULTS

Altered Maturation of N-Linked Chains in the BB_d Rat

Normal Wistar, BB_c and BB_d rats were fasted overnight and a jejunal loop prepared (cf. Materials and Methods). After a 5 min intraluminal pulse with 1 mCi of [^{35}S]methionine and a 30–60 min chase with non-radioactive methionine, S-D was solubilized and immunoprecipitated from ER-Golgi membranes, treated with Endo H, and analyzed by electrophoresis and autoradiography (Fig. 1). Measurement of the distance migrated as a ratio to the distance from origin to gel front (R_f) revealed that the immature precursor (P_i) migrated identically in Wistar, BB_c and BB_d rats with an apparent mass of ~220 kDa (Fig. 1, odd-numbered Endo H-lanes, R_f: 0.306–0.308). After the removal of immature N-linked glycans from P_i by Endo H, the deglycosylated protein, $P_{i(e)}$, again migrated comparably in all groups of rats (Fig. 1, even-numbered Endo H+lanes, R_f: 0.328–0.331) with an apparent mass of ~200 kDa. Thus, P_i appeared to be synthesized and assembled normally as a high-mannose glycoprotein in the BioBreed diabetic (BB_d) rat as compared to its controls: the BioBreed control (BB_c) and normal Wistar (Wist) rats.

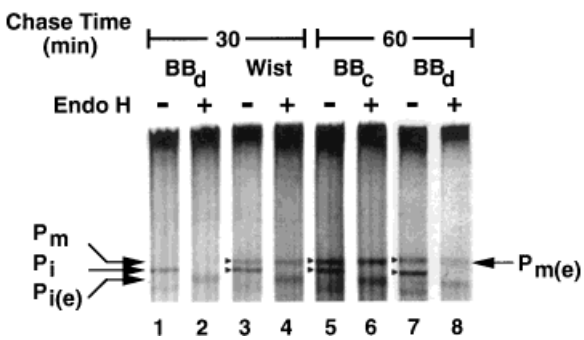

Fig. 1. Altered maturation of N-linked chains in the BB_d rat. Proximal jejunal loops of Wistar, BB_c and BB_d rats were pulse-chased intraluminally for 30 and 60 min. The S-D precursor (P) from ER-Golgi fractions (25–65 mU) was solubilized, immunoprecipitated, treated with Endo H (+) or with buffer alone (–), and analyzed by 6% SDS-acrylamide gel electrophoresis and autoradiography. P_i denotes the immature and P_m the mature form of the precursor. The figure is representative of two independent experiments.

Maturation of the carbohydrate chains (after 60 min of chase) yielded a larger species (P_m) in BB_d than in BB_c (Fig. 1, R_f: 0.279 in BB_d vs. 0.289 in BB_c, lane 7 vs. 5) and normal Wistars (Fig. 2A, R_f: 0.697 in BB_d vs. 0.752 in Wist, lane 1 vs. 3) (~240 vs. ~230 kDa). This can be appreciated by comparing the differential distance between the P_i–P_m doublet bands in Wistar or BB_c versus the BB_d rat (Figs. 1 and 2A, Endo H-lanes, internal arrows). Although Endo H had only a slight decrease (by less than 1 kDa) or an equivocal effect on the apparent mass of P_m in normal Wistar (Fig. 2A, R_f: $P_{m(e)}$ 0.758 vs. P_m 0.752; lane 4 vs. 3) and BB_c rats (Fig. 1, R_f: $P_{m(e)}$ 0.291 vs. P_m 0.289; lane 6 vs. 5), it reduced the mass of P_m by ~5 kDa in the BB_d rat (Fig. 1, R_f: $P_{m(e)}$ 0.287 vs. P_m 0.279, lane 8 vs. 7 and Fig. 2A, R_f: $P_{m(e)}$ 0.790 vs. P_m 0.697; lane 2. vs 1), suggesting that some of the high-mannose N-linked chains remain immature in the BB_d animal even after apparent maturation. Furthermore, the mature species in the brush border membrane of the BB_d rat retained Endo H sensitivity and migrated faster on SDS gels after Endo H treatment (Fig. 2B, 180 min of chase, $P_{(e)}$ vs. P, lane 2 vs. 1), as opposed to the P species in normal Wistars that became virtually Endo H-resistant (Fig. 2B, 180 min of chase, $P_{(e)}$ vs. P, lane 4 vs. 3). Based on the reduction in the apparent mass of P_m by Endo H (~5 kDa of the total of 20 kDa of the N-linked chains), approximately 25% of the N-linked chains fail to mature in the BB_d rat. Despite this persistence of immature high-mannose N-glycosylation in one quarter of the N-linked chains, there was no suggestion of inhibition of its transport to the brush border, as suggested by the detection of P_m in the brush border fraction after 180 min of chase (Fig. 2B).

