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High α-Amylase Activity in the Syncytiotrophoblastic Cells of First-Trimester Human Placentas

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The syncytiotrophoblastic brush border of the human placenta forms the maternal-fetal interface and is an important determinant of placental function. Electron micrographs of fresh brush border preparations isolated from first-trimester human placentas showed membrane vesicles, open-ended microvilli, and numerous glycogen particles. Analysis of the microvillar membranes for several plasma and intracellular membrane markers showed a high degree of purification, comparable to the results reported for the isolation of microvilli from full-term human placentas. The microvillar preparations from first-trimester placentas, however, also contained the enzymes necessary to synthesize and degrade glycogen. The degradation resulted in the accumulation of maltotriose and maltotetraose, apparently due to the action of a liver-type α-amylase. The occurrence of this enzyme and the enzymes for synthesizing glycogen in this brush border fraction is probably associated with the necessity for an extremely active glucose transport and liver-like storage system within the fetal tissue at this fetal-maternal membrane interface.

Key words: first-trimester, placenta, maltooligosaccharides, α-amylase, microvilli, brush border, maternal-fetal interface, membrane, glycogen

One of the functions of the fetal syncytiotrophoblastic brush border of the human placenta is the transport of glucose from the maternal blood, which constantly bathes the microvilli, to the fetal tissues. Some of the characteristics of this process have recently been described [1]. However, the exact fate of the glucose molecules within the fetal tissues is uncertain. Presumably glucose is either metabolized directly by the placenta and fetus or stored within the fetal tissues as glycogen.

We previously reported that brush border preparations isolated from first-trimester human placentas are rich in maltooligosaccharides, with a tri- and a tetra-

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saccharide as the most abundant members [2]. Although these oligosaccharides fractionated with the microvilli during the membrane isolation, they were free and not bound to other structural elements of the brush border. This series of oligosaccharides strongly resembled the products formed by the degradation of glycogen by rat liver α-amylase [3]. The enzymatic activity has been shown to copurify with the cellular fraction which contained microsomes and glycogen granules, but not lysosomes [4]. The aim of the work reported here was to determine whether the maltooligosaccharides were themselves a storage form of glucose within the placenta or were the products of the action of an endogenous liver-type α-amylase on a glycogen substrate.

MATERIALS AND METHODS

First-trimester human placentas were collected as previously described [2]. The microvilli were fractured from the syncytiotrophoblastic cell surface according to the method of Smith et al [5].

Membrane pellets which were processed for electron microscopy were fixed immediately after isolation and embedded in Epon 812 [6]. Thin sections were stained with lead citrate [7] and examined with a Philips 200 electron microscope.

All assays for enzyme markers were performed in triplicate and the activity determined for both the crude homogenate and the purified cell surface preparation. 5′-Nucleotidase [8], cytochrome c oxidase [9], cytochrome c reductase [10], and UDP-galactosyl transferase [11] were determined according to previously published procedures. Sialic acid content was measured as previously described [12].

To assess α-amylase activity 1 mg (based on protein content) of microvillar membranes was added to a 1 ml reaction mixture which contained: 2×10^{-2} M Tris/Cl(pH 7.4), 1.5×10^{-1} M NaCl, 2×10^{-4} M $MnCl_2$, 200 μm rabbit liver glycogen (Sigma, Type III), and 2 μm UDP-glucose. To determine if the rabbit liver glycogen preparation contained either (1) low molecular weight oligosaccharides or (2) a contaminating α-amylase activity, some control incubation mixtures contained no microvillar membranes. To determine the concentration of maltooligosaccharides present due to the action of α-amylase on glycogen present in the membrane fraction, some incubation mixtures contained no exogenous glycogen. The tubes were incubated for 30 min at 37°C in a shaking water bath. The reaction was stopped by boiling the tubes for 5 min. The reaction mixture was acidified by adding glacial acetic acid to a final concentration of 10%. After centrifugation (60 min, 100,000g) the supernatant was analyzed by gel filtration.

To determine the fate of UDP-glucose in brush border preparations, 200 μg of membrane protein in 50 μl of the Tris buffer previously described were incubated with 20 μCi of 3×10^{-5} M UDP-^{3}[H]glucose (3.26 Ci/mmol, New England Nuclear) for 1 min, then chased with a thousandfold excess of unlabeled UDP-glucose for 1, 2, 5, or 30 min. The reaction was stopped by boiling and the samples prepared for gel filtration as previously described.

