Glycodelin from seminal plasma is a differentially glycosylated form of contraceptive glycodelin-A

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Glycodelin-A is a human amniotic fluid-derived glycoprotein with contraceptive and immunosuppressive activities. An immunoreactive form of glycodelin was detected in seminal plasma over a decade ago, but definitive characterization of this glycoprotein was not pursued. We considered it unlikely that the seminal plasma of fertile men would contain an appreciable amount of contraceptive glycodelin-A. To address this issue we purified seminal plasma glycodelin (glycodelin-S) and performed comparative studies with glycodelin-A. Glycodelin-S behaved differently when compared with glycodelin-A during sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing but identically after enzymatic deglycosylation. N-terminal sequencing of glycodelin-A and glycodelin-S gave identical results, and digestion with trypsin gave identical peptide fragments. The glycoproteins were also found to be indistinguishable from each other based upon immunological analyses. These results indicate that glycodelin-S and glycodelin-A have similar overall protein structure, suggesting the likelihood that these glycoproteins are differentially glycosylated forms of very similar proteins. This latter possibility is supported by lectin binding studies indicating that, unlike glycodelin-A, glycodelin-S does not manifest any affinity for lectins from *Wisteria floribunda* or *Sambucus nigra*. The results of sugar analysis and neuraminidase digestion also lead us to conclude that glycodelin-S and glycodelin-A are differentially glycosylated forms of similar proteins. Our evidence indicates that glycodelin-A mediates its biological activities via its unusual oligosaccharide sequences that are not associated with glycodelin-S. In lectin-immunoblot assay no appreciable amount of contraceptive glycodelin-A was found in the 22 seminal plasma samples studied.

Key words: contraception/glycodelin/glycosylation/lectin/PP14

Introduction

Glycodelin-A (GdA) is a human glycoprotein with potent immunosuppressive (Pockley and Bolton, 1990; Okamoto *et al.*, 1991) and contraceptive activities (Oehninger *et al.*, 1995). GdA carries rare carbohydrate sequences (Dell *et al.*, 1995) whereas its protein backbone is homologous to the β-lactoglobulins of various species (Julkunen *et al.*, 1988). The highly unusual N-linked oligosaccharides associated with GdA have been previously implicated in immune and inflammatory responses (Grinnell *et al.*, 1994; Powell *et al.*, 1995) indicating that they may play a role in the known immunosuppressive effects of this glycoprotein. The human glycodelin gene is localized on chromosome 9q34 (Nguyen *et al.*, 1991). It spans over 5042 bp and consists of seven exons and six introns (Vaisse *et al.*, 1990). Although GdA was isolated from human amniotic fluid (Riittinen *et al.*, 1989), immunoreactive glycodelins, previously referred to as placental protein 14 (PP14) or progesterone-associated endometrial protein (Kämäräinen *et al.*, 1991), and/or their mRNAs have been found in the glandular epithelium of secretory/decidualized endometrium, Fallopian tube (Julkunen *et al.*, 1986; 1988; 1990), seminal vesicles (Julkunen *et al.*, 1984) and haematopoietic cells of the bone marrow (Kämäräinen *et al.*, 1994).

The temporal and spatial expression of GdA in reproductive organs of the human female combined with its biological activities *ex vivo* suggest that this glycoprotein probably plays an essential physiological role in the regulation of human fertilization and implantation. The absence of contraceptive GdA from the endometrium and uterine fluid at the time of ovulation and 3 days thereafter (Julkunen *et al.*, 1986; 1990) would permit successful fertilization during this restricted window. However, the subsequent increased secretion of contraceptive GdA into the uterine fluid (which spermatozoa must traverse on their way to the Fallopian tube) may block their fertilizing potential.

This enhanced expression of GdA may also serve another essential function. It has been shown that the number of natural killer cells increases in the endometrium during the latter half of the menstrual cycle and early pregnancy. These cells require no prior exposure to antigen to kill. The ability of GdA to potentently inhibit natural killer cell activity may provide a defence mechanism whereby the antigenically foreign embryo is protected (Okamoto *et al.*, 1991; Clark *et al.*, 1996).
Immunoreactive glycodelin, designated here as glycodelin-S (GdS), has been found in seminal plasma (Julkunen et al., 1984) but no definitive characterization of this protein has been carried out. We considered it highly unlikely that seminal plasma bathing fertile spermatozoa would contain an appreciable amount of contraceptive GdA. Therefore we speculated that the seminal plasma form of glycodelin must differ from GdA either in its protein or possibly in its carbohydrate sequences. To address this issue, we purified GdS and performed comparative studies with GdA, and developed a method to quantify GdA in the presence of GdS.

