Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6778–6782, September 1988
Cell Biology

Galactose at the nonreducing terminus of O-linked oligosaccharides of mouse egg zona pellucida glycoprotein ZP3 is essential for the glycoprotein's sperm receptor activity

(mouse sperm receptor/terminal galactose/exoglycosidases/galactose oxidase)

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Communicated by John J. Burns, May 25, 1988

ABSTRACT **During fertilization in mice, zona pellucida glycoprotein ZP3 mediates initial sperm–egg interactions by serving as receptor for sperm. Purified egg ZP3, as well as ZP3-derived O-linked oligosaccharides, exhibit sperm receptor activity *in vitro*. We report that treatment of purified egg ZP3 and ZP3-derived O-linked oligosaccharides with either α-galactosidase or galactose oxidase results in loss of sperm receptor activity. In the latter case, sperm receptor activity can be restored to the oxidized glycoprotein and O-linked oligosaccharides by treatment with sodium borohydride. We conclude that galactose, located in α-linkage at the nonreducing terminus of O-linked oligosaccharides, is at least one of the sugar determinants on ZP3 responsible for binding of sperm to the zona pellucida.**

The zona pellucida, a thick extracellular coat that surrounds all mammalian eggs and preimplantation embryos, performs a variety of functions during development (1–3). Perhaps paramount among these functions is its role during fertilization, since species-specific binding of sperm to unfertilized eggs is mediated by sperm receptors located in the zona pellucida. Following fertilization, the zona pellucida assists in prevention of polyspermy and protects preimplantation-stage embryos as they travel toward the uterus.

The mouse egg sperm receptor has been isolated and characterized. It is an 83,000 M_r glycoprotein (called zona pellucida glycoprotein ZP3) that is composed of a 44,000 M_r polypeptide chain, three or four complex-type N-linked oligosaccharides, and an undetermined number of O-linked oligosaccharides (4–6). The ability of ZP3 to serve as sperm receptor is attributable solely to certain of its O-linked oligosaccharides (6–8). Removal of these oligosaccharides from purified ZP3 by mild alkaline hydrolysis (β-elimination) inactivates the glycoprotein as a sperm receptor. On the other hand, O-linked oligosaccharides, recovered following β-elimination of ZP3, retain sperm receptor activity (SRA) and the activity is attributable to a population of oligosaccharides of about 3900 M_r.

Here, we describe results of experiments designed to analyze features of ZP3 O-linked oligosaccharides that account for their SRA *in vitro*. By enzymatic and chemical modification of purified ZP3 and ZP3-derived O-linked oligosaccharides, we have found that galactose, present in α-linkage at the nonreducing terminus of the oligosaccharides, is essential for SRA of ZP3. Removal or modification of terminal galactose results in loss of SRA.

MATERIALS AND METHODS

Collection and Culture of Mouse Gametes and Embryos. Eggs, sperm, and two-cell embryos were obtained from randomly bred, Swiss albino mice (CD-1; Charles River Breeding Laboratories) and cultured *in vitro* as described (4, 9).

Sperm Binding Assays. The extent of binding of sperm to eggs *in vitro* was determined as described (4, 7, 8). In some cases, eggs were fixed with 1.5% formaldehyde, rather than glutaraldehyde (10), in phosphate-buffered saline (PBS) for 3 hr and then washed extensively prior to use in the binding assay. SRA of glycoproteins and oligosaccharides was assessed by an *in vitro* "competition assay" as described (4). Briefly, capacitated sperm (≈5 × 10^5 per ml) were incubated for 40–60 min in 50 μl of culture medium containing the test substance, ovulated eggs and two-cell embryos were then added, and the cells were incubated an additional 10–12 min at 37°C. Sperm associated with, but not bound to, eggs and embryos were removed by repeated pipetting as described (4). Eggs with bound sperm were fixed with 1% glutaraldehyde in PBS and the number of bound sperm was scored as described (9, 11).

Purification of ZP3. Mass isolation of zonae pellucidae on Percoll (Pharmacia) gradients, purification of ZP3 by size fractionation on HPLC (TSK-250; Bio-Rad), and storage and handling of purified ZP3 were carried out as described (9, 11). Prior to use, frozen samples were thawed and then heated at 80°C for 20 min. Yields of ZP3 were estimated based on absorbance at 280 nm (≈0.7 mg/ml per OD unit).

