Uteroferrin has *N*-asparagine-linked high-mannose-type oligosaccharides that contain mannose 6-phosphate

(uterus/lysosomal hydrolase/glycoprotein/iron transport/acid phosphatase)

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ABSTRACT Uteroferrin is an iron-containing, progesterone-induced, acid phosphatase that is secreted in large amounts by the uterine endometrium of pigs. During pregnancy, it transports iron across the chorioallantois (placenta) for use in fetal hematopoiesis. In this paper, it is reported that uteroferrin synthesized by cultured endometrial explants possesses *N*-linked, high-mannose, oligosaccharide chains that contain 6-phosphomannose units. The latter is regarded as a possible recognition marker whereby acid hydrolases are targeted to the lysosome. On uteroferrin, however, the majority of the phosphate is in single diester linkages between the mannose and a covering *N*-acetylglucosamine. It is suggested that uteroferrin is a lysosomal enzyme that has assumed a role in iron transport and metabolism and is secreted because the covering *N*-acetylglucosamine is not removed.

Uteroferrin is a purple iron-containing basic glycoprotein synthesized under the influence of progesterone by the glandular epithelium of the porcine uterine endometrium (1). The midpregnant uterus is believed to secrete around 2 g of this glycoprotein per day (2). There is now good evidence that uteroferrin serves to transport iron across the placenta in pregnant pigs (3-5). Uteroferrin, however, also has enzymatic activity (6, 7) and belongs to a class of iron-containing, tartrate-insensitive acid phosphatases (8, 9). The purple color of uteroferrin and of the similar beef spleen enzyme (9) results from an iron center in which Fe is coordinated to one or more tyrosine residues (10, 11). Acid phosphatases having physical and enzymatic properties similar to uteroferrin have been isolated from a variety of sources, including lysosomes of mouse liver (12, 13), rat bone (14), human placenta (15), and the sera and spleens of human patients with Gaucher disease (β -glucocerebrosidase deficiency) (16) and with hairy cell leukemia (leukemic reticuloendotheliosis) (17). In the leukemic hairy cells, the tartrate-insensitive acid phosphatase is localized in intracellular vacuoles resembling lysosomes (18).

In this paper, we report that uteroferrin synthesized *in vitro* bears *N*-linked high-mannose-type oligosaccharide(s). Furthermore, oligosaccharides purified from uteroferrin recovered from culture medium possess mannose 6-phosphate residues, which have been proposed as recognition markers involved in targeting of lysosomal hydrolases to the lysosomal compartment of the cell (19). Thus, uteroferrin, a secreted glycoprotein apparently involved in transplacental iron transport in the pig, has many properties in common with acid phosphatases that may be of lysosomal origin.

METHODS

Materials. Tunicamycin, alkaline phosphatase (*Escherichia coli*), Pronase (type XIV), phosphomannose isomerase,

and phosphoglucose isomerase were obtained from Sigma. Radiochemicals [D -[6- 3H]glucosamine (238 Ci/mmol; 1 Ci = 37 GBq), D -[2- 3H]mannose (13.4 Ci/mmol), D -[U- ^{14}C]glucose (300 mCi/mmol), L -[1- ^{14}C]leucine (60 mCi/mmol), L -[^{35}S]methionine (1270 Ci/mmol), $H_3^{32}PO_4$, carrier free] were from Amersham. Endo- β -*N*-acetylglucosaminidase H (endo H) from *Streptomyces plicatus* was obtained from Miles or from New England Nuclear. Concanavalin A (Con A)-Seph-
arose was from Pharmacia. All other materials were reagent grade or better.

Animals. Gilts that had undergone two normal estrous cycles were mated to intact boars on the first day of estrus (day 0). Endometrial samples for *in vitro* culture were collected aseptically from anesthetized animals on days 90-110 of pseudopregnancy (20) or days 58-78 of pregnancy (21, 22); normal gestation period is 114 days.