Materials and methods

The study protocol was approved by the Institutional Review Board of the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Finland.

Materials

Based on informed consent, amniotic fluid samples were obtained from specimens examined for routine prenatal diagnosis of chromosome abnormalities at 15–17 weeks gestation at the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki, Finland. Seminal plasma samples were obtained from healthy male partners of infertile couples. Amniotic and seminal fluid samples were cleared of cells by centrifugation for 10 min at 1000 g. Delfia Europium-labeling reagent (Eu-chelate of isothiocyanato-benzylethylamidetetraacetic acid), enhancement solution (0.1 M acetate phosphate buffer, pH 3.2, containing 0.1 ml/l Triton X-100, 15 μM 2-naphthylthiourea and 50 μM tri-n-octylphosphine oxide) and streptavidin-coated microtitre wells were purchased from Wallac Ltd., Turku, Finland. Freund's complete and incomplete adjuvants were from Difco Labs., Detroit, MI. Sepharose CL-4B, Phenyl Sepharose CL-4B, Hitrap Q columns and the isoelectric focusing (IEF) kit were from Pharmacia LKB Biotechnology AB, Uppsala, Sweden. NHS-LC-Biotin was from Pierce (Rockford, IL, USA), and Immobilon-P Transfer Membranes from Millipore, Bedford, MA, USA. 3,3'-diaminobenzidine tetrahydrochloride and trifluoroacetic acid were from Fluka, Buchs, Switzerland. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and isoelectric focusing gels (pH 3–7) were from NOVEX, San Diego, CA, USA. The protein fingerprinting kit, containing alkaline protease, endoproteinase Lys C and endoproteinase Glu C, was obtained from Promega, Madison, WI, USA. Bovine serum albumin (BSA), neuraminidase and N-tosyl-L-phenylalanine chloromethyl ketone-treated trypsin were from Sigma, St. Louis, MO, USA. Aconitine [high performance liquid chromatography (HPLC) grade] was from Rathburn, Walkerburn, UK. Lectins from Wisteria floribunda (WFA), Sambucus nigra (SNA) and Bandeiraea simplicifolia (BS-II) were obtained from Sigma, St. Louis, MO, USA, and Lotus tetragonolobus (Lotus) and Limulus polyphemus (LPA) were from EY Laboratories, Inc., San Mateo, CA, USA. Peptide N-glycosidase F (PNGase F) was from New England BioLabs, Beverly, MA, USA. All the other reagents were of analysis grade.

Antibodies

Monoclonal antibodies (mAbs) against GdA and GdA-Keyhole limpet haemocyanin complex were generated and selected using procedures as described previously (Koistinen et al., 1994). In all, 67 clones were found after the fusions. Polyclonal antisera was produced by immunizing a rabbit with 100 μg purified GdA emulsified in Freund's complete adjuvant, and booster injections were given in Freund's incomplete adjuvant at biweekly intervals. The first blood specimen was collected 2 weeks after the fourth immunization. Peroxidase-conjugated swine anti-rabbit immunoglobulins and rabbit anti-mouse immunoglobulins were from Dakopatts, Glostrup, Denmark.

Purification of glycodelins

Seminal plasma was diluted 1:4 in Tris-buffered saline (TBS; 50 mM Tris–HCl, pH 7.7, containing 9 g/l NaCl and 0.5 g/l NaH2PO4). Triton X-100 (1% v/v) was added to amniotic fluid or diluted seminal plasma for affinity purification of the glycodelins using a monoclonal anti-GdA antibody (F25-9E6) column as described elsewhere (Riittinen et al., 1991). After elution GdA was dialysed against 100 mM sodium phosphate, pH 7.2. GdS was dialysed against 20 mM sodium phosphate, pH 7.2, and fractionated by Hitrap Q anion exchange column. This column was equilibrated and washed with 20 mM sodium phosphate, pH 7.2, and GdS was eluted from the column with 100 mM sodium phosphate, pH 7.2.