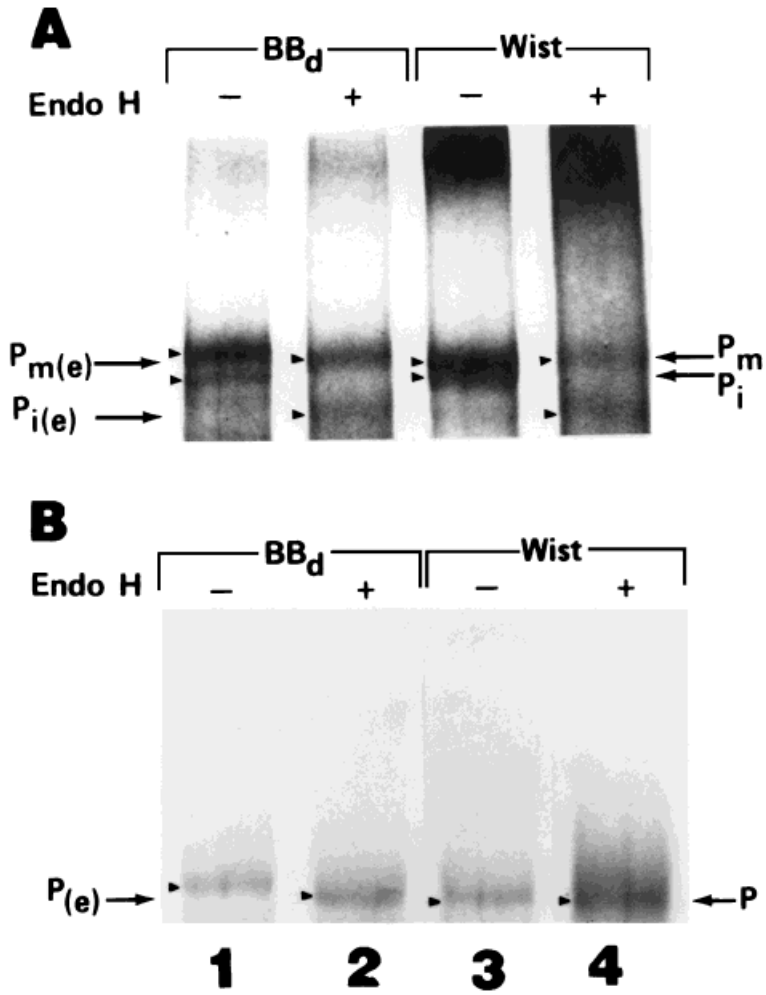

Fig. 2. Presence of immature N-linked chains of P_m in the BB_d rat. Wistar and BB_d rats were pulsed intraluminally for 5 min with 1 mCi [^{35}S] methionine and chased for 60 and 180 min with 1 mM methionine solution. S-D precursor (40–50 mU) was isolated from ER-Golgi fractions (**panel A**, 60 min) and brush border membranes (panel B, 180 min) treated with Endo H (+) or buffer alone (–) and applied to 6% SDS-acrylamide gel. $P_{i(e)}$ and $P_{m(e)}$ are the corresponding Endo H-treated species of P; (for brush border in **panel B**, the (m) subscript is omitted). Note the distinct increase in P migration after exposure to Endo H for the BB_d rat but not for the normal Wistar in both ER-Golgi (panel A) and brush border (panel B). The figure is representative of three independent experiments.

