Secretion of a lactosaminoglycan-containing glycoprotein by peri-implantation sheep conceptuses

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Sheep conceptuses from day 16 of pregnancy were cultured in the presence of [3H]glucosamine and [14C]leucine and a high-molecular-weight glycoprotein (HMWG) secreted into the culture medium was purified by a combination of anion-exchange and gel filtration chromatography. The HMWG was found to have a molecular weight between 800,000 and 900,000 and to be highly resistant to digestion with pronase. Characteristics of the carbohydrate portion of the purified glycoprotein were examined by selective chemical and enzymatic digestions and lectin binding studies. Mild alkaline reduction was ineffective in disassociating carbohydrate chains from the protein core. Furthermore, the protein was resistant to both O-glycanase and peptide:N-glycanase F. Harsh alkaline reduction caused the release of carbohydrates, however. After pronase digestion of these products, three molecular weight classes of carbohydrates were resolved by Sephadex G-25 chromatography. Two lines of evidence indicate that the HMWG contains lactosaminoglycan components. The intact molecule and two of the molecular weight classes of carbohydrates resolved by harsh alkaline reduction bind Datura stramonium lectin. Binding of HMWG to lectin could be partially inhibited by N-acetyllactosamine and completely inhibited by a mixture of N,N'-diacetylchitobiose and N,N',N''-triacetylchitotriose. Secondly, digestion with endo-β-galactosidase causes the release of 16% of the [3H]glucosamine from the intact molecule. Therefore, the HMWG of the sheep conceptus is the first reported example of secretion of lactosaminoglycan-containing glycoprotein by peri-implantation embryos.

Introduction

Lactosaminoglycans are polysaccharides characterized by Galβ1 → 4GlcNacβ1 → 3 repeats [1–3]. They exist as glycolipids or glycoproteins and have a significant heterogeneity in structure with respect to degree of branching and terminal structures [4–6]. Lactosaminoglycans have been found in a variety of cell types, frequently as cell surface or extracellular glycoproteins [2,4,7–10]. They may also play a functional role in fertilization and early embryonic development. Lactosaminoglycans have been identified as membrane components of sperm [11] and early embryonic cells [4,11,12] and have been shown to participate in cell adhesion of embryonic cells [12] and endometrial epithelium [13] by binding to cell-surface galactosyltransferase.

The peri-implantation conceptus of the sheep is one example of an embryonic tissue that is ac-
tively involved in the synthesis and secretion of glycoproteins [14]. Placentation in the sheep does not involve true implantation but rather interdigitation and interfolding of trophoblast and uterine epithelium. Contact between the conceptus and the caruncular surface of the endometrial epithelium is evident by day 14 of pregnancy and adhesion develops between days 16 and 18 [15]. Interdigitation of microvilli has been observed as early as day 18 [16] to day 20 [17] or early in the fourth week of gestation [18].

Around this time the conceptus is secreting a high-molecular-weight (Mr > 660,000) glycoprotein (HMWG) that is approximately 50% carbohydrate by weight [14]. Gas-liquid chromatography indicated that the major carbohydrates in this molecule are galactose and N-acetylglucosamine in a 1.57:1 ratio. These results suggest that the glycoprotein may carry lactosaminoglycan saccharides. Therefore, we examined the properties of the carbohydrate chains of HMWG to test whether this protein does indeed contain lactosaminoglycans.

Materials and Methods

Materials. All materials employed for tissue culture were supplied by the vendors noted by Godkin et al. [19]. Radionucleotides (D-[6-3H]-glucosamine, specific activity = 40 Ci/mmol; l-[1-14C]leucine, specific activity = 56.6 mCi/mmol) were supplied by ICN (Irvine, CA) and Amersham (Arlington Heights, IL), respectively. Genzyme Co., Boston, MA, was the supplier of N-glycanase (peptide:N-glycanase F from Flavobacterium meningosaminidase) and O-glycanase (Endo-α-N-acetylgalactosaminidase from Diplococcus pneumoniae). ICN Immunocochemicals supplied endo-β-galactosidase from Escherichia freundii. Chromatography supplies were obtained from Pharmacia, while the Zorbax GF-450 HPLC gel filtration column was from DuPont (Wilmington, DE). All other proteins and reagents were obtained from Sigma or Fisher and were of the highest grade available.