Preparation of Antibody to Uteroferrin. Antisera were raised in New Zealand White rabbits by injecting purified uteroferrin in Freund's adjuvant into multiple intradermal sites at monthly intervals. Antibodies specific for uteroferrin were purified on a column (1 $\times$ 5 cm) of uteroferrin coupled to CNBr-activated Sepharose CL-4B (3.7 mg of uteroferrin/ml of gel) (23).

Endometrial Explant Cultures. Endometrium (0.2 g of tissue/3 ml of medium) was cultured for 24 hr in Earle's modified Eagle's medium as described (22), except that phosphate-free medium was prepared for cultures to be labeled with $H_3^{32}PO_4$. For labeling experiments with D -[U- ^{14}C]glucose and D -[2- 3H]mannose, glucose-free medium (supplemented with L -glutamine at 5 g/liter) was used, a procedure that increased incorporation of radiolabel into oligosaccharide >5-fold (unpublished results). Tunicamycin was added from a 10 mg/ml stock solution in dimethyl sulfoxide to cultures 4 hr prior to the addition of radiolabel.

Purification of Uteroferrin. Uteroferrin from uterine flushings was purified as described (2, 6). Uteroferrin was purified from explanted culture medium as follows: tissue was removed by centrifugation at $10,000 \times g$ for 20 min at 4°C, and the medium was dialyzed against 10 mM Tris-HCl, pH 8.2/1 mM phenylmethylsulfonyl fluoride/0.02% sodium azide. A fraction enriched in basic proteins was prepared by batch adsorption to CM-cellulose in the same buffer and eluted with 0.5 M NaCl in 10 mM Tris-HCl (pH 8.2). These basic proteins were then applied at room temperature to a 1 $\times$ 10 cm column of anti-uteroferrin-Sepharose (2 mg of IgG/ml of gel). Bound uteroferrin was eluted with 0.05 M glycine, pH 2.3/0.15 M NaCl/0.02% sodium azide and neutralized with 1 M Tris-HCl (pH 8.2). The approximate yield of uteroferrin per ml of incubation medium was 10 μ g.

Immunoprecipitation of Uteroferrin from Tissue Extracts and Medium. Uteroferrin was extracted from tissue by sonication at 4°C (4 bursts $\times$ 15 sec using a Bronwill Biosonic

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Abbreviations: endo H, endo- β -*N*-acetylglucosaminidase H; Con A, concanavalin A.

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microprobe) in 5 vol of 0.3 M NaCl/1 mM EDTA/2% (vol/vol) Nonidet P-40/1 mM phenylmethylsulfonyl fluoride/0.2% sodium azide, buffered with 0.05 M Tris acetate (pH 7.5). Medium or solubilized tissue was clarified by centrifugation ($30,000 \times g$, 20 min). To each 100 μ l of medium or solubilized tissue was added (in order) 100 μ l of a solution containing uteroferrin at 50 μ g/ml, 200 μ l of the solubilizing buffer containing 0.1% (wt/vol) bovine serum albumin, and 100 μ l of whole antiserum to uteroferrin, diluted to equivalence. The precipitate was collected by centrifugation in a Microfuge (15 min) and washed twice. Recovery of 125 I-labeled uteroferrin tracer (prepared by the method of Markwell and Fox, ref. 24) using this procedure was $\approx 58\%$.

Preparation of Uteroferrin Glycopeptides. Uteroferrin was incubated with predigested Pronase according to the method of Varki and Kornfeld (25) in the presence of 0.1 M D-glucose 6-phosphate. Uteroferrin glycopeptides were isolated from the digest by gel filtration on Sephadex G-50 (1.5 $\times$ 100 cm) in 50 mM Tris-HCl, pH 7.5/0.3 M NaCl or Bio-Gel P-10 (1.5 $\times$ 110 cm) in 0.1 M acetic acid.

Con A-Sepharose Chromatography. Culture medium or uteroferrin glycopeptides were fractionated on columns of Con A-Sepharose (1 $\times$ 5 cm) in 25 mM Tris-HCl, pH 8/0.15 M NaCl/1 mM MnCl_2 /1 mM CaCl_2 . Glycoproteins that bound were eluted with 0.5 M methyl α -D-mannoside in buffer containing 0.1% Triton X-100. After enzymatic degradation, uteroferrin glycopeptides or oligosaccharides were eluted sequentially with 10 mM methyl α -D-glucoside and then at 60°C with 100 mM methyl α -D-mannoside (26, 27).