The increase in the apparent mass of P_m in the BB_d rat could be due to a change in the maturation of N-linked chains, altered structure or assembly of O-linked chains, or both. To address this question, newly synthesized S-D from ER-Golgi (ERG) and brush border (BBM) fractions were immunoprecipitated and treated with PNGase F prior to analysis on SDS-PAGE. Because PNGase F removes both high-mannose and complex N-linked carbohydrates, leaving any putative O-linked chains attached to the S-D protein chain, and Endo H only removes the high-mannose N-linked chains, comparison of the apparent mass of S-D before and after treatment with each of these enzymes provides considerable insight regarding the apparent mass attributed to N- and O-linked carbohydrates. Accordingly, the mass of N- and O-linked chains were derived from analysis of the P species in Figure 1 (Endo H treatment) and Figure 3 (PNGase F treatment), and summarized in Table I. Notably, the same mass for P_i was observed for Wistar and BB_d rats both before (~220 kDa) and after (~200 kDa) deglycosylation with Endo H (Fig. 1, 30 min, Endo H+vs-lanes) or PNGase F (Fig. 3, 15 min chase, PNGase F+vs-lanes, P_i vs. $P_{i(pf)}$; and Table I). Thus, the initial addition of N-linked high-mannose chains appeared to proceed normally in the BB_d rat. In contrast, whereas conversion of P_i to P_m in the normal Wistar resulted in a 10 kDa increase in mass ($220 \rightarrow 230$ kDa), twice the amount of carbohydrate (~20 kDa) was added in BB_d rats ($220 \rightarrow 240$ kDa) (Figs. 1, 3 and Table I). As estimated from deglycosylation experiments of P_m with PNGase F (Fig. 3, $P_m \rightarrow P_{m(pf)}$), the total mass of N-linked chains in P_m was comparable in Wistar and BB_d rats (Table I, CHO_n, 20 kDa each). The increase in the residual mass of the diabetic P_m after deglycosylation of its N-linked chains can be readily

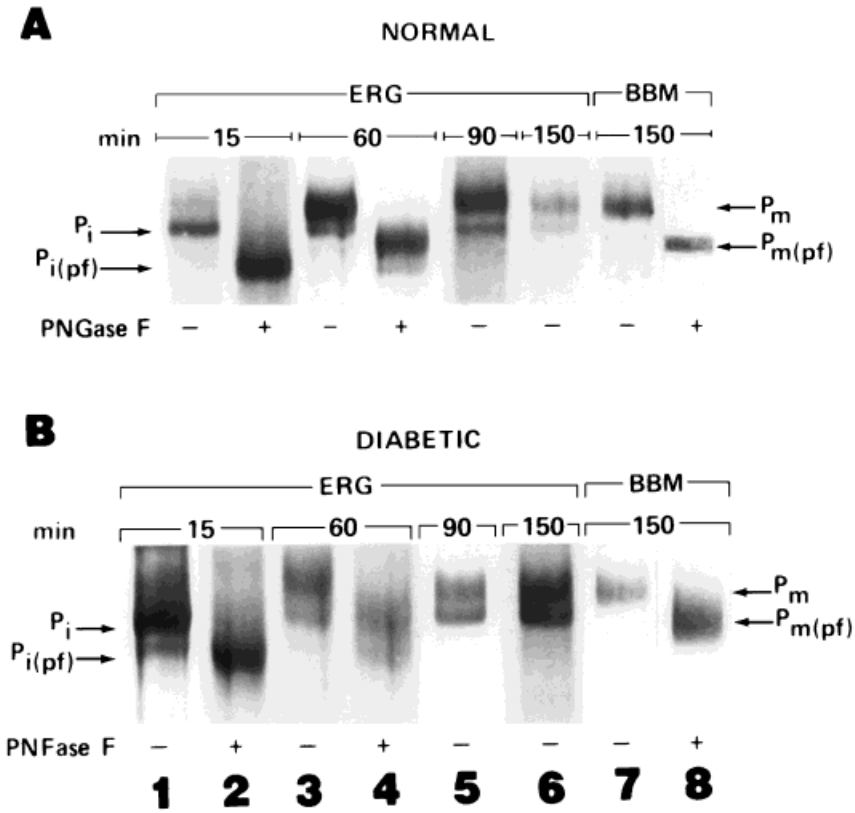

Fig. 3. Comparative kinetics of intracellular synthesis and assembly of S-D in intact rats. Wistar (**panel A**) and BB_d (**panel B**) rats were pulse-chased intrajejunally for 15, 60, 90, and 150 min; the P (10–70 mU), isolated from ER-Golgi (ERG) and brush border (BBM) after treatment with buffered PNGase F (+) or with buffer alone (–), was applied to 6% SDS-acrylamide gels for electrophoresis and autoradiography. (pf) subscript denotes species after PNGase F treatment. Other symbols are defined in Figure 1 legend. The figure is representative of two sets of experiments performed 30 days apart.