In vitro culture of conceptuses. Adult ewes, primarily of Rambouillet and Florida Native breeds, were checked for estrus every morning with a vasectomized ram. Ewes were bred to intact rams on the morning and afternoon of detected estrus and on the following morning. On day 16 post-estrus (estrus = day 0), conceptuses were flushed from uteri with sterile medium as previously described [19]. Conceptuses were individually cultured in 15 ml of a modified Eagle's minimum essential medium (custom formula No. 86-5007; Gibco Laboratories, Grand Island, NY; see Basha et al. [20] for modifications) with the l-leucine content reduced to one-tenth its normal level. One hundred microcuries of [3H]glucosamine and 25 µCi of [14C]leucine were added to each culture. Incubations were at 37°C in an atmosphere of 50% O2, 45% N2, and 5% CO2 (by volume). After 30 h, the medium was changed and the culture continued for a second 30 h period. Using this culture procedure, conceptuses continue to secrete de novo synthesized proteins for at least 4 days. Cultures were terminated by centrifugation at 12,000 x g for 10 min at 4°C. Culture supernatant fractions were harvested and frozen at −20°C.

Purification of dual-labelled HMWG. Conceptus-conditioned culture medium (15 ml) was dialyzed against four changes (16 liters total volume) of 10 mM Tris-HCl buffer (pH 8.2). The retentate was applied to an anion-exchange column of DEAE-Sepharose CL-4B (10 x 1.5 cm) that had been previously equilibrated with 10 mM Tris-HCl (pH 8.2). Protein bound was eluted with a linear salt gradient (300 ml; 0–0.5 M) in 10 mM Tris-HCl (pH 8.2). Fractions, usually 5 ml, were collected and assayed for radioactivity using a 1218 Rackbeta scintillation counter (LKB) programmed to count dual-labelled preparations. Counting efficiencies for dual-labelled preparations were 53% for 3H and 21% for 14C. The major [3H]glucosamine-labelled peak was concentrated using a Millipore immersible CX-10 ultrafiltration unit (cutoff = 10 kDa) and applied to a Sepharose CL-6B column (96 x 1.5 cm) with 10 mM Tris-HCl (pH 8.2), containing 0.33 M NaCl as eluent. The single [3H]glucosamine-labelled peak was dialyzed against distilled water, aliquoted and stored at −20°C.

Molecular weight estimation. Molecular weight of HMWG was estimated using Sepharose CL-6B with thyroglobulin (Mr = 660,000), bovine serum albumin (Mr = 69,000), and ovalbumin (Mr = 45,000) as standards. Molecular weight was also
estimated by high performance liquid chromatography using a Zorbax GF-450 gel filtration column calibrated with sheep IgM ($M_r = 900,000$), thyroglobulin ($M_r = 660,000$), bovine serum albumin ($M_r = 69,000$) and ovalbumin ($M_r = 45,000$). Column buffer used was 0.2 M sodium phosphate buffer (pH 8.0) with a flow rate of 0.7 ml/min.

Tube gel polyacrylamide electrophoresis was a third method used to estimate molecular weight and test for the presence of subunits. Samples of HMWG (approximately 5000 dpm [3H]glucosamine and 600 dpm [14C]leucine) were solubilized in 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% (w/v) sodium dodecyl sulfate (SDS), 15% (w/v) sucrose and 5% 2-mercaptoethanol (v/v). SDS-polyacrylamide electrophoresis was performed in tube gels (3 mm, i.d.) using the buffer system of Laemmli [21] with a 10 cm running gel of 5% (w/v) polyacrylamide and a 1 cm stacking gel of 4.5% polyacrylamide. Electrophoresis was performed at 3 mA/gel for 1-1.5 h. Gels containing HMWG were removed from the tubes, sliced in 2 mm sections using a gel slicer and solubilized in 0.4 ml hydrogen peroxide by heating at 60°C for 2 h. Scintillation fluid (4 ml) was added and samples were counted for radioactivity. Gels containing molecular weight standards were removed from the tubes and stained with Coomassie Blue R-250 (0.125%, w/v) dissolved in acetic acid/ethanol/water (7:40:53; v/v).