The products of each incubation were analyzed after chromatography on a 60 cm × 2.5 cm Bio-Gel P-2 (−400 mesh) column eluted with 10% acetic acid. One-milliliter fractions were collected. Maltotriose or [^{3}H]maltotriose were used as standards. Maltotriose was purified from a partial hydrolysis of starch. [^{3}H]maltotriose was prepared by incubating microvillar membranes with UDP-[^{3}H]glucose as previ-

ously described. Relative migration was determined by calibrating the column with the products of a partial hydrolysis of starch. In addition, oligosaccharides which had been purified by gel filtration were also analyzed by thin layer chromatography as previously described [2].

All glycohydrolase digests were carried out at 37°C for 2 hr using 0.1 U enzyme/1 μm hexose. α-Glucosidase (Yeast, Boehringer Mannheim) digestions were performed in 0.1 M acetate buffer at pH 6.0. After boiling, the products were desalted on a mixed bed ion exchange column (Bio-Rad AG 1 [acetate] and AG 50[H^+]) eluted with methanol/H_2O (1:1, v/v). The samples were dried under nitrogen and approximately 5 nm of each oligosaccharide applied to a Whatman silica-gel "high performance plate." The samples were chromatographed and the spots detected visually, as previously described [2].

Radioactive samples were analyzed following α-glucosidase digestion by chromatography on Bio-Gel P-2. Amyloglucosidase (Rhiszopus, Sigma) digestions were performed in 0.1 M sodium acetate buffer, pH 4.5. After boiling, the products were analyzed by chromatography on Bio-Gel P-2.

Protein was determined using the method of Lowry et al [13] with bovine serum albumin as a standard. Carbohydrate was determined using the method of Dubois et al [14] with maltotriose as a standard. Radioactivity was determined by mixing 1 ml of aqueous sample with 9 ml of scintillation fluid (67% toluene, 33% Triton X-100 containing 2-(4′-t-butylphenyl)-5-(4″-biphenylyl-1,3,4-oxadiazole and 2-(4′-biphenylyl)-6-phenylbenzoxazole) using a Packard 3330 scintillation counter.

RESULTS

Electron micrographs (Fig. 1) of the membrane pellet isolated from the brush border of first-trimester human placentas showed microvilli, some of which appear to have resealed, forming vesicles. In addition, large numbers of glycogen granules were also present. The relative proportion of glycogen granules to plasma membrane was significantly reduced if the membrane pellets were frozen before processing for electron microscopy. Storing at −70°C did not increase the survival of the glycogen particles. Other organelles such as mitochondria were rarely seen in the membrane pellets.

The enzymatic activities and sialic acid content of the placental homogenate and purified cell surface fraction are summarized in Table I. 5′-Nucleotidase activity and sialic acid content, both concentrated in the plasma membrane, were purified 50-fold and sixfold, respectively. Intracellular membrane markers (cytochrome c oxidase, cytochrome c reductase, UDP-galactosyl transferase) were decreased approximately tenfold.

No maltooligosaccharides were formed by incubation mixtures which contained only glycogen (Fig. 2). Therefore, the amount of oligosaccharides formed from the exogenous glycogen substrate during the incubation with the cell surface fraction was determined by subtracting the concentration of oligosaccharides present after a 30-min incubation of the membrane and particulate fraction to which no exogenous glycogen had been added. Incubation of the membrane and particulate fraction with authentic glycogen resulted in the formation of low molecular weight oligosaccharides, the concentration of which was significantly greater than the levels of maltooligosaccharides already present [2] in this preparation.

