Evidence for the presence of a large keratan sulphate proteoglycan in the human uterine cervix

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Profound changes occur in the uterine cervix during pregnancy. In particular, the extracellular matrix of the connective tissue is remodelled extensively. To elucidate the mechanisms involved in this process, we have analysed the proteoglycan pattern in the human cervix from pregnant and non-pregnant women. Proteoglycans of the cervix tissue specimen were extracted with 4 M guanidine hydrochloride and precipitated with 80 % ethanol. Purification of proteoglycans was performed by several chromatographic steps. Characterization of proteoglycans was done by SDS/PAGE before and after digestion with glycosaminoglycan-specific enzymes. Proteoglycans were detected by combined Alcian Blue/silver staining or, after blotting of biotin-labelled proteoglycans on to poly(vinylidene

difluoride) membrane, with peroxidase-conjugated avidin or by the use of keratan sulphate- or decorin-specific monoclonal antibodies. In contrast with previous reports, where only chondroitin/dermatan sulphate proteoglycans have been found in the uterine cervix, we have shown in the present study the existence of a large keratan sulphate proteoglycan with an $M_r > 220\,000$ in cervix samples from non-pregnant and pregnant women. This proteoglycan showed a strong reaction with the keratan sulphate-specific monoclonal antibody 5D4 and could be degraded by keratanases. The size of the core protein of this keratan sulphate proteoglycan was estimated to be about $M_r 220\,000$.

INTRODUCTION

Proteoglycans (PGs) are major components of the extracellular matrix that are responsible not only for the mechanical properties of the tissues but are also involved in cell–cell and cell–matrix interactions [1,2]. In general, PGs consist of a central core protein, substituted with at least one glycosaminoglycan (GAG) chain. Chondroitin sulphate (CS)/dermatan sulphate (DS), heparan sulphate (HS) and keratan sulphate (KS) are distinguished by the disaccharide units that build up the GAG chains [3,4]. During pregnancy major changes occur in the extracellular matrix of the uterine cervix [5–8], according to the altered specific functional requirements of the organ. It has been shown that cervical maturation during delivery is not only due to mechanical forces accomplished by uterine contractions, but rather an active process leading to major structural and biochemical alterations within the cervical extracellular matrix [5–9]. At present, these processes are not fully understood, but it is clear that collagens as well as PGs are involved. Previously, we have analysed changes of the GAG pattern during pregnancy and delivery [10–12]. The content of collagens and PGs in the uterine cervix during pregnancy decreased to about 70 % and 50 % respectively [6,7]. This decrease is paralleled by changes in the organization of the extracellular matrix affecting, for example, the degree of cross-linking between collagens [5]. So far, the existence of two types of small dermatan sulphate PGs [5,8,13,14] as well as a large CS/DS in the uterine cervix from non-pregnant and pregnant women [5] have been reported. Small amounts of HS, probably localized in the basement membrane of blood vessels, have been also described [15].

Whereas these studies have analysed either changes of [^{35}S]sulphate incorporation *in vitro* in response to a given stimulus or quantitative changes in the expression of one proteoglycan, mostly decorin, during pregnancy and delivery, there is no detailed analysis of the PGs in the uterine cervix during pregnancy. Moreover, analysis of changes in the PG composition during the time course of pregnancy is still lacking. Therefore, in the present study we have analysed in detail the PGs from human uterine cervix and have compared them in samples obtained from non-pregnant and pregnant women. An important novel finding of this study is the isolation and characterization of a large KS PG in the uterine cervix.

MATERIALS AND METHODS

Reagents

A Sepharose Q HP column (16 mm $\times$ 100 mm), a Sephacryl S400 HR column and preformed gels for SDS/PAGE (8–25 % and 4–15 % polyacrylamide gradients) with preformed buffer strips were obtained from Pharmacia/LKB (Freiburg, Germany). The BioSil Amino 5S column (250 mm $\times$ 4 mm) with guard column was from Bio-Rad Laboratories (Munich, Germany). Poly(vinylidene difluoride) (PVDF) blotting membrane (Immobilon N) was purchased from Millipore (Eschborn, Germany); nitrocellulose membrane was from Schleicher & Schuell (Dassel, Germany). High-molecular-mass standards, peroxidase-conjugated avidin, CHAPS, benzamidine hydrochloride, 6-aminohexanoic acid, PMSF and *N*-ethylmaleimide were purchased from Sigma (Deisenhofen, Germany). Diaminobenzamidine tetrahydrochloride (DAB), 1,9-dimethylmethylene

Abbreviations used: PG, proteoglycan; GAG, glycosaminoglycan; CS, chondroitin sulphate; DS, dermatan sulphate; HS, heparan sulphate; KS, keratan sulphate; PVDF, poly(vinylidene difluoride); DAB, diaminobenzamidine tetrahydrochloride; DMB, 1,9-dimethylmethylene blue chloride; mAb, monoclonal antibody; RT, room temperature.