appreciated by comparing its relative migration with that of P_i (which contains only N-linked but not O-linked chains); while the normal $P_{m(pf)}$ migrated appreciably faster than P_i, the diabetic $P_{m(pf)}$ migrated only slightly faster than P_i (compare Fig. 3A and 3B, lane 1 for P_i and lane 8 for $P_{m(pf)}$). Since O-linked chains were added during the maturation of P_i to P_m and maturation of the N-linked chains probably produced negligible change in their apparent mass, the difference in mass of P_m and P_i after the removal of N-linked chains provides an estimate of the O-linked carbohydrate mass in P_m. This comparison revealed a larger mass for the O-linked chains in the BB_d rat as compared to the normal Wistar (~20 vs 10 kDa, respectively) (Table I, CHO_o).

Retardation in the ER and Incomplete Maturation of P_i in the BB_d Rat

In all pulse-chase experiments (Figs. 1–3), there was an apparent dramatic difference in

TABLE I. Effect of Removal of N-linked Chains on the Apparent Mass of P[a]

	PNGase F– (kDa)	PNGase F+ (kDa)	CHO_n (kDa)	CHO_o (kDa)
Wistar				
P_m	230	210	20	10
P_i	220	200	20	0
BB_d				
P_m	240	220	20	20
P_i	220	200	20	0

[a]Data from replicate electrophoresis experiments (Fig. 3A and 3B) was subjected to log-linear plots of R_f vs. molecular weight after treatment of specific immunoprecipitates with PNGase F (PNGase F+) or buffer alone (PNGase F–). The total mass of N-linked chains (CHO_n) was calculated from the difference of the masses of the P species before and after removal of all N-linked (n) chains present by PNGase F. The putative O-linked (CHO_o) chains present in P_m were determined from the difference in mass of $P_{m(pf)}$ (N-linked chains removed; O-linked chains present) and $P_{i(pf)}$ (N-linked chains removed; O-linked chains not present).

the kinetics of intracellular membrane-associated maturation in the BB_d as compared to the normal Wistar rat. Although the initial appearance of P_i was readily detected at 15 min in both the normal and diabetic animals, the subsequent conversion to P_m was delayed in the diabetic animals (Figs. 1 and 3). An estimate of the rate of P_i to P_m conversion in ER-Golgi can be derived from the relative intensities of the P_i and P_m moieties on gel autoradiographs shown in Figure 3. Hence, the P_m:P_i ratio was plotted against chase time in Figure 4. Normal Wistar converted some P_i to P_m after 15 min of chase, and P_m became the predominant species, reaching a P_m:P_i ratio of ~6:1 at 150 min of chase. Similarly, BB_d converted some P_i to P_m after 15 min of chase. However, the P_m:P_I ratio did not reach higher than ~1:1 even after 150 min of chase in the BB_d rat (Fig. 4), suggesting incomplete maturation of P_i in the BB_d rat as compared to its normal counterpart.

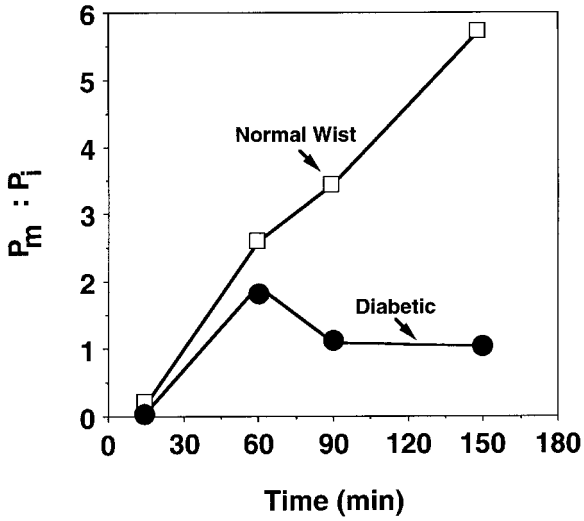

Fig. 4. Quantitation of the maturation of the precursor. [^{35}S]methionine-labeled immature (P_i) and mature (P_m) bands were excised from gels shown in Figure 3 above, and the ratio of P_m: P_i was calculated as measure of P maturation.