Pronase digestion. Pronase (10 mg/ml in 0.1 M sodium acetate buffer, pH 6.0) was self-digested for 30 min at 60°C [22]. Dual-labelled HMWG (approximately 43,000 dpm [3H]glucosamine and 1500 dpm [14C]leucine) was lyophilized and reconstituted in 1 ml of 0.1 M sodium acetate buffer (pH 6.0) containing 1 mg pronase. Incubation continued for 72 h with addition of 1 mg pronase every 24 h. Digestion products were separated by gel exclusion chromatography using Sepharose CL-6B (96×1.5 cm). The eluting buffer was 10 mM Tris-HCl buffer (pH 8.2) containing 0.33 M NaCl.

N-glycanase digestion. Purified HMWG (approximately 43,000 dpm [3H]glucosamine and 1500 dpm [14C]leucine) was lyophilized and reconstituted in 20 μl 0.5% (w/v) SDS containing 0.1 M 2-mercaptoethanol. The sample was further digested with 10.8 μl sodium phosphate buffer (pH 8.6), 3 μl 100 mM phenanthroline hydrate (in methanol) and 5 μl of 7.5% (w/v) Nonidet P-40. N-glycanase (0.5 unit) was added and the reaction mixture incubated at 37°C for 16 h. Ovalbumin and bovine serum albumin (20 μg) served as positive and negative controls, respectively. HMWG digestion products were separated by chromatography on Sepharose CL-6B (96×1.5 cm). Control reactions were monitored by SDS-polyacrylamide slab gel electrophoresis using 12.5% (w/v) polyacrylamide gels and the buffer system of Laemmli [21].

O-glycanase digestion. HMWG (approximately 43,000 dpm [3H]glucosamine and 1500 dpm [14C]leucine) was lyophilized and reconstituted in 20 mM Tris-maleate buffer (pH 6.0) containing 1 mM calcium acetate and 10 mM D-galactonolactone. Neuraminidase (0.1 unit) was added and the reaction incubated at 37°C for 60 min. O-glycanase (2.9 mU) was then added in some reactions and the incubation continued at 37°C for 16 h. Final reaction volume was 50 μl. Bovine serum albumin and fetuin (20 μg) were negative and positive controls, respectively. HMWG digestion products were separated by chromatography on Sepharose CL-6B (96×1.5 cm). Control reactions were monitored by polyacrylamide gel electrophoresis.

Endo-β-galactosidase digestion. HMWG (approximately 65,000 dpm [3H]glucosamine and 2100 dpm [14C]leucine) was lyophilized and reconstituted in 10 mM sodium acetate buffer (pH 5.8) containing 10 mM 7-galactonolactone. To this preparation 0.1 U endo-β-galactosidase was added. The final reaction volume was 50 μl. The reaction proceeded for 48 h at 37°C and was stopped by addition of 1 ml 10 mM Tris-maleate buffer (pH 8.2) containing 0.33 M NaCl. A second aliquot of HMWG (approximately 33,000 dpm [3H]glucosamine and 1050 dpm [14C]leucine) was lyophilized and incubated as described above except no enzyme was added. Reaction products were separated by gel exclusion chromatography on Sepharose CL-6B.

Alkaline borohydride treatment. HMWG (approximately 65,000 dpm [3H]glucosamine and 2100 dpm [14C]leucine) was lyophilized and reconstituted in 1 ml of either 1.0 M NaBH₄ in 1.0 M...
NaOH (mild alkaline reduction conditions) or 4.0 M NaBH₄ in 1.0 M NaOH (harsh alkaline reduction conditions). Mild alkaline reduction was carried out at 4 ° C for 48 h. Harsh alkaline reduction was performed at 60 ° C for 48 h. The reaction was terminated by the addition of 2 ml of 10 mM Tris-HCl buffer (pH 8.2) containing 0.33 M NaCl and neutralization with glacial acetic acid. Dissolved gasses were removed under reduced pressure. The reaction products were separated by gel chromatography on Sepharose CL-6B as described before. Peaks of [³H]glucosamine were pooled, lyophilized, reconstituted in distilled water (usually 2 ml) and digested with pronase as outlined earlier. These products were separated by gel chromatography on Sephadex G-25 superfine (90 x 1.5 cm) using 0.1 M acetic acid as a eluent. The column was calibrated with Man₉Glcitol[³H]NAc and Man₆Glcitol[³H]NAc as standards [23,24].