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Figure 1 Isolation and purification of the PGs from human uterine cervix

Blue chloride (DMB) and dialysis tubes (Spectrapor 3) were from Serva (Heidelberg, Germany). Peroxidase-conjugated goat anti-mouse IgG antibody was from Dianova (Hamburg, Germany). CS lyase AC (EC 4.2.2.5), CS lyase ABC (EC 4.2.2.4), KS hydrolyase (EC 3.2.1.103), KS endo- β -*N*-acetylglucosaminidase (keratanase II), the KS-specific monoclonal antibody (mAb) 5D4, the decorin core-protein-specific mAb 6B6 and the Δ -chondro-disaccharide kit standard (C-KIT) were obtained from Seikagaku (Tokio, Japan). The decorin core-protein-specific mAbs LN 1 and LN 3 were kindly provided by Dr. H. Kresse (University of Münster, Germany); the KS-specific mAbs 4B3/D10 and 3D12/H7 were established and characterized by our own group [16,17]. An affinity-purified antiserum against the lumican core protein was kindly provided by Dr. J.L. Funderburgh [18] (Division of Biology, Kansas State University, Manhattan, KS, U.S.A.). The digoxigenin-labelled lectin concanavalin A was from Boehringer Mannheim (Germany). Sulphosuccinimidyl 6-(biotinamido)hexanoate (NHS-LC-biotin) was from Pierce (Munich, Germany). For ultrafiltration, YM 10 membranes from Amicon (Witten, Germany) were used. All remaining reagents were of at least analytical grade.

Isolation and purification of PGs from human uterine cervix

Samples from the uterine cervix of a non-pregnant woman (wet weight 6.1 g) and a pregnant woman (wet weight 9.5 g) were obtained at hysterectomy for medical reasons. Isolation and purification of PGs from human uterine cervix was carried out as described previously [19–22] and is schematically shown in Figure 1.

Briefly, the frozen tissues were cut into small pieces (diameter of approx. 0.4 cm) and mechanically homogenized on ice by the

use of an Ultra Turrax (Janke & Kunkel, Staufen, Germany) for 5 min at 20000 rev./min in the presence of 4 mol/l guanidine hydrochloride and protease inhibitors (see below). PGs were extracted (2 $\times$ 24 h, 4 °C) with 4 mol/l guanidine hydrochloride/0.05 mol/l sodium acetate (pH 5.8) containing 0.5 % CHAPS and the protease inhibitors benzamidine hydrochloride (0.005 mol/l), 6-aminohexanoic acid (0.1 mol/l), PMSF (0.005 mol/l), *N*-ethylmaleimide (0.01 mol/l) and EDTA (0.05 mol/l). The 4 mol/l guanidine hydrochloride extracts were precipitated with cold ethanol [final concentration 80 % (v/v)] at 4 °C overnight. PGs were recovered after centrifugation (53 900 g, 4 °C, 2 h) and redissolved in 0.05 mol/l Tris/0.05 mol/l sodium acetate buffer (pH 5.8) containing 7 mol/l urea, 0.05 mol/l NaCl, 0.05 mol/l LiCl, 0.2 % (w/v) CHAPS and protease inhibitors (0.001 mol/l *N*-ethylmaleimide, 0.002 mol/l benzamidine hydrochloride and 0.001 mol/l PMSF).