Purification of ZP3-Derived O-Linked Oligosaccharides. Purified ZP3 (10–80 μg) was subjected to β-elimination in the presence of NaB^3H_4 essentially as described (8). Eluate from a Dowex 50W-400 column was lyophilized and fractionated according to size by HPLC on an Altex SW-2000 column in 60 mM sodium phosphate (pH 6.3), and fractions were tested for SRA. Active fractions were pooled, lyophilized, resuspended in H_2O, and desalted on a Bio-Gel P-2 column. Void volume fractions were pooled, lyophilized, resuspended in 10 mM phosphate buffer (pH 6.3), and subjected to HPLC on an Altex DEAE-5PW column using a 0–0.45 M NaCl gradient. Fractions exhibiting SRA were desalted on a Bio-Gel P-2 column and stored in H_2O at −20°C for up to 2 weeks before use. Prior to use, frozen samples were thawed and then heated at 80°C for 20 min. Yields of oligosaccharides were estimated based on the specific activity of NaB^3H_4.

Digestion of Substrates by Exoglycosidases. Individual exoglycosidases (Boehringer Mannheim) and mixed exoglycosidases (Miles) were used in appropriate buffers [β-galactosidase (*Escherichia coli*): 0.1 M Tris, pH 7.0/1 mM $MgCl_2$/1 mM $CaCl_2$/1 mM KCl/1 mg of bovine serum albumin per ml; α-galactosidase (coffee bean) and mixed exoglycosidases (*Charonia lampus*): 0.1 M citrate/phosphate, pH 6.0/1 mM $MgCl_2$/1 mM $CaCl_2$/1 mM KCl/1 mg of bovine serum albumin per ml; β-glucuronidase (*E. coli*),

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Abbreviations: SRA, sperm receptor activity; Dns, dansyl.
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α-fucosidase (beef kidney), β-*N*-acetylglucosaminidase (beef kidney), and neuraminidase (*Clostridium perfringens*): 0.1 M citrate/phosphate, pH 5.0/1 mM $MgCl_2$/1 mM $CaCl_2$/1 mM KCl/1 mg of bovine serum albumin per ml] at a concentration of 6.5×10^{-2} unit/ml. Digestions were carried out for 90 min at 37°C in a 10-μl volume containing about 600 zona pellucida equivalents of ZP3 or ZP3-derived oligosaccharides and were terminated by dilution with H_2O and heating at 100°C for 15 min. Each of the exoglycosidases was assayed under the appropriate conditions using *o*- and *p*-nitrophenyl sugars as substrates to determine the enzyme's specific activity. (Note: 1 unit of β-*N*-acetylglucosaminidase contained 0.2 unit of β-*N*-acetylgalactosaminidase activity.) All enzyme activities are expressed as μmol of product formed per min.

Oxidation of Substrates by Galactose Oxidase. Purified ZP3 or ZP3-derived oligosaccharides (≈500 zona pellucida equivalents per 4-μl reaction) were incubated for 1 hr at 20°C in the presence of galactose oxidase (4×10^{-3} unit; Sigma) and catalase (4×10^{-3} unit; Sigma) in 0.1 M phosphate buffer (pH 6). The reaction was terminated by heating at 100°C for 15 min, and substrates were tested for SRA after dilution with H_2O (final concentration, 20–40 zona pellucida equivalents per μl). In some cases, following heat inactivation, galactose oxidase-treated substrates were diluted with 9 vol of 0.1 M phosphate buffer (pH 7.8) containing 10 mM $NaBH_4$, incubated at 40°C for 30 min, and acidified with 2 M acetic acid followed by addition of an equal volume of methanol. In other cases, galactose oxidase-treated substrates were diluted with 5 vol of sodium acetate (pH 6) and 4 vol of 0.1 M NH_2OH and incubated at 40°C for 1 hr. Borohydride- and hydroxylamine-treated material was desalted on a Bio-Gel P-2 column in H_2O, and the void volume was lyophilized and then resuspended in H_2O (final concentration, ≈20–40 zona pellucida equivalents per μl). Specific activities of galactose oxidase and catalase were determined by the horseradish peroxidase-linked *o*-toluidine (Sigma) and hydrogen peroxide (Boehringer Mannheim) assays, respectively.