Purified glycodelins were also analysed by gel filtration using a Superdex 200 column (Pharmacia, Uppsala, Sweden). Glycodelin-containing fractions were identified by immunofluorometric assay for glycodelin (see above). Molecular weights were estimated using BSA, ovalbumin and soybean trypsin inhibitor as standards.

Peptide mapping by fingerprinting

Partial proteolytic digestion of the purified proteins, followed by SDS–PAGE (Cleveland et al., 1977) was performed using a protein fingerprinting kit according to the manufacturer's instructions. The enzymes were alkaline protease, endoproteinase Lys C and endoproteinase Glu C. The fingerprints were estimated by SDS–PAGE using a NOVEX X Cell II Mini-Cell apparatus. Soluble GdA and GdS were mixed with the proteases and loaded immediately into wells in 4–20% acrylamide gels. After electrophoresis for 35 min at a constant current of 10 mA, the current was interrupted for 20 min to allow digestion to take place. Electrophoresis was then completed at 18 mA. For fingerprinting of the gel-separated proteins GdS was first fractionated by SDS–PAGE. The bands were cut out and the gel slices were put into wells of another gel. Thereafter the proteases were loaded on top of the gel slices. The electrophoresis conditions of the proteolyzed fragments were the same as for soluble glycodelins.

Tryptic peptide mapping by HPLC

Deglycosylated GdS (20 μg) and GdA (20 μg) (see below) in 40 μl 0.1 M NaHCO3 were digested with trypsin (2.5 μg). After 20 h incubation at 37°C the resulting peptides were analysed by reversed phase-HPLC using Vydac C18 column. The column was equilibrated with 0.1% trifluoroacetic acid and eluted with a linear gradient of acetonitrile (0–60% in 60 min) containing 0.1% trifluoroacetic acid. Tryptic peptides were detected by absorbance at 218 nm and the chromatogram was recorded with a Shimadzu C-R3A integrator.

Measurement of total glycodelin concentrations

This was done by a sandwich-type immunofluorometric assay, essentially as described previously (Kämäräinen et al., 1994), but with two modifications to enhance sensitivity. First, to reduce non-specific binding labelled mAb (F25–9D8) was further purified by Phenyl Sepharose CL-4B chromatography. Second, solid phase mAb (F23–9G2) was biotinylated by incubating for 1 h at room temperature in 6-fold molar excess of NHS-LC-Biotin. Unreacted biotin was removed by Centricron Microconcentrator (Amicon, Beverly, MA, USA). Biotinylated mAb (0.5 μg/200 μl TBS) was allowed to adhere to the avidin-coated plates for 60 min at room temperature after which the plates were washed. The intra-assay variation was 3.4% at the level of 21.3 ng/ml, and interassay variation was 7.7% at the level of
Table 1. Selected lectins and their specificities and reactions with glycodelin-A (GdA) and seminal plasma glycodelin (GdS)

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Specificity</th>
<th>GdA</th>
<th>GdS</th>
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<tr>
<td>BS-II</td>
<td>GlcNAc</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LPA</td>
<td>NeuNAc (GaiNAc, GlcNAc)</td>
<td>-</td>
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<tr>
<td>Lotus</td>
<td>L-Fuc</td>
<td>+</td>
<td>+</td>
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<tr>
<td>SNA</td>
<td>NeuNAc2-6GalNAc</td>
<td>++</td>
<td>-</td>
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<tr>
<td>WFA</td>
<td>GalNAc</td>
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Lectins were from Bandeiraea simplicifolia (BS-II), Lilium polyphemus (LPA), Lotus serrganotholus (Lotus), Sambucus nigra (SNA) and Wisteria floribunda (WFA).

++ = strong reaction; + = weak reaction; - = no reaction.

22.7 ng/ml. The concentration of purified glycodelin was also measured by absorbance at 280 nm (Riittinen et al., 1989). The two methods gave similar results.

Protein labelling
MAbs and lectins were labelled with the Delfia Europium-labelling reagent by incubating the protein in 50-fold molar excess of Eu-chelate in 0.1 M NaHCO3 buffer, pH 9.3. After overnight incubation at room temperature the labelled protein was purified on a 1 x 50 cm Sephacryl S-200 column by elution with TBS.