**Binding to Datura stramonium lectin.** Lectin (1 mg) was coupled to Sepharose 4B by the cyanogen bromide method [25]. Coupling efficiency, determined by measuring protein concentration [26] in the supernatant following the coupling procedure, was greater than 75%. Intact HMWG was loaded onto a 2.5 ml column of coupled lectin previously equilibrated with 10 mM potassium phosphate buffer (pH 7.4) containing 0.9% (w/v) NaCl (PBS). The column was sequentially washed with 10 ml each of PBS, 10 mM potassium phosphate buffer (pH 7.4) containing 1 M NaCl, and 8 M urea. A control column was prepared as described above except no lectin was coupled to the Sepharose 4B. HMWG was loaded onto this column and eluted as described above. The ability of various low-molecular-weight sugars to inhibit binding of HMWG to *D. stramonium* lectin was evaluated. Fifty microliters of *D. stramonium* lectin coupled to Sepharose CL-4B (25% v/v in PBS) was incubated with an equal volume of PBS containing N-acetyllactosamine (400 mM) or *N*,*N'*-diacetylchitobiose (24 mM) and *N*,*N'*,*N'''*-triacetylchitotriose (16 mM). HMWG was then added and incubated for 2 h at room temperature on a tube turner. The reaction was terminated by centrifugation at 12 000 x g for 4 min and separation of supernatant from precipitate. The precipitate and an aliquot of the supernatant were counted for radioactivity. Counts bound to lectin were corrected for nonspecific binding to uncoupled Sepharose CL-4B and percent bound calculated relative to HMWG bound to *D. stramonium* without inhibitor.

Peaks of oligosaccharides isolated by gel filtration of harsh alkaline reduction products of HMWG were pooled, lyophilized and tested for ability to bind *D. stramonium* lectin. Pools of oligosaccharides were reconstituted in 1 ml of PBS. Two hundred microliters of a slurry of *D. stramonium* lectin coupled to Sepharose CL-4B was added contents rotated on a tube turner for 2 h at room temperature. Contents were centrifuged at 12 000 x g for 2 min and radioactivity was determined in both supernatant and precipitate fractions.

**Results**

**Purification of HMWG**

Dialyzed conceptus-conditioned culture medium was subjected to anion-exchange chromatography using DEAE-cellulose. The major [³H] glucosamine-labelled peak eluted early in the salt gradient (Fig. 1A). When this fraction was separated by gel exclusion chromatography on Sephadex G-25 superfine (90 x 1.5 cm) using 0.1 M acetic acid as a eluent. The column was calibrated with Man₉Glcitol[³H]NAc and Man₆Glcitol[³H]NAc as standards [23,24].

High performance liquid chromatography was also employed to generate an estimate of molecular weight. HMWG had a retention time of 10.66 min, a value intermediate to that of sheep IgM (10.52 min) and thyroglobulin (11.6 min). The calculated molecular weight was 896 000.

