Purification and characterization of a small dermatan sulphate proteoglycan implicated in the dilatation of the rat uterine cervix

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A small dermatan sulphate proteoglycan (DSPG) was extracted from rat cervixes and was purified by using DEAE-Sephacel ion-exchange chromatography, gel filtration on Sepharose CL-2B and CsCl-density-gradient centrifugation. Sedimentation-equilibrium centrifugation gave a weight-average M_r of 95000. Amino acid analysis showed a high content of aspartic acid, glutamic acid, glycine and leucine. The glycosaminoglycan chains had M_r 50000 as determined by gel filtration. Chondroitin AC lyase and chondroitin ABC lyase digestions of these chains showed that they were composed of 75% dermatan sulphate and 25% chondroitin sulphate. Chondroitin ABC lyase digestion produced a core protein of M_r 45000. The core protein, after treatment with HF, had M_r 37000. Amino acid sequences of the *N*-terminus and a CNBr-cleavage peptide showed similarity to the sequences of core proteins of small proteoglycans of bovine and human origin, but the *N*-terminal glycosaminoglycan-attachment site (Ser-Gly-Ile-Ile) differed from the consensus sequence (Ser-Gly-Xaa-Gly) [Bourdon, Krusius, Campbell, Schwartz & Ruoslahti (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3194–3198]. A polyclonal antibody against the rat cervical DSPG reacted with small proteoglycans from cervixes of human, mouse, dog, cow and sheep. DSPG is the major proteoglycan species present in the cervix. The amount of DSPG per cervix increases 4-fold during pregnancy, then falls precipitously within 1 day *post partum*. A role in cervical dilatation is postulated for this proteoglycan.

INTRODUCTION

In mammalian species softening and dilatation of the uterine cervix are indispensable for the delivery of offspring. Since 85% of the cervix is connective tissue (Danforth, 1954), dilatation depends on extensive alterations of the connective-tissue framework. Collagen fibres are the major tensile elements of the cervix. In the human cervix at term, collagen concentration decreases (Kleissl *et al.*, 1978) and collagenase activity increases (Rajabi *et al.*, 1987). However, in the rat cervix there is a continuing increase in total collagen content to the time of parturition (Harkness & Harkness, 1959), and we find no increase in collagenase activity (R. Kokenyesi & J. F. Woessner, Jr., unpublished work). Therefore we have considered the possibility that the proteoglycans may be important in dilatation, at least in the rat cervix. Golichowski *et al.* (1980) quantified various glycosaminoglycans in the rat cervix around the time of dilatation, but the proteoglycans have not hitherto been isolated or quantified. A small dermatan sulphate proteoglycan from human cervix has been characterized by Uldbjerg *et al.* (1983). The rat is a convenient animal in which to study cervical dilatation because the mechanical and biochemical properties of the entire cervix, not just a biopsy, can be studied at any stage of the pregnancy.

In the present paper we describe the isolation and characterization of the major proteoglycan of the rat uterine cervix, including the first report of amino acid

sequences of a small interstitial dermatan sulphate proteoglycan of rodent origin.

EXPERIMENTAL

Materials

Cervices were taken from non-gravid, parturient and 1-day-post-partum Sprague–Dawley rats. Although the highest amount of proteoglycan per cervix is found at parturition, cervixes at 1 day *post partum* were used for characterization of the proteoglycan, because uteri at this stage were needed for other projects in the laboratory. The cervix, defined as the tissue having parallel lumina and lying between the uterus and the vagina, was excised, trimmed of surrounding tissue and stored at -20°C . Sepharose, Sephadex and DEAE-Sephacel gels were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Papain, guanidinium chloride (practical grade), type III and type IV collagens and dipeptide standards (L-Asp-L-Glu, L-Ala-L-Ser and Gly-L-Ile) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Guanidinium chloride was treated with activated charcoal and filtered to give an A_{280} below 0.13 for a 4 M solution. Chondroitin AC lyase and chondroitin ABC lyase were obtained from Miles Laboratories (Elkhart, IN, U.S.A.). Dialysis tubing (M_r cut-offs of 12–14 kDa and 3.5 kDa) was purchased from Spectrum Medical Industries (Los Angeles, CA, U.S.A.). Large proteo-

Abbreviations used: DSPG, dermatan sulphate proteoglycan; GAG, glycosaminoglycan; PAGE, polyacrylamide-gel electrophoresis.