Immunochemical identity using monoclonal antibodies
Glycodelins (150 μg each of purified GdA and GdS) were biotinylated as above using 10-fold molar excess of NHS-LC-Biotin. The biotinylated glycodelins were diluted in 20 ml assay buffer (TBS containing 5 g/l BSA, 0.5 g/l bovine γ-globulin, 2 g/l diethylenetriamine-penta-acetic acid, and 0.1 g/l of Tween 20), and 200 μl diluted biotinylated glycodelins were allowed to attach to streptavidin-coated microtitre wells during 45 min incubation at room temperature. The wells were washed with TBS containing 0.5 g/l Tween 20. 25 μl of mAb-containing supernatants and 200 μl assay buffer were added to each well. After an overnight incubation at 4°C, the wells were emptied and washed twice in Tween 20-TBS. 10 ng Eu-labelled rabbit anti-mouse antibody was added in 200 μl assay buffer and incubated for 2 h at room temperature. The wells were washed four times and 200 μl enhancement solution was added. After 5 min of gentle shaking fluorescence was measured using a Model 1234 Delfia research fluorometer (LKB Wallac). This procedure was carried out for 67 different monoclonal antibodies.

Isoelectric focusing
Separation was performed using NOVEX IEF gels (pH 3–7, 5% polyacrylamide) according to the manufacturer’s instructions. The isoelectric points (pI) were estimated using pI markers from the IEF calibration kit.

Lectin-immunoassays to detect contraceptive GdA in the presence of GdS
Lectin-coated microtitre plates and Eu-labelled mAbs (F23–9G2 and F25–9D8) were used for the lectin assays. The microtitre plates were coated overnight at room temperature with 10 μg/ml lectin after which the wells were incubated with 10 g/l BSA for 3 h at room temperature. The lectins and their primary specificities are listed in Table 1. In the assay, 25 μl sample (dilutions of GdS and GdA) and 200 μl assay buffer supplemented with 1 mM CaCl2 2H2O, MnCl2 4H2O and MgCl2 6H2O were incubated overnight at 4°C in lectin-coated wells. After washing the wells twice with Tween 20-TBS, 50 ng Eu-labelled mAb was added in 200 μl assay buffer (with Ca/Mg/Mn) and incubated for 2 h at room temperature. The wells were washed four times and 200 μl enhancement solution was added. The fluorescence was measured after gentle shaking for 5 min. The SNA assay was performed using immobilized mAb (F23–9G2) and Eu-labelled SNA.

Immunoblotting
Immunoblotting after SDS–PAGE (under reducing and non-reducing conditions) was performed according to Towbin et al. (1979). After transferring the proteins to Immobilon-P Transfer Membrane the membrane was incubated with 10 g/l BSA in TBS overnight at 4°C and then with 1:200 diluted polyclonal anti-glycodelin antiserum for 2 h at 37°C. After washing the membrane was treated with 1:200 diluted peroxidase-conjugated anti-rabbit antibody using 3,3'-diaminobenzidine tetrahydrochloride, 0.3 g/l, as a substrate for the staining reaction.

N-terminal amino acid sequence analysis
After SDS–PAGE the sample was blotted onto a polyvinylidene difluoride membrane and the proteins were stained with Coomassie Brilliant Blue and sequenced using a Procise sequenator (Applied Biosystems, Foster City, CA, USA).

PNGase F and neuraminidase treatments
N-linked sugars of GdS and GdA were removed using PNGase F. GdS (20 μg) and GdA (20 μg) were denatured for 10 min at 100°C in 40 μl 0.5% SDS and 1% β-mercaptoethanol. Then 5 μl 0.5 M sodium phosphate, pH 7.5, 5 μl 10% NP-40, and 15 IUB mlU PNGase F enzyme were added and incubated for 1 h at 37°C. For trypsin digestion GdA and GdS (20 μg each) were treated with PNGase F (15 IUB mlU) without denaturation for 20 h at 37°C and the sugars were removed by an Ultrafree-MC filter unit (utoff 10 000, Millipore, Bedford, MA, USA). For desialylation experiments GdA (17 μg) and GdS (17 μg) were treated with neuraminidase (2.4 μlU) in 11 μl 0.1 M Tris, pH 7, for 1.5 h at room temperature.

Sugar analysis
This was performed on trimethylsilyl methyl glycosides as described previously (Khoo et al., 1995).