Analysis of Sugars by Analytical HPLC. Purified ZP3-derived O-linked oligosaccharides (≈50–250 pmol) were incubated with exoglycosidases (0.06 unit/ml; 20-μl reaction, under toluene) at 37°C for 24 hr. The reaction mixture was diluted with H_2O to 100 μl, 50 μl of 5% dansyl (Dns)-hydrazine (in acetonitrile) and 10 μl of 10% trichloroacetic acid was added, and the mixture was incubated at 65°C for 20 min. Separation of Dns-sugars was carried out by reverse-phase HPLC (12).

RESULTS

Purification of ZP3-Derived O-Linked Oligosaccharides Exhibiting SRA. Purified ZP3 was subjected to β-elimination in the presence of NaB^3H_4. Released O-linked oligosaccharides were fractionated by HPLC according to size, and individual fractions were assayed for SRA by using an *in vitro* competition assay (Fig. 1*A*). Fractions exhibiting SRA (≈3900 M_r) were pooled and subjected to ion-exchange chromatography, and, once again, individual fractions were assayed for SRA (Fig. 1*B*). Oligosaccharides exhibiting SRA were resolved by ion-exchange chromatography as a single peak of material that was recovered, desalted, lyophilized, and stored at −20°C. These purified ZP3-derived O-linked oligosaccharides exhibiting SRA were used in the experiments that follow.

Effect of Exoglycosidases on SRA of Purified ZP3 and ZP3-Derived O-Linked Oligosaccharides. As previously reported (4, 8), preincubation of mouse sperm with either purified egg ZP3 or ZP3-derived O-linked oligosaccharides inhibits binding of sperm to unfertilized eggs *in vitro* (Fig. 2). To evaluate the requirement for individual sugars in SRA, purified ZP3 and ZP3-derived O-linked oligosaccharides

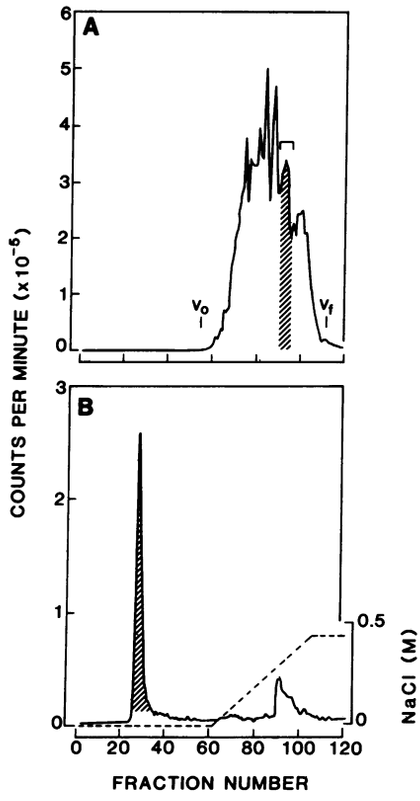

FIG. 1. Purification of ZP3-derived O-linked oligosaccharides possessing SRA. ZP3, purified from ≈80,000 zonae pellucidae, was subjected to β-elimination in the presence of NaB^3H_4, and radiolabeled oligosaccharides were fractionated (≈90 μl loaded) according to size by HPLC on an SW-2000 column (*A*). Fractions (100 μl) were assayed for radioactivity and tested for SRA, and active fractions were pooled (*A*; hatched region-fractions 89–95), desalted, lyophilized, resuspended, and subjected to ion-exchange chromatography (≈50 μl loaded) by HPLC on a DEAE-5PW column (*B*). Fractions (100 μl) were assayed for radioactivity and tested for SRA, and active fractions were pooled (*B*; hatched region-fractions 27–31) and stored frozen prior to use. V_o, void volume; V_f, final volume.

were treated with a variety of exoglycosidases and the resultant products were tested for the ability to inhibit binding of sperm to eggs *in vitro* (i.e., SRA).