DISCUSSION

We have reported previously that the structure of the 140-kDa α and 125-kDa β subunit products of pro (S-D) in the brush border membrane of intact rats are altered in the BB_d rat [Najjar et al., 1991]. The β subunit (which carries the dextrinase active site) uniformly displays a larger mass (+5 kDa) than that of the normal Wistar rat and in some of the diabetic animals, the α-subunit (sucrase) is smaller (−5 kDa) or normal [Najjar et al., 1991]. These differences in the S-D structure in Wistar and BB_d rats disappeared when carbohydrate chains were removed by chemical deglycosylation, indicating that either N- or O-linked chains may be responsible for the apparent mass changes [Najjar et al., 1991].

In this paper, we have examined the structure of the single chain S-D precursor, P, as it is synthesized and processed in the ER and Golgi compartments in the intestine of the intact rat. P_i, the immature species carrying high-mannose N-linked chains, appears to be synthesized efficiently in the BB_d rat and to have a mass indistinguishable from normal. Thus, the initial synthesis and assembly of N-linked high mannose chains appear to proceed normally in congenital diabetes. This is consistent with the fact that dolichol-phosphate mannosyl and N-acetylglucosamine transferases are maintained at normal levels in human diabetes mellitus [Alhadeff and Watkins, 1983]. But the conversion to P_m, which involves removal of glucose and mannose and attachment of galactose and *N*-acetylglucosamine residues to N-linked chains, as well as addition of O-linked chains in Golgi, was prolonged and resulted in the production of a larger P_m in the BB_d than in normal Wistars (240 vs. 230 kDa) (Fig. 3 and Table I). Because O-linked chains are added in Golgi concomitantly with maturation of P_i to P_m, the higher mass of the diabetic versus normal $P_{m(pf)}$ produced by the removal of all N-linked chains with PNGase F treatment of P_m, may be explained by a larger total mass of O-linked chains in the BB_d rat. Thus, the apparent doubling of the putative O-linked carbohydrate mass from 10 to 20 kDa probably reflects the presence of enhanced O-linked glycosylation in diabetes. This could be accounted for either by addition of hexose residues at normal sites or by glycosylation of additional sites. Further structural study of the glycopeptide chains of S-D will be required to distinguish between these possibilities.

In contrast to P_m which underwent complete maturation in the normal Wistar, ~25% of the N-linked glycans of P_m in the BB_d rat failed to attain full complex structure in Golgi (Figs. 1 and 2). Evidence of essentially complete conversion to complex N-linked chains in P_m had been noted in the intestinal organ culture experiments of normal pig [Danielsen, 1982].

Hence, congenital diabetes in the rat involves persistence of immature N-linked chains even after transfer to the brush border surface (Fig. 2B). Despite the presence of these immature N-linked constituents in P_m in the BB_d rat, its transport to the brush border membrane appeared to be comparable to that of the normal Wistar rat, suggesting that full maturation of N-linked glycans is not necessary for optimal vectorial transport to the enterocyte's surface. Absence of an absolute requirement for N-linked glycosylation for transfer of S-D to the surface membrane has been shown in intestinal extracts [Danielsen and Cowell, 1986]. Similarly, complete glycosylation of the insulin receptor, of its close relative, the insulin-like growth factor receptor, and of one of its substrate, pp120, was not required for their insertion in the plasma membrane [Duronio et al., 1986; Choice et al., 1999].

There are reasons to have anticipated interference with the normal processes of glycosylation in diabetes mellitus. Both the substrates and enzymes required for the synthesis of carbohydrate O- and N-linked chains may be changed in diabetes. Galactose-containing residues, particularly N-acetylgalactosamine, a common constituent of O-linked chains [Funakoshi and Yamashina, 1982], are elevated in renal membrane glycoproteins in human diabetes [Srinivasan et al., 1970] and galactose containing proteins are increased appreciably in STZ-diabetic rats [Jacobs, 1981]. Recently, STZ has been shown to stimulate O-glycosylation of a 135 kDa protein (p135) in pancreatic β-cells, perhaps by altering the levels of *O-N*-acetylglucamine-selective *N*-acetyl-β-D-glucosaminidase and its transferase [Konrad et al., 2000]. Altered activities of carbohydrate biosynthetic enzymes and their regulation by insulin have been examined in liver slices of STZ-diabetic rats [Sharma et al., 1987]. Although dolichol-phosphate mannosyl transferase activity of membrane glycoproteins in STZ-diabetic rats was unaltered, the enzymes responsible for the glycosylation of secretory glycoproteins were reduced and could be restored to normal levels by 3 h in vitro incubation with 10^{-5} M insulin. However, no direct effect of insulin therapy on S-D assembly was noted in normal or BB_d rats [Najjar et al., 1991]. Examination of the function of processing enzymes involved in N- and O-linked glycosylation may help to elucidate the exact mechanism of abnormal carbohydrate chain structure and assembly of S-D in the BB_d rat.