Molecular weight was also estimated using polyacrylamide tube gel electrophoresis on 5% polyacrylamide gels in the presence of SDS with or without 2-mercaptoethanol. The majority of the radioactivity failed to enter the gel under both conditions, indicating that the glycoprotein was of large molecular weight (> 500 000) and was composed of a single polypeptide chain (data not shown).
Fig. 1 Isolation of HMWG. Conceptus-conditioned culture medium (15 ml) was applied to an anion-exchange column of DEAE-Sepharose CL-4B (10 × 1.5 cm) previously equilibrated with 10 mM Tris-HCl buffer (pH 8.2). Protein bound was eluted with a linear salt gradient (G = 300 ml; 0–0.5 M) in 10 mM Tris-HCl (pH 8.2) (panel A; 5 ml/fraction). Aliquots (100 µl) from each fraction were analyzed for radioactivity. The major [3H]glucosamine-labelled peak (HMWG) was applied to a Sepharose CL-6B column (96 × 1.5 cm) with 10 mM Tris-HCl buffer (pH 8.2) containing 0.33 M NaCl as eluent (panel B; 3.7 ml/fraction). Aliquots (100 µl) from each fraction were counted for radioactivity. The major dual-labelled peak (Kay = 0.165) was identified and utilized in subsequent experiments. The void volume (V0) and elution volumes of thyroglobulin (T), bovine serum albumin (B) and ovalbumin (O) are shown by arrows. Panel C represents HMWG (peak 1), purified as described above, from a conceptus cultured with L-[4,5-3H]leucine (specific activity = 146 Ci/mmol). Only the CL-6B profile is shown. HMWG eluted as a symmetrical peak with a Kay of 0.17. GlcN, glucosamine.

Pronase digestion of intact HMWG

HMWG was treated with self-digested pronase and digestion products separated by chromatography on Sepharose CL-6B. While most (71.8%) of the [14C]leucine was in the salt volume, all of the [3H]glucosamine was partially excluded from the column. Pronase digestion caused a shift in the elution volume of [3H]glucosamine label associated with HMWG from a Kay of 0.165 to 0.434, indicating that large segments of the molecule are resistant to pronase.

Digestion with endoglycosidases

Enzymatic hydrolysis of N- [27] and O-linked [28] carbohydrate side chains was used to determine the type of linkages present on HMWG. Neither N- nor O-glycanase (with or without prior treatment with neuraminidase) had an effect on the size or glycosylation of HMWG as determined by gel filtration chromatography. There was no shift in molecular weight caused by these digestions nor was there any disassociation of [3H]glucosamine into the salt volume (results not shown). Utilizing identical reaction conditions, a decrease in the molecular weight of ovalbumin (N-glycanase) and fetuin (O-glycanase plus neuraminidase) was demonstrated by polyacrylamide gel electrophoresis, indicating that the enzymes were active. Furthermore, bovine serum albumin, a protein with no oligosaccharide side chains, was unaltered by these treatments.

Alkaline borohydride reduction

Since neither N- nor O-glycanase was effective in determining the type of carbohydrate linkages of HMWG, borohydride treatment was employed [31,32]. Mild (1.0 M NaBH₄ in 1.0 M NaOH) alkaline reduction, carried out at 4°C for 48 h, caused only a slight decrease in molecular weight of HMWG (Kay = 0.376). Only a single peak of [3H]glucosamine was detected, suggesting the absence of O-linked oligosaccharides. When harsh reduction (4.0 M NaBH₄ in 1.0 M NaOH) was carried out at 60°C for 48 h, one to three peaks of [3H]glucosamine were present near or at the salt volume of the column (Fig. 2A), indicating hydrolysis of Asn-GlcNAc bonds and/or extensive destruction of peptide bonds. These fractions were pooled separately, digested with pronase to insure...
that all products were oligosaccharides or monoamino acid glycopeptides, and each separated by Sephadex G-25. Three peaks were consistently resolved (Fig. 2B). One peak ($K_{av} = 0.029$) was resolved at or near the void volume of the column, one near the salt front ($K_{av} = 0.727$), and there was an intermediate peak with a $K_{av}$ of 0.323. The relative size of each peak varied between digestions: the latest eluting peak was sometimes quantitatively less than the first peak (Fig. 2B) while, for other digestions, it was the major peak resolved.