Of six exoglycosidases tested for their ability to destroy SRA of purified ZP3 and ZP3-derived O-linked oligosaccharides, only α-galactosidase and α-fucosidase had a significant effect; β-galactosidase, β-glucuronidase, β-*N*-acetylglucosaminidase (containing β-*N*-acetylgalactosaminidase), and neuraminidase were completely ineffective (Fig. 2). In three independent experiments, each employing a different preparation of ZP3 and ZP3-derived O-linked oligosaccharides, only α-galactosidase and α-fucosidase had a significant effect on SRA, in each case, reducing it to almost undetectable levels. Furthermore, neither inactivated α-galactosidase nor α-fucosidase (inactivated at 100°C for 15 min) had a significant effect on SRA of either substrate (Fig. 2). Consistent with these results, much lower levels of sperm binding were observed with formaldehyde-fixed, unfertilized eggs that had been exposed to either α-galactosidase (12 ± 4 sperm per egg) or α-fucosidase (12 ± 4 sperm per egg) than with untreated eggs and eggs exposed to each of the other exoglycosidases (29 ± 6 sperm per egg) (see *Materials and Methods*). Collectively, these results suggest that removal of either terminal galactose or fucose from ZP3 and ZP3-derived O-linked oligosaccharides destroys their SRA.

In view of the observations just described, purified ZP3-derived O-linked oligosaccharides were treated with either

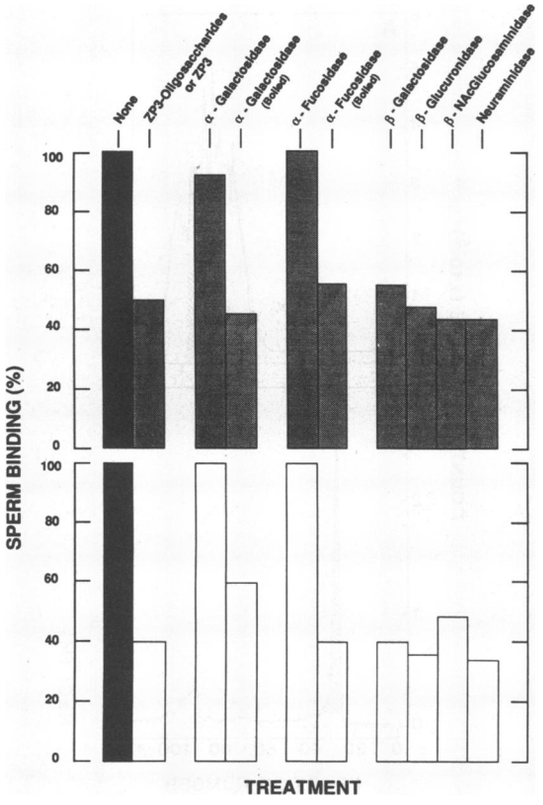

FIG. 2. Effect of exoglycosidases on SRA of ZP3 and ZP3-derived O-linked oligosaccharides. In these experiments, the number of sperm bound to eggs *in vitro* was determined under control conditions—i.e., in the absence of added ZP3 or ZP3-derived O-linked oligosaccharides (black bars), in the presence of untreated or exoglycosidase-treated ZP3-derived O-linked oligosaccharides (stippled bars), or in the presence of untreated or exoglycosidase-treated ZP3 (white bars). The number of sperm bound to eggs in the presence of either ZP3 or ZP3-derived O-linked oligosaccharides was compared with the number bound under control conditions (range, 27–57 sperm per egg; four individual experiments) and expressed as a percentage of the control value.