Enzyme Digestions and Mild Acid Hydrolysis. Uteroferrin glycopeptides or intact uteroferrin were incubated with 1–4 milliunits of endo H in 50 mM citrate phosphate buffer (pH 5.5) for 24 hr at 37°C (25). Oligosaccharides were incubated with 0.5–1 milliunit alkaline phosphatase in 0.1 M borate buffer (pH 9.8) for 24 hr at 37°C both before, and after, mild acid hydrolysis for 30 min in 0.1 M HCl at 100°C.

Gel Filtration of Oligosaccharides. After incubation of uteroferrin with endo H, oligosaccharides were separated from polypeptide on Bio-Gel P-4 (200–400 mesh, 110 $\times$ 1.5 cm). Different size classes of uteroferrin oligosaccharides were identified by gel filtration on Bio-Gel P-4 superfine (<400 mesh, 110 $\times$ 1.5 cm). After mild acid hydrolysis or incubation with alkaline phosphatase, oligosaccharides were separated from low molecular weight components by gel filtration on Sephadex G-15 (40- to 120- μ m mesh, 55 $\times$ 1.5 cm). All three columns were run at room temperature in 0.1 M acetic acid/0.02% sodium azide.

QAE-Sephadex Ion-Exchange Chromatography. Oligosaccharides were dissolved in 1 mM ammonium acetate (pH 5.4) then applied to a 0.7 $\times$ 15 cm column of QAE-Sephadex A-25 equilibrated with 2 mM ammonium acetate (pH 5.4) (28, 29). The column was washed with the equilibration buffer and bound oligosaccharides were eluted with a 200-ml linear gradient of acetate (2–350 mM).

Identification of Mannose 6-Phosphate. Phosphorylated oligosaccharides were bound to QAE-Sephadex A-25 in 2 mM Tris base, eluted with 0.5 M HCl, and then hydrolyzed in 1 M HCl for 4 hr at 100°C to yield monosaccharides (30). The hydrolysate was lyophilized and passed through a column of Dowex 50-X8 (H^+ form), and the unbound material was dried. Some hydrolysates were incubated with phosphomannose isomerase and phosphoglucose isomerase (31). Hydrolyzed sugars and sugar standards were analyzed by descending chromatography for 18–22 hr on Whatman no. 1 paper in either solvent A (ethyl acetate/pyridine/acetic acid/ H_2O , 5:5:1:3; ref. 29) or solvent B (ethanol/1 M ammonium acetate, pH 7.5, 7.5:3 for 18–24 hr). Neutral sugars were detected with alkaline silver nitrate stain (32) and phosphate esters, with antimony-molybdate stain (33). The chromatograms were cut into 1-cm strips and radioactivity was counted.

Polyacrylamide Slab Gel Electrophoresis. Immunoprecipitates were subjected to electrophoresis in 10% (wt/vol) acrylamide slab gels (34). Alternatively, the samples were analyzed by two-dimensional PAGE (22, 35, 36). Gels were stained with Coomassie blue. Radioactive polypeptides were detected using Kodak XAR or XRP film by direct autoradiography or by fluorography (37).

RESULTS

In Vitro Biosynthesis and Glycosylation of Uteroferrin.