Purification of PGs

PGs were isolated by anion-exchange chromatography on Sepharose Q HP (16 mm $\times$ 100 mm). The column was equilibrated with 0.05 mol/l Tris/0.05 mol/l sodium acetate buffer (pH 5.8) containing 7 mol/l urea, 0.05 mol/l NaCl, 0.05 mol/l LiCl, 0.2 % (w/v) CHAPS and protease inhibitors (0.001 mol/l *N*-ethylmaleimide, 0.002 mol/l benzamidine hydrochloride and 0.001 mol/l PMSF). For elution of PGs, NaCl gradients (0.05–3.6 mol/l; 1 ml/min) were applied; the fraction size was 3 ml. Elution profiles were monitored at 280 nm; in addition, aliquots of each fraction were tested with DMB [23], and analysed by a semi-quantitative slot-blot enzyme-immunoassay for the presence of KS PG and decorin. Fractions were pooled as indicated by the bars (see Figure 2), concentrated by ultrafiltration (YM 10 membranes; final volume about 3 to 4 ml) and submitted to gel-permeation chromatography on Sephacryl S-400 HR. A sample of 3 ml was applied to the column (16 mm $\times$ 600 mm), which had been equilibrated with 0.05 mol/l NaH₂PO₄ (pH 5.8) containing 0.3 mol/l NaCl and 0.05 % (w/v) CHAPS. Elution was performed with a flow rate of 0.5 ml/min; fractions of 1 ml were collected. The elution profiles were monitored at 210 nm and 280 nm; in addition, aliquots of each fraction were tested with DMB. PG-containing fractions were pooled according to the DMB and UV signals (see bars in Figure 3) and were further analysed for the presence of KS PG and decorin, using an semi-quantitative slot-blot enzyme-immunoassay.

For amino acid and amino sugar analysis, samples were treated with 3 mol/l HCl at 105 °C for 15 h. Analysis was performed on a LKB-Alphaplus amino acid analyser (Pharmacia/LKB).

Derivatization of the PGs with biotin was carried out as described previously [24]. Biotin-labelled PGs and core proteins were detected after SDS/PAGE and diffusion blot on to PVDF membranes (Immobilon N; Millipore) by incubation with peroxidase-conjugated avidin [24]. Staining with DAB was performed as described below.

Enzymic deglycosylation

Enzymic digestion with CS lyase AC (EC 4.2.2.5), CS lyase ABC (EC 4.2.2.4), KS hydrolyase (EC 3.2.1.103) and keratanase II was performed in the presence of proteinase inhibitors essentially as described previously [25–28].

Analysis of the disaccharide components was carried out on an HPLC compact system from Waters (Eschborn, Germany) equipped with a BioSil Amino-5S column (4 mm $\times$ 250 mm) with guard column from Bio-Rad. The column was equilibrated with

0.1 mol/l NaH_2PO_4 (pH 3.9); elution was performed with a gradient from 0.1 mol/l to 0.7 mol/l NaH_2PO_4 (pH 3.9) at a flow rate of 0.5 ml/min. The elution profile was monitored at 232 nm.

Immunochemical characterization of the PGs

For immunoblots, native PGs as well as PGs treated with CS lyases AC/ABC, with KS hydrolyases or with a combination of these enzymes were submitted to SDS/PAGE on preformed 4–15% or 8–25% gradient gels (Pharmacia Phast system; Pharmacia/LKB). Subsequently, the gels were either stained with Alcian Blue/silver [29] or submitted to diffusion blot on to PVDF membranes (Immobilon N; Millipore) and/or nitrocellulose membranes (Schleicher & Schuell) [24]. Non-specific binding sites of the PVDF membrane were blocked with 2% (w/v) blotto in Tris-buffered saline (0.02 M Tris/HCl and 0.3 M NaCl, pH 7.5) for 1 h at room temperature (RT) before incubation with the mAb (diluted appropriately in blocking solution; 4 h at RT) was performed. Bound antibodies were detected by a peroxidase-conjugated goat anti-mouse antibody and detected by the use of 1.26 mmol/l DAB and 0.005% (v/v) H_2O_2 in 0.05 mol/l Tris/HCl, pH 7.4. For detection of lumican after electrophoretic separation, samples were transferred on to nitrocellulose membranes (see above). Non-specific binding sites were blocked with 5% (w/v) BSA in PBS (1 h, RT), and incubation with the antiserum against lumican core protein, appropriately diluted in blocking solution, was performed overnight. Bound antibody was detected by a peroxidase-conjugated swine anti-rabbit antibody as described above.

RESULTS

In the present study we have analysed in detail the PG composition of the human uterine cervix. For comparison of PGs present in the uterine cervix, two samples from a non-pregnant and a pregnant woman, taken at hysterectomy for medical reasons, were analysed.

About 6.1 g (wet weight) of tissue of uterine cervix from the non-pregnant woman and 9.5 g of tissue of uterine cervix from the pregnant woman (first trimester) were obtained and further processed as schematically shown in Figure 1.