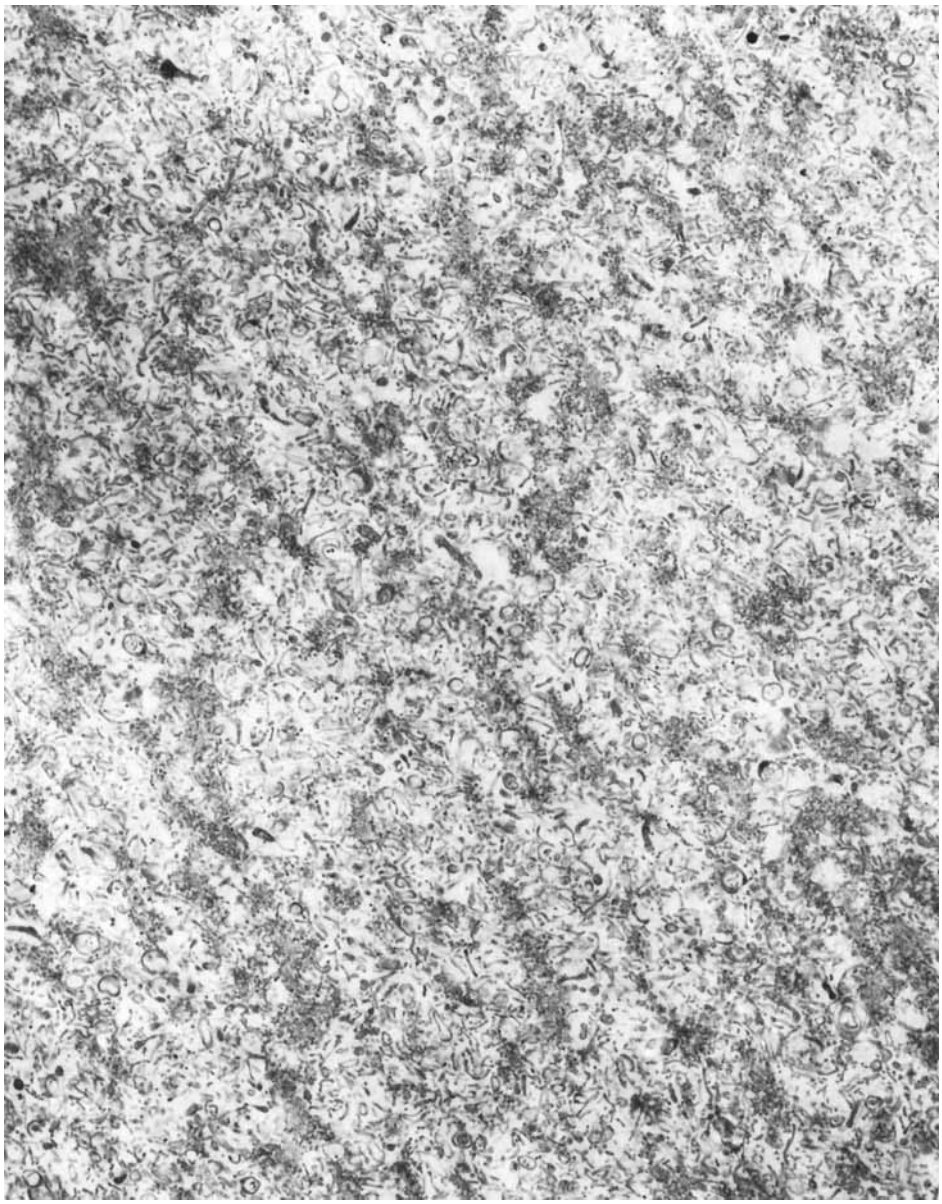

Fig. 1. Electron micrograph of the cell surface fraction isolated from first-trimester human placentas. Microvilli, as well as large numbers of glycogen particles (G) are visible (×5,500).

The major oligosaccharides formed from the exogenous glycogen substrate cochromatographed on Bio-Gel P-2 with maltotetraose and maltotriose (Fig. 2). Thin layer chromatography of the purified oligosaccharides showed identical migration relative to the maltooligosaccharide standards. After digestion of the products with yeast α-glucosidase, only free glucose could be detected by thin layer chromatography, suggesting that the oligosaccharides were $\alpha(1\rightarrow4)$oligoglycosides.

TABLE I. Comparison of Plasma and Intracellular Membrane Markers in Homogenates of First-Trimester Human Placentas and Cell Surface Preparations

Enzyme	No. of determinations	Homogenized* first-trimester placenta	Microvilli	Ratio (microvilli/ homogenate)
5′-Nucleotidase (μmol/min)	3	0.090 ± 0.004	4.623 ± 0.271	51.31
Sialic acid (nm)	2	12.93 ± 0.92	82.63 ± 2.10	6.39
Cytochrome oxidase (μmol/min)	3	0.0100 ± 0.003	0.001	0.10
Cytochrome c reductase (nm/min)	3	47.2 ± 4.7	3.1 ± 0.2	0.07
UDP/galactosyl transferase (nm/min)	2	1.30 ± 0.12	0.12 ± 0.03	0.09

*Mean ± SEM/mg protein [Lowry et al, 1951].