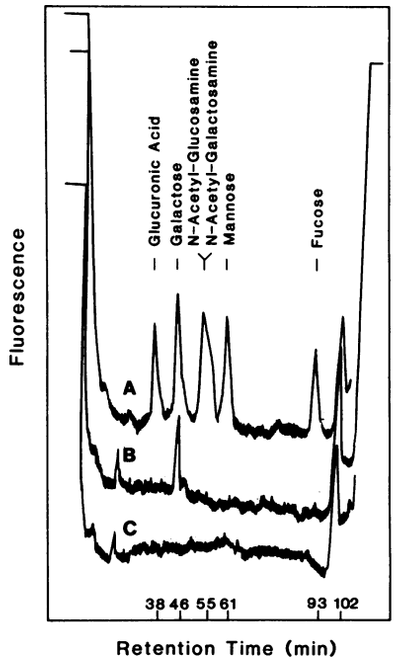

FIG. 3. HPLC analysis of sugars released from ZP3-derived O-linked oligosaccharides by α-galactosidase. ZP3-derived O-linked oligosaccharides (≈94 pmol), purified by ion-exchange chromatography (fractions 26–31, Fig. 1*B*), were incubated with either active or heat-inactivated α-galactosidase under conditions described in the text. Released sugars were allowed to react with Dns-hydrazine and resolved by reverse-phase HPLC, measuring relative fluorescence as a function of retention time. Profile A, sugar standards (50 pmol) dansylated in the presence of α-galactosidase. Profile B, sugars released from O-linked oligosaccharides by treatment with α-galactosidase. Profile C, sugars released from O-linked oligosaccharides by heat-inactivated α-galactosidase. The retention time of the peak seen in profile B, as well as the fact that this peak preceded elution of Dns-*N*-acetylgalactosamine by about 10 min, strongly suggest that it represents Dns-galactose.

α-galactosidase or α-fucosidase, sugars released from the oligosaccharides were allowed to react with Dns-hydrazine, and the Dns-sugars were analyzed by HPLC. (Note: Comparable analyses were not carried out with the other four exoglycosidases since they had no effect on SRA.) Under conditions that resulted in loss of SRA, α-galactosidase released only galactose from the purified oligosaccharides (Fig. 3), whereas neither fucose nor any other sugar was detected in samples treated with α-fucosidase (data not shown). To date, we have no satisfactory explanation for the latter results. [Note: When sugar standards (50 pmol) were dansylated in the presence of α-fucosidase and subjected to HPLC, a profile identical to that shown in profile A of Fig. 3 was obtained.] However, results obtained with α-galactosidase suggest that release of galactose, located in an α-linkage at the nonreducing terminus of ZP3 oligosaccharides, is responsible for loss of SRA.

Effect of Galactose Oxidase on SRA of Purified ZP3 and ZP3-Derived O-Linked Oligosaccharides. In view of the results obtained with α-galactosidase, additional experiments were carried out to examine the potential role of galactose in SRA. Purified ZP3 and ZP3-derived O-linked oligosaccharides were treated with galactose oxidase, an enzyme that converts the C-6 position alcohol of terminal galactose and *N*-acetylgalactosamine residues in oligosaccharides to an aldehyde (13), and SRA was assayed *in vitro*. Whereas inactivated galactose oxidase (inactivated at 100°C for 15 min) had no effect on SRA of either substrate, active enzyme completely destroyed SRA of ZP3 and ZP3-derived oligosaccharides (Fig. 4). However, SRA could be restored to galactose oxidase-treated samples by regeneration of a C-6 position alcohol on galactose (not *N*-acetylgalactosamine; see below) by reduction with $NaBH_4$ (14) (Fig. 4)—i.e., the inhibitory effect of galactose oxidase on SRA is reversible. (Note: Unlike results obtained with $NaBH_4$, conversion of the sugar aldehyde to an oxime adduct in the presence of NH_2OH did not restore SRA to ZP3-derived oligosaccharides treated with galactose oxidase; data not shown.) Thus, conversion of a ZP3 sugar alcohol to an aldehyde is sufficient to preclude binding of sperm to ZP3.

Analysis of Terminal Sugars of ZP3 O-Linked Oligosaccharides Following Treatment with Galactose Oxidase. To determine whether terminal galactose was modified by galactose oxidase in the experiments described above, ZP3 was treated with galactose oxidase, and oxidized sugars of ZP3-derived O-linked oligosaccharides were identified by analytical HPLC. In these experiments, purified ZP3 was incubated first with galactose oxidase and then with NaB^3H_4, and then radiolabeled ZP3 was separated from other components by size fractionation and subjected to β-elimination. (Note: No radiolabel was incorporated into ZP3 first treated with heat-inactivated galactose oxidase and then incubated with NaB^3H_4, indicating that ^3H incorporation was galactose oxidase-dependent.) Released oligosaccharides were subjected to size fractionation on HPLC, fractions were assayed for SRA (Fig. 5*A*), and material exhibiting SRA (Fig. 5*A*,