Results
Physicochemical comparison of the glycodelins
GdS was initially purified by the same antibody affinity chromatography procedure as that used for the purification of GdA (Riittinen et al., 1991). After anion exchange chromatography SDS–PAGE of GdS indicated a major band at 27 kDa and a minor band at 30 kDa (Figure 1A). These bands were reactive with anti-GdA antibodies (Figure 1B). Unlike GdS, GdA migrated as a single band at 28 kDa (Figure 1). The results were the same under reducing and non-reducing conditions. In gel filtration, GdA and GdS eluted at 55 kDa and 47 kDa respectively, suggesting that in non-denaturing conditions GdA and GdS were dimeric proteins.

During isoelectric focusing, GdS separated into several bands with pIs of 4.9–5.6, with a single major band at pI 5.3 (Figure 2). GdA was more acidic, yielding two major bands with pIs of 4.7 and 4.9 and other minor bands with pIs between 4.5–5.2 (Figure 2). All the bands separated by this technique were immunoreactive as determined by immunoblot analysis.

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Figure 1. Molecular weight estimation and immunoblot analysis of human glycodelins. Glycodelin-A (GdA) from human amniotic fluid and glycodelin-S (GdS) from human seminal plasma were purified as described in Materials and methods. (A) Affinity-purified GdA (3.0 µg, lane 1), affinity-purified GdS (2.7 µg, lane 2), affinity-purified GdS after anion exchange chromatography (2.9 µg, lane 3), purified GdA (3.4 µg, lane 4) and GdS (3.4 µg, lane 5) after PNGase F treatment, and PNGase F (6.2 IUB mlU, lane 6) were analysed by SDS-gel electrophoresis on 4–20% polyacrylamide gels under reducing conditions and stained with Coomassie Brilliant Blue. (B) Amniotic fluid (diluted 10-fold, lane 1), seminal plasma (diluted 10-fold, lane 2), purified GdA (18.8 ng, lane 3), GdS before (22.5 ng, lane 4) and after anion exchange chromatography (18.1 ng, lane 5), and purified GdA (21 ng, lane 6) and GdS (21 ng, lane 7) after PNGase F treatment were analysed by SDS-polyacrylamide gel (4–20%) under reducing (except lanes 1 and 2 non-reducing) conditions and transferred to polyvinylidene difluoride membrane. The membrane was incubated with rabbit anti-human glycodelin antiserum, washed and probed with peroxidase-conjugated anti-rabbit antibody using 3,3’-diaminobenzidine tetrahydrochloride as substrate.

Figure 2. Isoelectric points of glycodelins. Isoelectric focusing (IEF) of desialylated GdA (7.7 µg, lane 1) and GdS (7.7 µg, lane 2), and native GdA (5.0 µg, lane 3) and GdS (5.0 µg, lane 4) was done using NOVEX IEF gels (pH 3–7, 5% polyacrylamide) according to the manufacturer’s instructions. The isoelectric points (pI) were estimated using pI markers from the IEF calibration kit.

Figure 3. Protein fingerprinting of glycodelins. Partial proteolytic digestion of soluble glycodelins. Lanes 1 and 2, undigested GdA and GdS (4 µg) respectively; lanes 3 and 4, digested by alkaline protease (15 ng); lanes 5 and 6, digested by endoproteinase Lys C (400 ng); lanes 7 and 8 digested by endoproteinase Glu C (300 ng).

Figure 4. Comparative tryptic peptide mapping of glycodelins by reversed phase-HPLC. Glycodelins were digested by trypsin as described in Materials and methods. Peptides of GdA (upper) and GdS (lower) showed identical chromatographic pattern as detected from a Vydac C18 column by absorbance at 218 nm (—) using a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (—).

Thus, there were major differences in the net charge between GdS and GdA.

Partial proteolytic digestion with alkaline protease, endoproteinase Lys C and endoproteinase Glu C of the glycodelins was carried out in SDS-PAGE to determine differences in the proteins. The majority of the bands obtained after digestion were similar between GdS and GdA (Figure 3). However, the 30 kDa form of GdS was unaffected by this treatment. When the 27 kDa and 30 kDa bands were preparatively isolated from the gel and digested separately, the 30 kDa band remained resistant to proteolysis.

Although the differences between GdS and GdA were significant, other results indicated similarity in their protein sequences. Comparative tryptic peptide mapping by reversed phase-HPLC analysis showed the same retention times for both glycodelins (Figure 4), indicating that the protein backbones of GdA and GdS are similar. The results of N-terminal sequencing of GdS indicated complete homology between GdS and GdA in their first 20 amino acids.