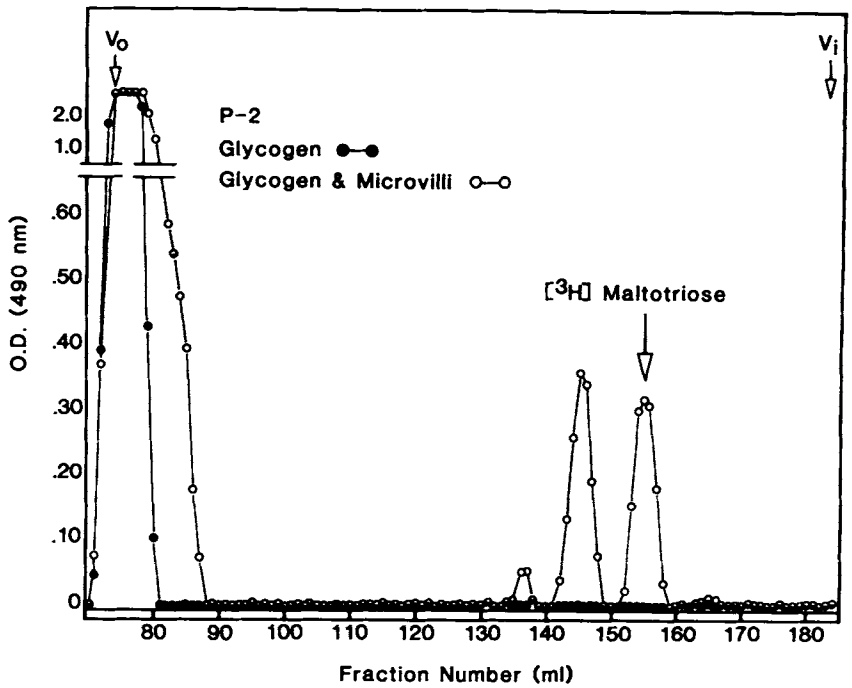

Fig. 2. Bio-Gel P-2 chromatography of the products from the incubation of the membrane and particulate fraction with authentic glycogen. The major products cochromatographed with maltotetraose and maltotriose.

The enzymatic activity present in this cellular fraction was determined by calculating the mean ± SEM of the μmol of maltooligosaccharides formed/mg protein/min for three separate experiments. The average brush border preparation isolated from first-trimester human placentas contained 24 mU ± 1 mU of activity.

Incubation of these preparations with UDP-^{3}[H]glucose (Fig. 3) for 1 min resulted in the labeling of a high molecular weight product which chromatographed