The elution profiles of the initial anion-exchange chromatographies are given in Figure 2 (sample from the non-pregnant woman shown in Figure 2A; sample from the pregnant woman shown in Figure 2B). Since a partial separation of PGs was seen in the first sample analysed (Figure 2A), the slope of the gradient was modified for the second sample in order to achieve a better separation (Figure 2B). Immunochemical analysis of the fractions by a semi-quantitative slot-blot assay revealed the presence of decorin- and KS-containing PGs between 0.4 M NaCl and 1.2 M NaCl (Figure 2A) and between 0.6 M NaCl and 1.1 M NaCl (Figure 2B) respectively. Slot-blot analysis indicated that the relative content of KS PG was higher in the later eluting fractions. Fractions were pooled as indicated by the bars and were further analysed by SDS/PAGE (4–15% polyacrylamide gels) and combined Alcian Blue/silver staining (results not shown). Since all samples contained PGs of different sizes, further purification using gel-permeation chromatography was performed. The elution profiles of KS PG-enriched pools (pool 2 in Figure 2A and pool 2 in Figure 2B) are given in Figure 3. Elution patterns of both samples are similar (Figure 3) with respect to the first two peaks; however, in the sample from the non-pregnant woman (Figure 3A), a third peak near the total volume appears. Furthermore, the amounts and distribution of larger and smaller PGs are different in the pools obtained by anion-exchange chromatography. Aliquots of the fractions obtained after gel

Figure 2 Initial anion-exchange chromatography of extracts of the uterine cervix from a non-pregnant woman (A) and a pregnant woman (B)

Samples were applied to the column (Sephacrose Q HP; 16 mm $\times$ 100 mm), and the column was equilibrated with buffered 7 M urea. PGs were eluted with a linear NaCl gradient (---) (A: 0.05–1.5 M NaCl; B: 0.3–1.5 M NaCl) in the same buffer. Elution profiles were monitored at 280 nm (—). Fractions were pooled as indicated by the bars 1 and 2 and were submitted to further analysis.

chromatography were analysed with DMB. PGs were seen to elute in the void volume and with either $0.22 \leq K_{av} \leq 0.57$ (Figure 3A) or with $0.22 \leq K_{av} \leq 0.6$ (Figure 3B). The relative amount of large PGs eluting in the void volume was increased in the sample obtained from a pregnant woman (Figure 3B). In the sample from the non-pregnant woman (Figure 3A) the third peak, eluting at $0.6 \leq K_{av} \leq 1$, contained mainly smaller proteins, fragments from degraded PGs and low-molecular-mass contaminations, as judged by SDS/PAGE and combined Alcian Blue/silver staining (results not shown). Fractions were pooled as indicated by the bars (Figures 3A and 3B) and were dialysed.

Immunochemical analysis using a semi-quantitative slot-blot assay showed KS-containing PGs in pool 1 and decorin in pool 2 of both samples from the gel-permeation chromatography shown in Figures 3A and 3B. In addition, small amounts of decorin were eluted in pool 1 obtained from the uterine cervix of a pregnant woman (Figure 3B). The large KS PG-containing pools (pool 1, Figures 3A and 3B) were taken for further characterization. For a rough estimation of the proportion of GlcN- and GalN-containing PGs, amino acid and hexosamine analyses were performed. In the sample prepared from the uterine cervix of a non-pregnant woman, the ratio of GlcN:GalN was about 2:1, whereas in the sample from the uterine cervix of the pregnant woman, the ratio of GlcN:GalN was about 1:2. The molar ratios of protein to hexosamine were in the region of 7:1 for both samples. The molecular-mass distribution of the large

Figure 3 Gel-permeation chromatography of PGs isolated from the uterine cervix of a non-pregnant woman (A) and a pregnant woman (B)

PG-containing pools (pools 2, Figures 2A and 2B), obtained after anion-exchange chromatography, were concentrated and submitted to gel-permeation chromatography on Sephacryl S-400 HR (16 mm $\times$ 600 mm). The column was equilibrated and eluted with 0.05 M NaH_2PO_4 buffer (pH 5.8) containing 0.3 M NaCl and 0.05% (w/v) CHAPS. Elution profiles were monitored at 210 nm; fractions eluting in the void volume (V_0) were pooled as indicated by the bars 1, 2 and 3 and taken for further characterization. The total volume (V_t) is also indicated.