**Immunological identity**

A number of polyclonal and monoclonal antibodies were generated against GdA. Using polyclonal antibodies reactions of
immunological identity between GdS and GdA were obtained in experiments using immunodiffusion and tandem-crossed immunoelectrophoresis (Figure 5). Also the dose-response curves of GdS and GdA were identical in immunofluorometric assay using monoclonal antibodies (Figure 6). The reactivity of immobilized GdS and GdA was tested in a sandwich-type assay with monoclonal anti-GdA antibodies. Complete immunological identity between GdS and GdA was again indicated by similar reactivity of the monoclonal antibodies in this assay. Therefore, immunological analyses did not indicate any differences between GdS and GdA despite their observed physicochemical differences.

**PNGase F and neuraminidase digestion of GdS and GdA**

Results of the immunological analyses, tryptic peptides and the N-terminal sequencing of GdS suggested that the physicochemical differences between GdA and GdS could result from their different post-translational modifications. We have previously demonstrated that GdA is glycosylated at Asn-28 and Asn-63 (Dell et al., 1995). GdS and GdA were therefore treated with PNGase F under reducing conditions to remove the N-linked glycans from GdS and GdA. An identical single 20 kDa band was observed following SDS-PAGE of deglycosylated GdS and GdA (Figure 1).

Approximately 60% of GdA-derived glycans are sialylated (Dell et al., 1995). To determine whether the differences in behaviour during isoelectric focusing were due to sialylation, both GdS and GdA were digested with neuraminidase. Following this treatment a single major band at pI 5.3 was obtained for both GdA and GdS (Figure 2), suggesting that the differences in GdS and GdA observed in SDS-PAGE and isoelectric focusing are due to differential glycosylation (see below).

**Sugar analysis and development of a lectin-immunoassay for detection of GdA in seminal plasma**

A distinguishing feature of GdA-derived oligosaccharides is the presence of antennae with terminal GalNAcβ1-4GlcNAc type sequences in their intact or fucosylated forms (Dell et al., 1995). Oligosaccharides of this type have been shown to react with the lectin from *Wisteria floribunda* (WFA). The lectin from *Sambucus nigra* (SNA) should also react with GdA because of its terminal NeuAcα2-6Gal(NAc) sequences. These potential GdA-binding lectins and other lectins (Lotus, LPA and BS-II) were immobilized into polystyrene wells and their interactions with glycodelins were measured in a solid-phase sandwich assay system. GdS and GdA did not react with either BS-II or LPA. However, GdA reacted with both SNA and WFA, whereas GdS, even at a concentration of 10 μg/ml, did not react with either of these GdA-reactive lectins (Table I, Figure 7). Both glycodelins show some binding to Lotus, GdS being more reactive than GdA. These results indicate that there are substantial differences in glycosylation between GdS and GdA. In addition, sugar analysis of trimethylsilyl methyl glycosides indicated that GdS has only Fuc, Man, Gal and GlcNAc and, unlike GdA, it contains no GalNAc or sialic acid.

The presence of GdA-like reactivity was investigated in the individual seminal plasma of 22 healthy male partners of infertile couples. The assay used immobilized GdA-binding lectin to enrich the possibly existing small amounts of reactive GdA from the vast amount of GdS. As the lectin-immunoassay using immobilized WFA was found to be more sensitive than SNA-immunoassay, a combination of immobilized WFA and Eu-labelled mAb (F25-9D8) was selected for the development of a GdA-detecting assay. This assay has a detection limit of 0.1 μg GdA/ml. No WFA-reactive glycodelin was found in any of the native seminal plasma samples studied. However, we could not rule out that competing glycoproteins in the seminal plasma could have blocked glycodelin binding to the immobilized WFA. Therefore, affinity purified and concentrated total glycodelin (10 μg/ml or more) from individual seminal plasmas were analysed separately for GdA; <2% of glycodelin in seminal plasma was found to be WFA-reactive (2%, n = 1; 1%, n = 6; <1%, n = 15). This result indicates that normal seminal plasma contains little if any GdA-like reactivity.
Recent studies indicate that glycodelin expression is not limited to female reproductive tissues (Julkunen et al., 1984; Kämäräinen et al., 1994; Morrow et al., 1994). In addition, it seems that the glycodelins are differentially processed in a tissue-specific manner, suggesting potential divergent physiological roles for these glycoproteins.