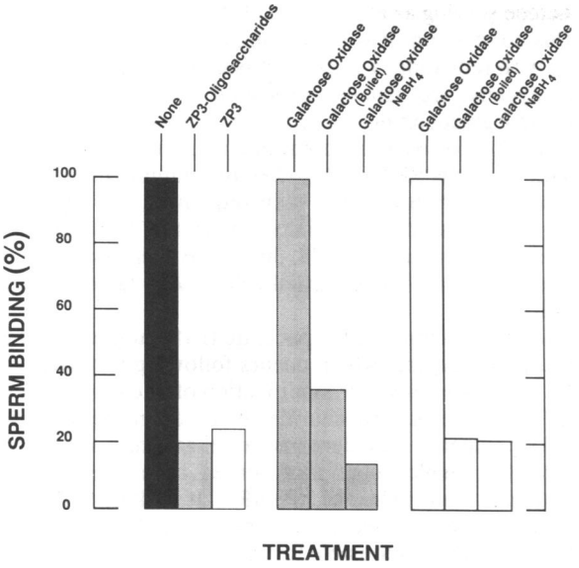

FIG. 4. Effect of galactose oxidase on SRA of ZP3 and ZP3-derived O-linked oligosaccharides. In these experiments, the number of sperm bound to eggs *in vitro* was determined under control conditions—i.e., in the absence of added ZP3 or ZP3-derived O-linked oligosaccharides (black bar), in the presence of untreated or galactose oxidase-treated (with or without $NaBH_4$) ZP3-derived O-linked oligosaccharides (stippled bars), or in the presence of untreated or galactose oxidase-treated (with or without $NaBH_4$) ZP3 (white bars). The number of sperm bound to eggs in the presence of either ZP3 or ZP3-derived O-linked oligosaccharides was compared with the number bound under control conditions (range, 27–40 sperm per egg; three individual experiments) and expressed as a percentage of the control value.

peak ii) was treated with a mixture of exoglycosidases (including α-galactosidase) to remove radiolabeled terminal sugars from the oligosaccharides. Released sugars were then reacted with Dns-hydrazine and the radiolabeled Dns-sugars were analyzed by analytical HPLC.

As seen in Fig. 5*B*, following reaction of exoglycosidase-released sugars from peak ii with Dns-hydrazine, the only radiolabeled sugar adduct identified by HPLC was [^{3}H]Dns-galactose. Indeed, when peaks i–iii seen in Fig. 5*A* were pooled and treated with exoglycosidases, and released sugars were allowed to react with Dns-hydrazine, only [^{3}H]Dns-galactose was identified by HPLC (data not shown). These results lend further support to the involvement of galactose, located at the nonreducing terminus of ZP3 O-linked oligosaccharides, in SRA.

Two peaks of radiolabel (Fig. 5*B*, a and b) were found in the region corresponding to that of Dns-sulfonic acid. These peaks do not represent either free, unreacted sugars or undigested oligosaccharides—e.g., redigestion of both peaks, followed by reaction with Dns-hydrazine, did not alter their behavior on HPLC. Furthermore, when mucin was treated in the manner described above for ZP3 and analyzed by HPLC, in addition to peaks representing [^{3}H]Dns-galactose and [^{3}H]Dns-*N*-acetylgalactosamine, peaks corresponding to a and b in Fig. 5*B* were observed (Fig. 5*C*). The latter remain as yet unidentified products of the Dns-hydrazine reaction.