Uteroferrin purified from uterine secretions has a characteristic molecular weight of 35,000 when analyzed by Na-DodSO₄/PAGE (Fig. 1, lane 1). Uteroferrin synthesized by explanted endometrium *in vitro* in the presence of L-[35 S]methionine had a molecular weight of 37,000 when immunoprecipitated from either tissue (lane 2) or medium (lane 3). Pulse-radiolabeling experiments failed to demonstrate conversion of the M_r 37,000 form to the M_r 35,000 polypeptide in tissue after 48 hr of culture (data not shown). When tissue was incubated in the presence of tunicamycin, an immunoprecipitable uteroferrin polypeptide of M_r 33,000 was found (Fig. 1, lane 4). The appearance of this lower molecular weight polypeptide was dependent on the concentration of tunicamycin and was maximal, but still not complete, at concentrations of antibiotic between 5 and 10 μ g/ml (data not shown). The presumed aglycouteroferrin was also secreted into the medium (Fig. 1, lanes 5 and 6). The medium from explants cultured in the presence of tunicamycin at 10 μ g/ml and L-[14 C]leucine was fractionated on a column of Con A-Sepharose, and uteroferrin was collected by immunoprecipitation from the unbound and retained fractions. Although the bound fraction contained only the M_r 37,000 form (lane 8), the M_r 33,000 uteroferrin (aglycouteroferrin) was not retarded on the Con A-Sepharose column (lane 7).

Phosphorylation of Uteroferrin. Endometrial explants were cultured in medium containing either $^{32}\text{P}_i$ or L-[14 C]leucine in the presence or absence of tunicamycin at 20 μ g/ml. Uteroferrin, immunoprecipitated from the medium, was analyzed by two-dimensional PAGE (Fig. 2). In the presence of tunicamycin, phosphorylated uteroferrin was not detected.

FIG. 1. Polyacrylamide gel electrophoresis of uteroferrin synthesized in the presence and absence of tunicamycin. Lane 1, uteroferrin standard purified from uterine secretions and stained with Coomassie blue. Lanes 2 and 3, uteroferrin immunoprecipitated from solubilized tissue and from medium, respectively, after supplying endometrial explants (300 mg of tissue/5 ml of medium) from mid-pregnant pigs with L-[35 S]methionine (10 μ Ci/ml) for 24 hr. Lanes 4 and 5, uteroferrin immunoprecipitated from solubilized tissue and from medium, respectively, after culture in the presence of tunicamycin (20 μ g/ml) and L-[35 S]methionine. Lane 6, uteroferrin immunoprecipitated from medium after incubation of endometrium (500 mg/15 ml of medium) in the presence of L-[14 C]leucine (5 μ Ci) and tunicamycin (10 μ g/ml) for 24 hr. Lane 7, material equivalent to that in lane 6 that was not retained by Con A-Sepharose. Lane 8, material equivalent to that in lane 6 that was retained by Con A-Sepharose and then eluted with 0.5 M methyl α -D-mannoside. Numbers on the left indicate $M_r \times 10^{-3}$.

camycin, the majority of the ^{14}C -labeled uteroferrin had, as expected, an apparent M_r of 33,000 with only traces of the M_r 37,000 form present (Fig. 2A). However, with ^{32}P , only the M_r 37,000 form was detectable, whether or not tunicamycin was present in the medium (Fig. 2B and C). These results suggest that the ^{32}P was incorporated into the oligosaccharide and not into the polypeptide portion of uteroferrin.

Analysis of Uteroferrin Glycopeptides on Con A-Sepharose. A mixture of immunoaffinity-purified [^3H]glucosamine- and ^{32}P -labeled uteroferrin was digested with Pronase and the glycopeptides were separated from amino acids on Sephadex G-50. The glycopeptides were passed over Con A-Sepharose in an attempt to classify the structure of their oligosaccharide chains (Fig. 3). Approximately 20% of the ^3H -labeled glycopeptides were eluted with methyl α -D-glucoside and were therefore presumed to have a complex structure (27). However, greater than 70% of the ^3H -labeled and 95% of the ^{32}P -labeled glycopeptides bound avidly to Con A and could only be eluted with warm methyl α -D-mannoside.

Cleavage of ^{32}P -Labeled Glycopeptides by Endo H. Fractionation of the ^{32}P -labeled glycopeptides on Bio-Gel P-4 gave a single radioactive peak with a slight leading shoulder (Fig. 4). After incubation of the glycopeptides with endo H the ^{32}P peak eluted with a lower apparent molecular weight. This labeled material bound to Con A-Sepharose and could be eluted only with warm methyl α -D-mannoside (results not shown). The ^{32}P radiolabel was, therefore, present on an oligosaccharide and not on a peptide fragment.