Fig. 2. Harsh alkaline reduction of HMWG. The glycoprotein was incubated for 48 h at 60 °C in a mixture of 4.0 M NaBH$_4$ and 1.0 M NaOH. Reaction products were separated by gel chromatography on Sepharose CL-6B (96 × 1.5 cm) with 10 mM Tris-HCl (pH 8.2), containing 0.33 M NaCl as eluent (panel A; 3.7 ml/fraction). $V_0$ represents the column void volume and I the elution volume of intact HMWG. Aliquots (400 μl) from each fraction were analyzed for radioactivity and peaks of $[^3]$Hglucosamine were pooled, lyophilized, reconstituted in distilled water and digested with pronase. These products were separated by gel chromatography on a column of Sephadex G-25 superfine (90 × 1.5 cm) previously calibrated with Man$_9$Glcitol$[^3]$HNAc (Man$_9$) and Man$_6$Glcitol$[^3]$HNAc (Man$_6$). Eluent was 0.1 M acetic acid and aliquots (400 μl) from each fraction were analyzed for radioactivity.

Panel B illustrates a representative profile of elution from Sephadex G-25. Three molecular weight classes of oligosaccharides with $K_{av}$ values of 0.029, 0.323 and 0.727 were resolved. Elution profiles of other digestions from Sepharose CL-6B, as resolved by Sephadex G-25, gave similar results except that the relative size of the latest eluting peak was sometimes larger than shown here. GlcN, glucosamine.

Fig. 3. Digestion of HMWG by endo-$\beta$-galactosidase. HMWG was reacted with 0.1 U enzyme in 200 μl sodium acetate buffer (pH 5.8), containing 0.01 M \( \gamma \)-galactonolactone. The reaction proceeded for 48 h at 37 °C. Reaction products were separated by gel exclusion chromatography on Sepharose CL-6B (96 × 1.5 cm; 3.7 ml fractions) with 10 mM Tris-HCl (pH 8.2), containing 0.33 M NaCl as eluent. $V_0$ represents the column void volume and I the elution volume of intact HMWG. Aliquots (400 μl) from each fraction were analyzed for radioactivity (panel A). Endo-$\beta$-galactosidase resulted in dissociation of approximately 16.3% of the $[^3]$Hglucosamine from the protein core. The carbohydrates released in this manner (peak 2) were separated by gel chromatography on a column of Sephadex G-25 superfine (90 × 1.5 cm; 2.5 ml/fraction) previously calibrated with Man$_9$Glcitol$[^3]$HNAc (Man$_9$) and Man$_6$Glcitol$[^3]$HNAc (Man$_6$). Eluent was 0.1 M acetic acid and aliquots (400 μl) from each fraction were analyzed for radioactivity. Four peaks were resolved: one near the void volume of the column ($K_{av} = 0.025$) and peaks at $K_{av}$ values of 0.198, 0.372 and 0.447. GlcN, glucosamine.
Digestion with endo-β-galactosidase

This enzyme hydrolyzes β-galactosidic linkages of various substrates [33-35]. Endo-β-galactosidase resulted in dissociation of approximately 22.6% of the [3H]glucosamine from the protein core (Fig. 3A). The major peak of [3H]glucosamine was shifted to a \(K_a\) of 0.30. This peak was followed by a broad, smaller peak with a \(K_a\) of approximately 0.80 (Fig. 3A). When corrected for [3H]glucosamine present in the region of the smaller peak following chromatography of nondigested HMWG, specific cleavage of 16.3% of the [3H]glucosamine was attributed to endo-β-galactosidase. The carbohydrates released in this manner (peak 2) were separated by Sephadex G-25 column chromatography (Fig. 3B). Four peaks were resolved: one at or near the void volume of the column (\(K_a = 0.025\)) and peaks at \(K_a\) values of 0.198, 0.372 and 0.447.

Lectin binding

All the radioactivity associated with intact HMWG could be bound to a column of *D. stramonium* lectin coupled to Sepharose CL-4B (results not shown). Radioactivity could be eluted with 8 M urea, but not with 1 M NaCl. Preincubation of lectin with equal volumes of N-acetyllactosamine (400 mM in PBS) or PBS containing \(N, N', N''\)-triacetylchitotriose (16 mM) inhibited HMWG binding by 30% and 100%, respectively. Results suggest that each molecule of HMWG contains at least one carbohydrate chain with repeating β1,4-linked oligomers of N-acetylglucosamine.