DISCUSSION

Involvement of carbohydrates in cellular adhesion is indicated in a variety of instances, including that of sperm–egg interaction in plants and animals (15, 16). In this context, previously, we reported that certain O-linked oligosaccharides of mouse egg ZP3 account for the glycoprotein's SRA *in vitro* (6, 8). Here, we have analyzed the O-linked oligosaccharides further to identify specific sugars involved in binding of sperm to ZP3, the mouse sperm receptor. Evidence presented strongly suggests that galactose, located in

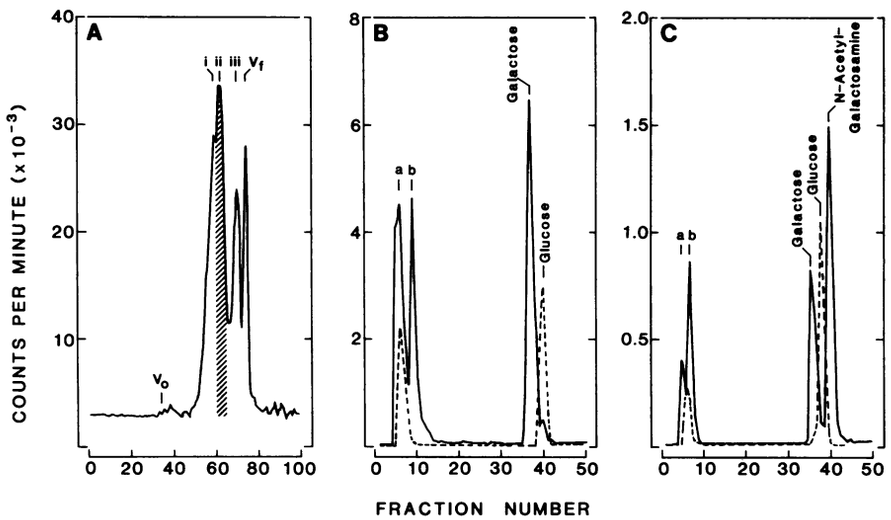

FIG. 5. HPLC analysis of sugars released by exoglycosidases from galactose oxidase-treated ZP3 and submaxillary mucin. ZP3 (≈50 μg) was treated first with galactose oxidase and then with NaB^3H_4 (10 Ci/mmol; 1 Ci = 37 GBq) to radiolabel galactose oxidase-modified terminal sugars; ZP3 was then subjected to β-elimination in the presence of unlabeled $NaBH_4$, and released O-linked oligosaccharides were fractionated according to size by HPLC as described in the legend to Fig. 1*A*. Fractions were assayed for radioactivity and for SRA (Fig. 5*A*), and active fractions (peak ii, hatched region) were pooled and desalted. An aliquot of the pooled fractions (≈3 × 10^4 cpm) was digested with a mixture of exoglycosidases (including α-galactosidase and α-*N*-acetylgalactosaminidase), [^{14}C]glucose and sugar standards (≈500 pmol) were added, the mixture was allowed to react with Dns-hydrazine, and Dns-sugars were resolved by reverse-phase HPLC, as in Fig. 3. Fluorescence was detected to establish retention times of Dns-sugars, and column fractions were assayed for radioactivity to determine relative positions of ^{3}H-Dns-sugars (released from peak ii, Fig. 5*A*) and [^{14}C]Dns-glucose (Fig. 5*B*). Bovine submaxillary mucin (≈25 μg) was treated with galactose oxidase followed by NaB^3H_4 and digested with exoglycosidases as described for ZP3. Sugars released from mucin were allowed to react with Dns-hydrazine, [^{14}C]Dns-glucose was added, and Dns-sugars were resolved by reverse-phase HPLC. As before, fluorescence was detected and column fractions were assayed for radioactivity (Fig. 5*C*). The positions of [^{3}H]Dns-galactose and [^{3}H]Dns-*N*-acetylgalactosamine were clearly distinguishable from that of [^{14}C]Dns-glucose. V_o, void volume; V_f, final volume.

α-linkage at the nonreducing terminus of ZP3 O-linked oligosaccharides, plays an essential role in the process of sperm binding to receptor. Treatment of ZP3 and ZP3-derived O-linked oligosaccharides with α-galactosidase resulted in release of galactose from both substrates and in loss of SRA (Figs. 2 and 3). A much less convincing case can be made for the participation of terminal fucose in sperm–egg binding. Although α-fucosidase treatment of ZP3 and ZP3-derived oligosaccharides destroyed their SRA (Fig. 2), we were unable to detect release of fucose, or any other sugar, from either substrate.