Although the immature precursor, P_i is synthesized promptly in the BB_d rat and appears to have a normal mass of both its protein and carbohydrate constituents (Figs. 1, 3A, and 4), it persists in the BB_d rat as a major intracellular species with a 1:1 ratio relative to its mature counterpart (Fig. 4). In contrast, P_i appears to undergo complete conversion to P_m in the normal rat, as suggested by the 1:6 ratio of P_i:P_m (Fig. 4). Although some of this apparent delay in the conversion of P_i to P_m in the BB_d rat may reflect more rapid removal of P_m from the Golgi compartment, the prolonged persistence of P_i in association with ER-Golgi membranes is sufficient to propose that the movement from ER to Golgi or the carbohydrate modification process itself is delayed in the diabetic rat. Several alternative explanations could result in prolonged residence of P_i in the ER. Among these are (1) delay in synthesis and attachment of the high-mannose N-linked chains, (2) retarded cleavage rate of terminal glucose residues or subsequently of the uncovered terminal mannose residues, (3) retarded acquisition of an appropriate shuttling signal for vesicular-mediated transport to Golgi, and (4) slowing of the rate of movement of transport vesicles carrying the P_i from ER to Golgi.

Stieger et al. [1988] noted that the maturation in and transit through the Golgi apparatus was rate-limiting in the assembly of P, the S-D precursor, in the Caco-2 human colon adenocarcinoma cell line. Our findings of an increased mass for P_m, apparently due to addition of O-linked chains exceeding those found in the normal rat verify that P_m must be transported to and processed in the Golgi compartment in the BB_d rat. Similar to our observations, Koh et al. [1994] reported delayed processing of the insulin proreceptor to mature insulin receptor in hepatocytes derived from STZ-diabetic rats. Thus, coordinate changes in the kinetics of intracellular assembly of membrane glycoproteins may constitute a common phenomenon in diabetes.

The discrete changes in S-D structure are the result of modifications in the intracellular trafficking of the precursor followed by transfer to the brush border surface membrane of a slightly enlarged protein harboring an increase in the mass of O-linked glycans, and retaining some of its immature N-linked carbohydrate

chains. This culminates in altered apparent mass of S-D at the apical surface membrane [Najjar et al., 1991]. We have previously reported normal sucrase activity in this congenital diabetic rat model of type 1 diabetes [Najjar et al., 1991]. This suggests that the glycosylation defect in sucrase is not correlated with significant functional alterations in congenital diabetes. Conversely, the increase in sucrase activity in STZ-induced diabetes was not correlated with altered apparent mass [Najjar et al., 1991]. Thus, it appears that despite the putative importance of carbohydrate chains for orderly intracellular assembly, molecular folding, protection from intracellular degradation, and transfer to the cell surface, the exact role for N- and O-linked carbohydrate side-chains of sucrase-α-dextrinase in its function is not yet well established as is in other glycoproteins with structural changes in diabetes, including the insulin receptor. Using Swainsonine, an inhibitor of mannosidase II in Golgi, Duronio et al., [1986] showed that incomplete processing of most of the branches of N-Glycans of the insulin receptor precursor did not alter the function of the receptor. However, site-directed mutagenesis revealed that some glycosylation sites are more required for the proper function of the insulin receptor than others [Leconte et al., 1992; Bastian et al., 1993; Caro et al., 1994]. This suggests that the assembly of some N-linked glycans, but not their complete maturation to more complex structures is required for the normal function of the insulin receptor. Thus, it appears that more direct studies, such as site-directed mutagenesis that targets specific glycosylation sites on sucrase-α-dextrinase are required to depict the exact regulatory role of its glycosylation on its function. Because only few glycoproteins have been examined, additional information about the structural alterations of other membrane glycoproteins and possibly, their functional correlation may enhance our understanding of the underlying metabolic mechanisms leading to altered glycosylation in diabetes.

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