Several lines of evidence indicate that the protein component of GdS and GdA is similar. The two glycoproteins have identical amino terminal sequences. Proteolytic digestion of GdS and GdA produces identical patterns of peptide fragments. Deglycosylation of GdS and GdA with PNGase F yields a single band with the predicted size of the GdA sequence deduced from the cDNA data (Julkunen et al., 1988). However, the two proteins show heterogeneity on SDS–PAGE and isoelectric focusing. Comigration of the PNGase F-treated forms of GdS and GdA is a strong indication that differential glycosylation is responsible for the differences observed on SDS–PAGE. Isoelectric focusing of neuraminidase-treated GdS and GdA indicates that the differences in pI of the two glycodelins are dependent on sialylation. It is significant that the isoelectric points of GdS and desialylated GdA are the same as that predicted from the protein sequence of GdA using the Wisconsin Package (Version 8.0-UNIX; Genetics Computer Group, Madison, WI, USA).

Our results indicate that the differences in GdS and GdA are due to changes in glycosylation. We used lectin immunoassay systems to determine specific changes in oligosaccharide expression between GdS and GdA and found that WFA and SNA demonstrate selective binding to GdA but not to GdS. Lotus lectin bound to both GdS and GdA, suggesting that both glycoproteins have terminal α-linked fucose on their oligosaccharides.

There are several significant implications associated with these findings. WFA has previously been shown to react with either laciNAc or fucosylated laciNAc-type sequences including those associated with GdA (Srivatsan et al., 1992). N-linked oligosaccharides with terminal fucosylated laciNAc type sequences have been shown to potently inhibit selectin-mediated adhesions (Grinnell et al., 1994). SNA binds to N-linked oligosaccharides terminated with NeuAca2–6Gal(NAc) sequences. Oligosaccharides with such terminal sequences also bind to CD22 (Powell et al., 1995), the human B cell receptor glycoprotein.

Sugar analysis was carried out to determine differences in composition between GdS and GdA. Unlike GdA glycans, GdS oligosaccharides contain no GalNAc or sialic acid. Since WFA and SNA require terminal GalNAc and sialic acid for binding, respectively, the sugar analysis supports the results of our lectin binding studies. Therefore the absence of WFA and SNA binding coupled to the results of sugar analysis and neuraminidase treatment confirms that GdS lacks the unusual laciNAc sequences and terminal sialylation associated with GdA.

The precise physiological role of GdS in the seminal plasma is unknown at this time. Some of the inhibitory effect of seminal plasma on human lymphocyte proliferation could be inactivated by incubation with a monoclonal anti-GdA antibody-based immunoabsorbent (Bolton et al., 1987). However, more investigation needs to be carried out to define the precise role of GdS as a potential immunosuppressive agent in human seminal plasma.

GdA has been shown to suppress human sperm binding to the zona pellucida, the specialized extracellular membrane surrounding the oocyte (Oehninger et al., 1995). Because carbohydrates play a crucial role in sperm–oocyte recognition (Wassarman 1990; Oehninger et al., 1991), it is likely that the glycans associated with GdA are also required for its contraceptive action. In our preliminary studies, we have found that GdS lacks contraceptive activity in the hemizona assay system (Morris et al., 1996). This result adds more supportive evidence that the oligosaccharides play a key role in the expression of the biological activities of GdA.

Structural dissimilarity between the two isoforms described in this study could be of potential interest in the clinical setting. There is no GdA in the endometrium at the time of conception and this contraceptive glycoprotein appears in endometrium 4–5 days after ovulation (Julkunen et al., 1986).
Before these studies were initiated, we considered it unlikely that a contraceptive substance such as GdA would dominate in human seminal plasma. Our hypothesis turned out to be correct. However, it will be of great interest to determine if contraceptive glycoforms of glycodelin are found in the seminal plasma of infertile men.

Acknowledgements

This work was supported by grants from the Finnish Cancer Foundation, the Academy of Finland Finnish Federation of Life and Pension Insurance Company and the University of Helsinki (to M.S., R.K. and H.K.), the Medical Research Council and Wellcome Trust (to H.R.M. and A.D.), and the Jeffress Memorial Trust and the American Cancer Society (G.F.C.). We thank Ms Anu Harju for technical assistance.

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Received on June 5, 1996; accepted on August 15, 1996