Perhaps most germane is our finding that galactose oxidase-catalyzed modification of terminal galactose on either ZP3 or ZP3-derived O-linked oligosaccharides destroyed their SRA; however, receptor activity was restored by $NaBH_4$ reduction (Figs. 4 and 5). Thus, conversion of the C-6 alcohol of galactose to an aldehyde apparently is sufficient to abolish binding of ZP3 to sperm. Similar behavior following treatment with galactose oxidase has been reported for a number of other carbohydrate-mediated interactions, including binding of lectins to human erythrocytes (17) and platelet-aggregating activity of human von Willebrand factor (factor VIII) (18). These results are not unexpected, since interactions between carbohydrate binding proteins and sugar ligands are stabilized largely by hydrogen bonds and van der Waals contacts, with the former providing the major contribution to binding (19). Sugar hydroxyls participate extensively in such hydrogen bonding, probably accounting for the inhibitory effects of galactose oxidase treatment on carbohydrate-mediated sperm–egg interaction.

In addition to our own work, presented here and elsewhere, several other studies suggest that sugars play a role in binding of mammalian sperm to eggs. For example, binding of sperm to eggs *in vitro* has been inhibited by certain lectins (e.g., wheat germ agglutinin) (20), glycoconjugates (e.g., fucoidin) (21), and monosaccharides (α-methylmannoside) (21, 22), and pretreatment of eggs with various glycosidases (e.g., α-mannosidase, α-fucosidase, or β-*N*-acetylglucosaminidase) has led to decreased binding of sperm to eggs *in vitro* (21, 23). Although relevant to experiments from our own laboratory, results of these other studies must be interpreted cautiously. Since the zona pellucida is composed of several glycoproteins, each with multiple oligosaccharides, the inhibitory effect of lectins on sperm binding could be attributable to masking of sperm receptor sites indirectly, rather than to binding of lectins specifically to oligosaccharides responsible for SRA. Previous studies involving glycosidases did not include analyses of sugars released from the zona pellucida and, in most cases, did not assess whether or not contaminating enzymatic activities were present in the glycosidase preparations. Finally, although certain monosaccharides and glycoconjugates inhibit sperm binding *in vitro*, millimolar concentrations are required for inhibition and, in some cases, sperm motility, an important factor in sperm binding, is affected deleteriously. On the other hand, ZP3 and ZP3-derived O-linked oligosaccharides inhibit sperm binding at nanomolar concentrations without affecting sperm motility.

It has been suggested that galactosyl transferase, associated with plasma membrane overlying the sperm head, serves as an egg binding protein during sperm–egg interaction in mice (23, 24). Presumably, the enzyme mediates such interaction by binding to certain ZP3 oligosaccharides that terminate with an *N*-acetylglucosamine, the usual acceptor sugar for UDP-galactose. Although this remains an appealing possibility, our present results suggest that galactosyl transferase may not be the sole mediator of sperm–egg interaction in mice. It is tempting to speculate that in mice, as in sea urchins (25, 26), a lectin-like protein associated with sperm binds to sperm receptor oligosaccharides, with terminal galactose serving as at least one of the binding determinants. In this connection, it is interesting to note that oligosaccharides with repeating *N*-acetyllactosamine units (i.e., lactosaminoglycans) are usually associated with developmentally regulated glycoconjugates (27, 28). Apparently, lactosaminoglycans are present on a porcine zona pellucida glycoprotein that exhibits SRA *in vitro* (29). In a number of cases (e.g., neurons and erythrocytes), such sequences are often terminated by a galactose in α-linkage with the galactose of the *N*-acetyllactosamine unit (28, 30, 31). Experiments are necessary to examine the potential role of ZP3 lactosaminoglycans in SRA.

Finally, it is tempting to speculate that inactivation of ZP3 as a sperm receptor, which occurs following fertilization (1, 2, 4), is due to removal or modification of one or more sugars of ZP3 O-linked oligosaccharides involved in sperm binding. Results presented here demonstrate that removal or modification of a single sugar that serves as a sperm binding determinant leads to loss of ZP3 SRA. It is likely that cortical granule enzyme(s), released into the zona pellucida following fertilization, are responsible for inactivation of sperm receptors (1, 2).

We are grateful to Dr. Jacques Baenziger for helpful advice and to members of our laboratory for constructive criticism.

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