PGs was influenced by degradation with CS lyase AC/ABC and keratanase/keratanase II (see below). In contrast, treatment with heparinase/heparitinase had no effect regardless of whether the large PGs were isolated from the uterine cervix of a pregnant or non-pregnant woman (results not shown).

Characterization of the large KS PG isolated from the uterine cervix of a non-pregnant woman

Aliquots of the large PGs (pool 1, Figure 3A) were labelled with biotin before degradation with CS lyases AC/ABC and keratanases. The native (Figure 4, lanes 1) and enzymically digested PGs (Figure 4, lanes 2–4) were submitted to SDS/PAGE followed by diffusion blot on to PVDF membrane (Figure 4).

After staining with peroxidase-conjugated avidin (Figure 4A), a core protein of about M_r 55 000 showed up in the CS lyases AC/ABC-treated sample (Figure 4A, lanes 2 and 4). After treatment with keratanase/keratanase II, a core protein of about M_r 220 000 was detected (Figure 4A, lane 3). Incubation with the KS-specific mAb 5D4 (Figure 4B) confirmed the presence of KS. After keratanase/keratanase II treatment (Figure 4B, lanes 3 and 4), only a weak signal with the mAb 5D4, most probably due to incomplete enzymic digestion, was obtained. Moreover, this KS

Figure 4 SDS/PAGE of the native and enzymically digested large PGs isolated from the uterine cervix of a non-pregnant woman

Purified large PGs (pool 1, Figure 3A) were labelled with biotin before digestion with CS lyases AC/ABC, keratanase and keratanase II, or a mixture of these enzymes. The samples were submitted to SDS/PAGE on 4–15% polyacrylamide gradient gels. For detection of PGs the gels were blotted to PVDF membrane. Core proteins liberated by degradation of the GAG chains were detected with peroxidase-conjugated avidin (A). For detection of KS-containing PG the mAb 5D4 was used (B). Lane 1, native PGs; lane 2, PGs after digestion with CS lyases AC/ABC; lane 3, PGs after digestion with keratanase/keratanase II; lane 4, PGs after digestion with a combination of CS lyases AC/ABC and keratanase/keratanase II. Molecular masses are indicated on the left.

PG showed only very faint reaction with the KS-specific mAbs 4B3/D10 and 3D12/H7, described recently by our group [16,17]. This is most probably due to a relative specificity of these antibodies for KS species from cartilage.

The CS PG-derived core protein showed no reaction with the decorin core-protein-specific mAbs 6B6 and LN1/LN3. However, when a sample from human articular cartilage was analysed as a control, these antibodies recognized the decorin core protein (results not shown). In addition the molecular-mass distribution of the native molecule with an M_r of $> 200\,000$ is clearly distinct from that of decorin with an M_r of about 100 000. Moreover, analysis of disaccharides liberated from this sample by digestion with CS lyase AC and CS lyases AC/ABC revealed the presence of only chondroitin O-sulphate and chondroitin 6-sulphate, thus excluding the existence of a dermatan sulphate-containing PG in this preparation (results not shown). These data clearly indicate the existence of a CS PG, which is distinct from decorin. In contrast, in the cervix from the pregnant woman large amounts

Figure 5 SDS/PAGE of the native and enzymically digested large PGs isolated from the uterine cervix of a pregnant woman

Purified large PGs (pool 1, Figure 3B) were labelled with biotin before digestion with CS lyases AC/ABC, keratanase and keratanase II, or a mixture of these enzymes. Samples were submitted to SDS/PAGE on 4–15% polyacrylamide gradient gels. For detection of PGs the gels were stained with Alcian Blue/silver (A) or blotted to PVDF membrane (B and C). Core proteins liberated by degradation of the GAG chains were detected with peroxidase-conjugated avidin (B). For detection of KS-containing PG (C) the mAb 5D4 was used. It should be noted that the intense bands at about M_r 200 000 and 40 000 (A, lanes 2, 3 and 4) are derived from the enzyme preparations and do not represent CS-PG and KS-PG core proteins. Furthermore, slightly different amounts of the samples (Figures 5A and 5B) were applied in lanes 3 and 4. Lane 1, native PGs; lane 2, PGs after digestion with CS lyases AC/ABC; lane 3, PGs after digestion with keratanase/keratanase II; lane 4, PGs after digestion with a combination of CS lyases AC/ABC and keratanase/keratanase II. Molecular masses are indicated on the left of each panel.