Identification of Mannose 6-Phosphate. ^{32}P -labeled oligosaccharides were hydrolyzed with 1 M HCl and analyzed by paper chromatography in solvent A. Three peaks of ^{32}P were detected, one of which (27% of total cpm) remained at the origin and was presumed to be incompletely hydrolyzed oligosaccharides. The second major peak ($R_{\text{man}} = 0.23$; 64% of total cpm) coincided exactly with the mannose 6-phosphate standard, while a third, faster migrating peak ($R_{\text{man}} = 0.48$; 9% of total cpm) co-migrated with P_i . This solvent clearly separates mannose 6-phosphate from glucose 6-phosphate ($R_{\text{man}} = 0.18$). Similar results were obtained using solvent B.

If the hydrolyzed mixture was treated prior to chromatography with phosphomannose isomerase and phosphoglucose isomerase, there was a 70% reduction in the ^{32}P associated with the putative mannose 6-phosphate and a corresponding increase in ^{32}P in the regions of the chromatogram to which glucose 6-phosphate and fructose 6-phosphate migrated. Omission of phosphomannose isomerase resulted in no conversion.

High-mannose oligosaccharides were also prepared from uteroferrin isolated from cultures provided with D-[2- ^3H]mannose. They were chromatographed on a column of

FIG. 2. Two-dimensional PAGE of uteroferrin synthesized in the presence (A and C) and absence (B) of tunicamycin using L-[^{14}C]leucine (A) and $^{32}\text{P}_i$ (B and C) as labeled precursors. Endometrium (500 mg) was incubated with L-[^{14}C]leucine (5 μCi) or $^{32}\text{P}_i$ (2.1 mCi) in the presence or absence of tunicamycin (20 $\mu\text{g}/\text{ml}$). Uteroferrin was immunoprecipitated from the medium, solubilized, and analyzed by two-dimensional PAGE. Numbers on the left indicate $M_r \times 10^{-3}$.

FIG. 3. Con A-Sepharose chromatography of uteroferrin glycopeptides. Endometrium (50 g) was cultured in the presence of 100 μCi of D-[6- ^3H]glucosamine or 20 mCi of $^{32}\text{P}_i$ in 500 ml of medium. Uteroferrin was purified from the medium, digested with Pronase, desalted, and applied to Con A-Sepharose (1 $\times$ 5 cm). Arrowheads: W, start of washing with application buffer; G, elution with 10 mM methyl α -D-glucoside; M, elution with 100 mM methyl α -D-mannoside at 60°C. One-milliliter fractions were collected.

Bio-Gel P-4 and the phosphorylated components that eluted between fractions 52 and 58 (see Fig. 4) were pooled. After hydrolysis with 1 M HCl and complete removal of the acid, mannose 6-phosphate was trapped on a column of QAE-Sephadex equilibrated with 2 mM acetate. A total of 13% of the ^3H remained bound to the column and it was eluted with 100 mM acetate. This result suggests that approximately one in eight of the mannose residues was phosphorylated.

Diester Nature of the Incorporated $^{32}\text{P}_i$. Uteroferrin, labeled with D-[^3H]glucosamine and $^{32}\text{P}_i$ was treated with endo H, and high-mannose oligosaccharides were isolated by chromatography on Con A-Sepharose. After separation of the oligosaccharides from any remaining uteroferrin on a column of Bio-Gel P-4, they were then subjected to ion-exchange chromatography on QAE-Sephadex (Fig. 5). Approximately 40% of the ^3H failed to bind to the column (peak A) and it was assumed to represent nonphosphorylated neutral oligosaccharides. Peak B, which eluted early in the gradient (at about 25 mM acetate), and peak C, which eluted at 40 mM acetate, contained both ^{32}P and ^3H .

In a subsequent experiment, dual-labeled oligosaccharides representing peak B were incubated with alkaline phosphatase.