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glycan monomer was prepared from bovine nasal cartilage as described previously (Nagase & Woessner, 1980). Type I collagen was purified by the method of Ryhänen *et al.* (1982).

Analytical methods

Protein was determined by the method of Bradford (1976), with bovine serum albumin as standard; uronic acid was determined by the method of Bitter & Muir (1962). Sulphated glycosaminoglycans were quantified by the dimethylMethylene Blue method (Farndale *et al.*, 1986), based on a standard of pig skin dermatan sulphate (Sigma Chemical Co.).

Amino acid and hexosamine analyses were done as described previously (Whitney *et al.*, 1986). The dipeptide standards and the cathepsin C digestion mixture were analysed on the amino acid analyser according to the procedure of Chopra *et al.* (1985).

Extraction and isolation of proteoglycan

Cervices were thawed, split open, rinsed in 0.9 % NaCl and blotted with filter paper. A batch of 25–30 cervices were minced and extracted for 24 hours at 4 °C with 4 M-guanidinium chloride containing proteinase inhibitors as described by Uldbjerg *et al.* (1983); 1 mM-phenylmethanesulphonyl fluoride was also included in the mixture. The residue was removed by centrifugation at 1000 *g* for 10 min. The supernatant was chromatographed on Sephadex G-25 in 7 M-urea, and the excluded fractions were subjected to further purification on DEAE-Sephacel (Yanagishita & Hascall, 1983), with a stepwise elution with 2 M-NaCl. This fraction was analysed on a Sepharose CL-2B column (Vogel *et al.*, 1984). The pooled fractions were subjected to CsCl-density-gradient centrifugation in a Beckman type 40 rotor as described previously (Cöster & Fransson, 1981), and 1 ml fractions were collected from the bottom of the tubes.

Cervices excised at different stages of pregnancy were extracted separately, and the extracts were subjected to desalting, ion-exchange chromatography and gel filtration as described above. DSPG from individual cervices was quantified as glycosaminoglycan in the Sepharose CL-2B fractions by the dimethylMethylene Blue method.

Degradation methods

Alkaline degradation was performed as described by Carlson (1968). HF degradation was done in an apparatus designed for Merrifield peptide synthesis (Mort & Lamport, 1977). Freeze-dried proteoglycan (200 µg) was stirred with 1 ml of anisole and 4 ml of anhydrous HF for 1 h at 4 °C, then the HF was removed with a water pump. The residue was dissolved in 0.5 M-sodium acetate containing 0.5 % CHAPS (a zwitterionic detergent) and dialysed.

Digestions with chondroitin AC lyase (EC 4.2.2.5) and chondroitin ABC lyase (EC 4.2.2.4) were done in accordance with Vogel *et al.* (1984). Papain digestion was performed as described by Golichowski *et al.* (1980). Digestion with bovine spleen cathepsin C (dipeptidyl aminopeptidase, EC 3.4.14.1; Sigma Chemical Co.) followed the method of Chopra *et al.* (1985).

CNBr degradation was performed as described by Brew & Hill (1970). The resultant peptides were separated by h.p.l.c. (model 1330 apparatus; Bio-Rad Laboratories, Richmond, CA, U.S.A.) on a C₄ column (Chromtec,

Lake Worth, FL, U.S.A.) eluted with a 2–98 % (v/v) acetonitrile gradient.

Antibody production and immunoassays

Immunization of rabbits and the e.i.s.a. were performed in accordance with Heinegård *et al.* (1985), with the exception that the micro-titre plates (Costar, Cambridge, MA, U.S.A.) were coated with the antigen in the presence of 0.1 M-carbonate buffer, pH 9.0. Electrophoretic transfer to Zeta-Probe (Bio-Rad Laboratories) and subsequent treatment with bovine serum albumin was done as described by Gershoni & Palade (1982). The membranes were first incubated with the anti-DSPG serum (diluted in 20 mM-Tris/HCl buffer, pH 7.5, containing 0.15 M-NaCl and 1 % bovine serum albumin) and then with the alkaline phosphatase-conjugated goat anti-(rabbit immunoglobulin) antibody (Zymed Laboratories, San Francisco, CA, U.S.A.). BCIP/NBT substrate (containing 0.15 mg of 5-bromo-4-chloroindol-3-yl phosphate/ml and 0.3 mg of Nitro Blue Tetrazolium/ml, both from Sigma Chemical Co.) to stain the membranes.