Figure 6 SDS/PAGE and Western blot of native and enzymically digested decorin isolated from the uterine cervix of a pregnant woman

Purified decorin was submitted to SDS/PAGE on an 8–25% polyacrylamide gradient gel. For detection the mAbs LN1/LN3 were used. Lane 1, native PGs; lane 2, PGs after digestion with CS lyases AC/ABC; lane 3, PGs after digestion with keratanase/keratanase II; lane 4, PGs after digestion with a combination of CS lyases AC/ABC and keratanase/keratanase II.

of decorin could be detected in addition to the larger CS PG (Figure 5B, Figure 6).

Characterization of the large KS PG isolated from the uterine cervix of a pregnant woman

Aliquots of the large PGs (pool 1, Figure 3B) were labelled with biotin before degradation with CS lyases AC/ABC and keratanases. The native (Figure 5, lanes 1) and enzymically digested PGs (Figure 5, lanes 2–4 in each panel) were submitted

Figure 7 SDS/PAGE and Western blot of extracts from uterine cervix of non-pregnant or pregnant women

Extracts from the uterine cervix of a non-pregnant woman (lane 1) and a pregnant woman (lane 2) were submitted to SDS/PAGE on an 8–25% polyacrylamide gradient gel and blotted on to nitrocellulose membrane. For detection an antiserum against the lumican core protein was used.

to SDS/PAGE followed by either combined Alcian Blue/silver staining (Figure 5A) or diffusion blot on to PVDF membrane (Figures 5B and 5C).

The native sample contained PGs in the range of $M_r > 220\,000$ – $100\,000$ and several contaminating proteins of $M_r \leq 50\,000$. After treatment with CS lyases AC/ABC, a large proportion of the PGs was degraded. Several core proteins and probably core-protein fragments in the range of about M_r 55 000– $< 20\,000$ are detected (Figure 5A, lanes 1 and 2). However, interpretation of the data shown in Figure 5A was difficult due to the presence of numerous proteins from the enzyme preparation. It should be noted that the intense bands at about M_r 200 000 and 40 000 in Figure 5A, lanes 2, 3 and 4, are derived from the enzyme preparations and do not represent CS PG and KS PG core-proteins.

Analysis of the M_r distribution in the biotin-labelled samples showed that by degradation with keratanase/keratanase II only a minor, but significant, part of the PG smear in the high-molecular-mass region disappears (Figure 5B, lane 3) and a rather sharp band at about M_r 220 000 is seen. These observations are further confirmed by immunoblotting. The large KS PG detected in the sample from the cervix of the non-pregnant woman (Figure 4B) is present in the tissue of the pregnant woman, too (Figure 5C). After degradation with keratanase/keratanase II a core protein in the range of M_r 220 000 is seen (Figure 5A and B, lanes 3 and 4), and the signal obtained with the mAb 5D4 is almost completely abolished (Figure 5C).

Whereas from the Western blots shown in Figure 4B and Figure 5C it might be assumed that the human uterine cervix does not contain small KS PGs, Northern-blot analysis using a lumican-specific probe indicated the expression of lumican (results not shown). To clarify this discrepancy, Western-blot analysis using an antiserum against the lumican core protein,

kindly provided by Dr. Funderburgh, was performed. Figure 7 clearly shows that lumican is expressed in the human uterine cervix as a free core protein.

The PG with an M_r of about 100 000 and a core protein of about 40 000 has been identified as decorin by the use of mAbs LN1/LN3 (Figure 6). This was also supported by analysis of disaccharides liberated through the action of CS lyase AC and CS lyases AC/ABC respectively. The content of dermatan sulphate was calculated to be 87% (results not shown). These results are in good agreement with those reported by the groups of Norman et al. [5,8] and Uldbjerg et al. [13].

DISCUSSION

During pregnancy, the extracellular-matrix composition of the uterine cervix undergoes major changes according to the specific functional requirements of the organ. Even though PGs might be involved in these processes, a detailed analysis of the PGs in the uterine cervix during pregnancy and especially an analysis of the changes in PG expression at different stages of pregnancy are still lacking.

Therefore, in the present study we have analysed the PGs of the human uterine cervix. In an attempt to elucidate the changes in the PG expression during pregnancy we have compared the PG expression in the uterine cervix of a non-pregnant and a pregnant woman.