FIG. 4. Gel filtration of ^{32}P -labeled uteroferrin glycopeptides and oligosaccharides on a column of Bio-Gel P-4. Phosphorylated glycopeptides, prepared as described in the legend to Fig. 3, were applied to a column of Bio-Gel P-4 (<400 mesh) in 0.1 M acetic acid (-----; dpm $\times 10^{-3}$). Fractions indicated by the open bar were pooled, lyophilized, incubated with endo H (1 milliunit), and rechromatographed (—; dpm $\times 10^{-2}$). Fraction size in each experiment was 2 ml. The arrow head indicates the elution volume of [^3H]Man $_9$ GlcNAc $_1$ (M_9) standard.

FIG. 5. Analysis of dual-labeled uteroferrin oligosaccharides by QAE-Sephadex ion-exchange chromatography. Uteroferrin was purified from the culture medium after supplying endometrium (10 g) with 50 ml of medium containing D-[^3H]glucosamine (100 μCi) and $^{32}\text{P}_i$ (5 mCi) for 24 hr. Oligosaccharides were prepared by endo H digestion (4 milliunits for 48 hr at 37°C) of the intact uteroferrin and purified by gel filtration on Bio-Gel P-4. Oligosaccharides were dried by rotary evaporation to remove acetic acid, dissolved in 1 mM ammonium acetate, and applied to the column of QAE-Sephadex. The arrow head indicates the start of the linear gradient of ammonium acetate (200 ml; 2–350 mM). Fraction size was 1.4 ml.

tase both before and after mild acid hydrolysis; oligosaccharides were separated from compounds of low molecular weight by using Sephadex G-15. Alkaline phosphatase alone failed to cleave any ^{32}P from the oligosaccharides, suggesting that the phosphate was protected and most probably, therefore, in diester linkage. Mild acid hydrolysis removed 26% of the ^3H radioactivity as a low molecular weight component, presumably an amino sugar derivative, but none of the ^{32}P . The procedure, however, rendered 79% of the ^{32}P susceptible to subsequent hydrolysis by alkaline phosphatase.

Peak C material, like peak B, was also resistant to alkaline phosphatase until after it had been treated with hot dilute acid, suggesting that the oligosaccharides in this peak contained two phosphodiester groups (data not shown).

Identification of the Covering Sugar. High-mannose phosphorylated oligosaccharides were prepared from uteroferrin that had been isolated from cultures incubated with D-[^{14}C]glucose to provide uniform labeling of the carbohydrate. Material containing 7700 dpm of ^{14}C was subjected to mild acid hydrolysis to release any covering sugars, which were then separated from oligosaccharide by gel filtration on Sephadex G-15. About 12% (i.e., ≈ 1000 dpm) of the ^{14}C eluted as a low molecular weight fraction which was lyophilized. Upon chromatography of the dried material in solvent A, more than 90% of the ^{14}C chromatographed with the *N*-acetylglucosamine standard in solvent A ($R_{\text{man}} = 1.19$). A trace of ^{14}C was also associated with glucosamine ($R_{\text{man}} = 0.63$). No radiolabeled sugars that comigrated with glucose, mannose, galactose, or *N*-acetylgalactosamine standards were detected.

DISCUSSION

Our experiments have shown that uteroferrin, the major progesterone-induced secretory protein of the porcine endometrium, carries phosphorylated oligosaccharide chains when released by explanted endometrium in culture. These oligosaccharides were *N*-linked, since their formation was inhibited by tunicamycin. Furthermore, the phosphorylated oligosaccharides were of the high-mannose type as they bound to Con A with great avidity and were displaced only by methyl

α -D-mannoside at elevated temperature (27). They were also susceptible to cleavage by endo H (29). The oligosaccharides appeared to be relatively large as they eluted from Bio-Gel P-4 ahead of an authentic $\text{Man}_5\text{GlcNAc}_1$ standard. However, they may have migrated anomalously because of their negative charge and behaved as if they were larger than their true molecular mass.