Fractions isolated by Sepharose CL-6B following harsh alkaline reduction were digested with pronase and separated by Sephadex G-25 chromatography. The three peaks isolated in this manner (Fig. 2B) were evaluated for ability to bind *D. stramonium* lectin. The majority of the radioactivity present in peak 1 (78.9%) and peak 2 (84.6%) bound to lectin while very little of the radioactivity present in peak 3 was bound (7.8%).

Discussion

Results presented here suggest that the major secretory glycoprotein of day 16–17 ovine conceptus is of high molecular weight (765 000–896 000), highly resistant to chemical and enzymatic degradation and bears N-linked polylactosamine groups. The protein was fairly resistant to pronase, since only a small portion of the molecule was cleaved, leaving the bulk of the glycoprotein intact. In addition, \(N\)- and \(O\)-glycanase were unable to disassociate carbohydrate label from the protein backbone, indicating that the active sites for these enzymes may be masked by the extensive glycosylation occurring on the protein. Though mild alkaline reduction cleaved a considerable portion of the molecule, no specific release of carbohydrate was evident. Only harsh alkaline conditions succeeded in dissociating carbohydrate moieties from the protein core, suggesting that chains are N-linked. Data obtained from Sephadex G-25 chromatography of products released by harsh alkaline reduction indicate the presence of three molecular weight classes of oligosaccharides. The size of fraction 1, which eluted near the void volume, is not known but the second fraction appeared to be of relatively small size (approximately 7 units) and may represent carbohydrates that were partially cleaved by the digestion procedure [31]. Fraction 3, of lowest molecular weight, probably represents extensively digested saccharide chains, since the relative magnitude of radioactivity in this peak was variable and the peak eluted near the salt volume.

Three lines of evidence suggest that the HMWG contains lactosaminoglycan components. The carbohydrate composition of HMWG, determined by gas-liquid chromatography [14], indicates that the major sugars present are D-galactose and \(N\)-acetyl-D-glucosamine in molar ratios of 1.57:1.00. Furthermore, 16.3% of the carbohydrate on HMWG was removed by endo-β-galactosidase, an enzyme that cleaves \(\beta\)-galactosidic bonds on certain types of lactosaminoglycans. The portion of carbohydrate released by endo-β-galactosidase is similar to results of Masters et al. [14]. That more extensive cleavage was not observed may be further evidence that the sugar units are extensively branched [35] or contain fucose near the cleavage site [34]. Finally, the intact molecule and two of the three molecular weight classes of oligosaccharides isolated by harsh alkaline reduction bind *D. stramonium* lectin. *D. stramonium* lectin recognized oligomers of \(N\)-acetylglucosamine [36,37].
Binding of intact HMWG could be inhibited by preincubation of lectin with N-acetyllactosamine (30%) or a mixture of N,N’-diacetylchitobiose and N,N’,N’’-triacetylchitotriose (100%). Therefore, each molecule of HMWG contains oligomers of N-acetylglucosamine. The susceptibility of HMWG to endo-β-D-galactosidase indicates that some of these oligomers are substituted with galactose to form N-acetyllactosamine units.

The role of HMWG is unknown. It may protect the conceptus from the uterine immune system because Murray et al. [38] have shown that HMWG can inhibit blastogenesis of mitogen- and mixed-lymphocyte-stimulated lymphocytes. In addition, proteinases can be present in uterine secretions [39] and the local presence of HMWG around the conceptus may protect it from proteolytic actions of these enzymes. In other systems, lactosaminoglycan-containing glycoproteins participate in cell adhesion in embryonic carcinoma and uterine epithelial cells through binding to cell surface galactosyltransferase [12,13]. Sheep blastocysts are spherical between day 4 and 10 and then elongate to a filamentous form by day 15 [40], through processes that probably involve extensive cell migration. Similarly, contact between the conceptus and the caruncular surface of the uterine lumen has been reported around day 14, with adhesion developing around day 16–18 [15]. Perhaps rearrangement of embryo structure or uterine epithelium–trophoblast interactions leading to placentation involve cell adhesion mechanisms mediated by embryonal lactosaminoglycans such as HMWG.

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