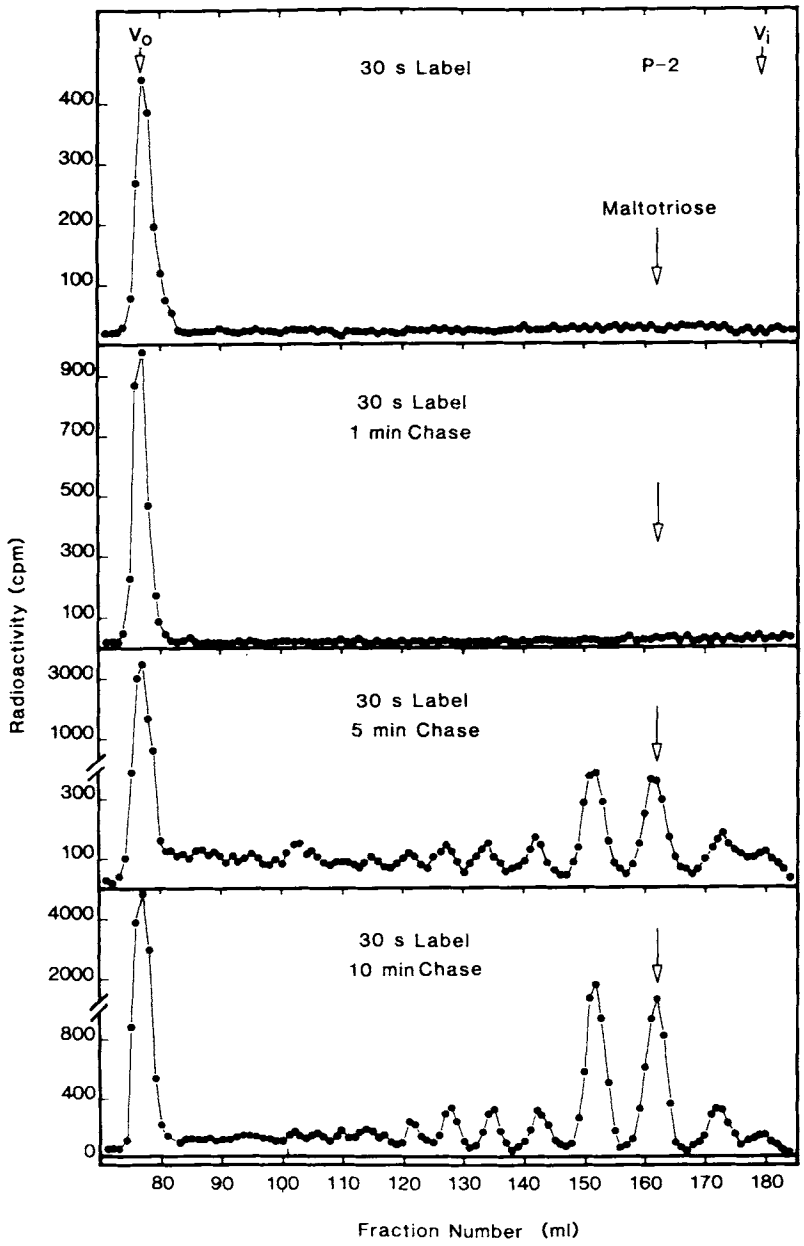

Fig. 3. Bio-Gel P-2 chromatography of the products formed by incubation of the membrane and particulate fraction with UDP-[^{3}H]glucose for one minute, followed by the addition of unlabeled UDP-glucose. Initially glycogen is labeled, followed by rapid degradation to maltotetraose and maltotriose.

in the void volume of Bio-Gel P-2. This, and all subsequent labeled products which were voided on P-2, was identified as glycogen by susceptibility to digestion with amyloglucosidase, yielding free glucose. One minute after the addition of cold UDP-glucose, only glycogen was labeled. From 5 to 30 min after this addition, the percentage of radioactivity found in the low molecular weight oligosaccharides increased. These oligosaccharides cochromatographed with maltotetraose and maltotriose and were identified as α-1,4-glucans by their susceptibility to α-glucosidase digestion.

DISCUSSION

Brush border preparations isolated from term human placentas have been shown to contain only microvilli and membrane vesicles [5]. In contrast, an identical cell surface fraction isolated from first-trimester human placentas contained both microvilli and a large number of glycogen particles. Since the glycogen particles fractionated along with microvilli which were sheared from the syncytiotrophoblastic cell surface without homogenizing the tissue, it is likely that in vivo glycogen is synthesized within the fetal portion of the placenta and deposited in close apposition to the placental brush border.

Relative to homogenized first-trimester human placenta, the cell surface fraction was greatly enriched in plasma membrane markers and decreased in enzymes which are characteristic of intracellular organelles. The data indicate that this preparation is more highly purified than microvilli prepared by this same technique from term placentas [5] and equivalent to preparations of term microvilli which are further purified by sucrose density gradient centrifugation [15].

There are two probable major reasons for the increased purity of the cell surface preparation, isolated by fractionation, from the first-trimester human placenta. First, the 8–10-wk placenta is still vigorously growing as compared to the term placenta, which shows many signs of necrosis such as amyloid deposition and narrowing of the syncytiotrophoblastic layer. Second, the first-trimester placenta can be collected intact and blood and decidua totally removed without homogenization or disruption of the tissue.

Previous work has shown that, in contrast to human term placental microvilli [5], those isolated from first-trimester placentas contained a high concentration of glucose [16]. We have reported that the glucose molecules can be found in the form of low molecular weight oligosaccharides, principally maltotetraose and maltotriose, rather than glycogen. In addition, little free glucose or maltose is present [2].

The work described here suggests that the maltooligosaccharides are formed due to the action of a liver-type α-amylase on glycogen particles, which we found by electron microscopy to be abundant in preparations of first-trimester human placental brush border if examined immediately after isolation. In homogenized rat liver, this enzymatic activity has been shown to be concentrated in the microsomal fraction [3], which contains both microsomes and glycogen granules [4]. In first-trimester placenta we have found that the enzyme and its products occur in the microvillar fraction, which also contains glycogen granules, and which was isolated without homogenization of the tissue.

Incubation of the membrane and particulate fraction isolated from first-trimester placentas with UDP-[^{3}H]glucose resulted in the initial labeling of glycogen. Presum-

ably glucose which is transported from the maternal circulation is added to the outer branches of a preformed molecule in the fetal portion of the placenta by glycogen synthetase. Subsequently, the labeled glucose residues are rapidly removed from the exterior of the glycogen molecule by the competing action of α-amylase, with the resultant production of maltotetraose and maltotriose. Since the synthetic and degradative activities appear to involve the same pool of glycogen molecules, the α-amylase activity, like the synthetase activity, is probably associated with the glycogen granules. This activity may serve to supply the fetus with a constant supply of glucose when the maternal blood glucose levels vary.

Although these results clarify the in vitro fate of glucose within the first-trimester human placental brush border the relationship of these events to in vivo fetal metabolism has not been investigated. However, the concentration of glycogen in the syncytiotrophoblastic brush border of the human placenta may indicate that this organ, in addition to its many other functions, acts as a fetal liver during early development [17], a time when the fetus itself appears to lack the enzymes necessary for glycogen storage and metabolism [18,19].

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