The incorporated ^{32}P was recovered as mannose 6-phosphate after strong acid hydrolysis, and about one in eight of the mannose residues in the oligosaccharide fraction carried a phosphate group. The majority of the phosphates were in diester linkage since the ^{32}P became susceptible to alkaline phosphatase hydrolysis only after mild acid treatment. It is possible that some uncovered phosphate groups were lost during the course of the workup due to phosphatase action, including that of uteroferrin itself. However, uteroferrin acid phosphatase activity is maximal at pH 4.9 and very low above pH 6.0 (6). Moreover, attempts were made to reduce any contaminating phosphatase activities by including inhibitors (either glucose 6-phosphate or P_i) in the enzyme digests. For these reasons we consider the majority of the ^{32}P -labeled oligosaccharides to contain a single phosphodiester group.

The sugar covering the phosphate group and protecting it from hydrolysis by alkaline phosphatase was identified as *N*-acetylglucosamine after its release from [^{14}C]glucose-labeled oligosaccharides with hot dilute acid. This experiment confirmed that other species of covering sugar, including glucose itself, were not present to any significant extent. Such a determination was important because an extracellular glycoprotein has recently been described that carries mannose 6-phosphate on its high-mannose oligosaccharide chains but on which the covering sugar is glucose and not *N*-acetylglucosamine (38).

Hot dilute acid treatment, however, removed only about one-quarter of the ^3H label from the most abundant phosphorylated oligosaccharide isolated from uteroferrin labeled in culture with both $^{32}\text{P}_i$ and D-[^3H]glucosamine. Release of 50% of the glucosamine label would have occurred if the oligosaccharides resulting from endo H cleavage had contained a single remaining "core" *N*-acetylglucosamine moiety and a single terminal amino sugar residue covering the phosphate. Lower values would occur only if the mild acid hydrolysis were incomplete or if some of the oligosaccharides were partially processed to hybrid chains. The latter explanation is the most likely considering the reported susceptibility of terminal *N*-acetylglucosamine to acid hydrolysis (25).

The incorporation of the phosphate group into the oligosaccharide chain of uteroferrin presumably occurs in a manner similar to that demonstrated for a number of lysosomal enzymes. In this process, a residue of *N*-acetylglucosamine 1-phosphate is transferred to one or two of several potential mannose acceptors by UDP-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine 1-phosphate transferase ($\text{GlcNAc-1-P transferase}$) (39) within the Golgi apparatus (40). The reaction results in a diester linkage between position 1 of *N*-acetylglucosamine and position 6 of mannose. In the case of lysosomal enzyme processing, however, a phosphodiesterase associated with the Golgi complex (40, 41) cleaves the *N*-acetylglucosamine exposing the phosphate, which remains in 6-monoester linkage to mannose (41). It is then postulated that the oligosaccharide becomes bound to receptors specific for mannose 6-phosphate residues, and these receptors then mediate the movement of the enzyme to primary lysosomes (42). In the case of uteroferrin, the covering *N*-acetylglucosamine residues are not removed, and it may be for this reason that the glycoprotein is secreted rather than sequestered within the cell.

There are few other examples known where glycoproteins are secreted with phosphorylated oligosaccharide chains.

One is a M_r 35,000 glycoprotein that is released by virus-transformed 3T3 fibroblasts (43). In untransformed cells, the glycoprotein is not secreted unless the cell is treated with the lysosomotropic agent, ammonium chloride (44), suggesting that it may be an as yet unidentified lysosomal enzyme. The secreted form of this glycoprotein bound to purified mannose 6-phosphate receptors and unlike in uteroferrin the phosphate was probably not, therefore, in diester linkage.

It is clear that uteroferrin has many of the characteristics of a lysosomal enzyme. It is synthesized bearing the so-called lysosomal recognition marker (although this remains masked), it has an acid pH optimum, and it resembles other tartrate-insensitive acid phosphatases that appear to have a lysosomal location (13, 18). We suggest that uteroferrin, whose synthesis during pregnancy is under progesterone control, may in the uterus represent an overproduced lysosomal enzyme that is directed along a secretory pathway rather than transported into lysosomes.

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