Here we report for the first time on the presence of a large KS PG in the human uterine cervix. This KS PG was isolated and partially separated from other PGs by anion-exchange chromatography of tissue extracts (Figure 2). Immunochemical analysis of the fractions obtained revealed that the majority of the KS PG was eluted with NaCl concentrations of about 0.8 M, pointing towards a strong anionic character. Further purification of PGs was performed by gel-permeation chromatography (Figure 3). The KS PG was eluted mainly in the void volume of the column, indicating a high molecular mass of this KS PG.

PGs were further characterized by SDS/PAGE before and after enzymic digestion with GAG-specific enzymes. Analysis by combined Alcian Blue/silver staining was difficult due to contaminating proteins from the enzyme preparations (Figure 5A, lanes 2–4). Therefore aliquots of the isolated PGs were labelled with biotin before enzymic degradation and analysis by SDS/PAGE (Figures 4A and 5B). Whereas treatment with CS lyases AC/ABC had no effect on the molecular-mass distribution of the large KS PG isolated from the uterine cervix (Figure 4A, lane 2; Figures 5A and 5B, lanes 2), treatment with keratanase/keratanase II revealed the presence of a rather sharp core-protein band with an M_r of about 220 000 (Figure 4A, lane 3; Figures 5A and 5B, lanes 3). It should be noted that the intense bands at about M_r 220 000 that are clearly detectable after Alcian Blue/silver staining (Figure 5A, lanes 3 and 4) resulted from superposition of contaminating proteins from the enzyme preparation. However, labelling of PGs with biotin before degradation with CS lyases AC/ABC and keratanase/keratanase II clearly showed that the M_r 220 000 band has to be identified as core protein of the large KS PG (Figure 5B, lanes 3 and 4). These findings were further confirmed by immunochemical analysis using the KS-specific mAb 5D4 (Figures 4B and 5C). Both samples showed strong reactivity with the mAb 5D4. After incubation with either keratanase/keratanase II or a combination of CS lyases AC/ABC and keratanase/keratanase II, the epitope recognized by mAb 5D4 was nearly complete degraded (Figure 4B, lanes 3 and 4; Figure 5C, lanes 3 and 4). These data clearly indicate the presence of a KS PG in the human uterine cervix,

regardless of whether the tissue was taken from a non-pregnant woman or a pregnant woman.

The different sensitivity to treatment with keratanase/keratanase II of the KS PGs isolated from the uterine cervix of either a non-pregnant (Figure 4) or a pregnant (Figure 5) woman is most probably due to incomplete digestion of the KS PG in the experiment shown in Figure 4.

A more detailed characterization of this KS PG is at present under study. In preliminary experiments this KS PG failed to react with concanavalin A, thus pointing towards O-linked GAG-chains (results not shown); but, clearly, for confirmation a more detailed analysis is needed.

Whether in the human uterine cervix small KS PGs, such as lumican, are also expressed has to be analysed in more detail. Whereas at the protein level only a large KS PG was detectable (Figures 4B and 5C), on the mRNA level at least lumican could be detected (results not shown). Using an antiserum against the lumican core protein [18] a rather sharp core-protein band in the range of M_r 37 000 was detectable after SDS/PAGE and Western blot in the crude cervix extracts (Figure 7), but not in samples obtained after anion-exchange chromatography. These data indicate that lumican is expressed as a free core protein and most probably lost during anion-exchange chromatography. Moreover these data explain why lumican was not detectable by the KS-specific mAb 5D4 (Figures 4B and 5C). The expression of free lumican core protein has been recently described for several tissues by Funderburgh et al. [18].

This report is the first one describing the presence of a large KS PG in human uterine cervix. This PG, with a molecular-mass distribution of M_r > 220 000 and a core protein of about M_r 220 000, was recognized by the well-characterized KS-specific mAb 5D4, which recognizes a highly sulphated domain in the KS-chain [30]. Neither the native PG nor the enzymically degraded one is recognized by the mAb 4G4/A10 [16], which is specific for the aggrecan core protein from human articular cartilage (results not shown), indicating that this KS PG and the respective core protein are distinct from aggrecan. This is also supported by the fact that the core protein of the large KS PG is not substituted with CS chains. The expression of this KS PG seemed not to be changed in the uterine cervix during early pregnancy. Even though this KS PG is not a major PG in the human cervix, it might have a specific function in the extracellular matrix of the uterine cervix.

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