Additional methods

SDS/PAGE was done under reducing conditions according to the procedure of Laemmli (1970). All gels were stained with AgNO₃ (Morrissey, 1981).

The amino acid sequences were determined by use of an Applied Biosystems 470-A gas-phase sequencer and standard programs supplied by the manufacturer. The amino acid phenylthiohydantoin derivatives were identified and quantified by use of a Hewlett-Packard 1040 h.p.l.c. system with an Applied Biosystems narrow-bore phenylthiohydantoin analyser column eluted with solvent supplied by Applied Biosystems.

The HF-degraded core protein was sequenced off an Immobilone membrane (Millipore Corp., Bedford, MA, U.S.A.) after SDS/PAGE and subsequent electrophoretic transfer on to the membrane (Matsudaira, 1987).

Sedimentation-equilibrium measurements (Yphantis, 1964) were performed in a Beckman model E analytical ultracentrifuge at 22000 rev./min with an An-H rotor and interference optics. Purified DSPG was dissolved in 50 mM-Tris/HCl buffer, pH 7.0, containing 0.5 M-KCl, to give concentrations of 0.25 mg/ml, 0.5 mg/ml and 1.0 mg/ml.

The aggregation of DSPG with hyaluronic acid was studied by the method of Heinegård *et al.* (1981).

RESULTS

Extraction and isolation of the cervical DSPG

Analysis of the cervical extracts and residues after papain digestion indicated that 85 % of the proteoglycans was extracted by 4 M-guanidinium chloride. The net recovery of the extracted proteoglycans after sequential chromatography on Sephadex G-25 and DEAE-Sephacel was 95 %. The bulk of the cervical proteoglycans (90 %) chromatographed as a peak positioned in the region K_{av} 0.55–0.85 on the Sepharose CL-2B column (results not shown). Approx. 10 % of the proteoglycans was eluted in the region K_{av} 0.2–0.55. The dimethylMethylene Blue-reactive material that was eluted in the void volume probably contained mucin-type glycoproteins, because it was not digested by chondroitin ABC lyase and, after alkali elimination, the dimethylMethylene Blue-reactive material passed through dialysis tubing with cut-off at

Fig. 1. SDS/PAGE of core protein of rat cervical DSPG

(a) lane 1, M_r markers; lane 2, D1 fraction from CsCl gradient; lane 3, D1 fraction digested with chondroitin ABC lyase; lane 4, D6 fraction digested with chondroitin ABC lyase; lane 5, chondroitin ABC lyase. The 7.5% gel was stained with AgNO_3 . (b) lane 1, M_r markers; lane 2, DSPG degraded with HF. The 10% gel was stained with AgNO_3 .

M_r 3500. The fractions containing the major proteoglycan were pooled and subjected to CsCl-density-gradient centrifugation. The bottom fraction (D1) contained purified DSPG as determined by SDS/PAGE before (Fig. 1a, lane 2) and after (Fig. 1a, lane 3) chondroitin ABC lyase digestion. This bottom fraction, containing 25% of the applied material, was used for characterization of this DSPG. The remaining fractions contained decreasing amounts of proteoglycan and increasing amounts of protein towards the top of the tube (D9). Twenty-five cervixes (3.5 g wet wt.) provided 0.9 mg of pure DSPG with an overall yield of 15%.

Physical characterization of the purified DSPG

The M_r of the DSPG was determined by sedimentation-equilibrium ultracentrifugation. The partial specific volume was taken as 0.69 ml/g from the average buoyant density of this DSPG (1.45 g/ml). The plot of $\ln(\text{fringe displacement})$ versus radius² was linear at three DSPG concentrations: 0.25, 0.5 and 1.0 mg/ml. A plot of $\bar{M}_w$ versus concentration was linear, with negative slope; when extrapolated to zero concentration it gave $\bar{M}_w = 95000$.

The rat cervical DSPG did not form detectable aggregates with high- M_r hyaluronic acid, as judged by Sepharose CL-2B column chromatography under associative conditions (50 mM-sodium acetate buffer, pH 5.8; results not shown).

Characterization of the GAG chains

The proteoglycan nature of the material purified by CsCl-density-gradient centrifugation was confirmed by alkali degradation in the presence of NaBH_4 . The released GAG chains chromatographed with K_{av} 0.28 on Sepharose CL-6B (Fig. 2). This elution position corresponds to M_r 50000 on the basis of the calibration method of Wasteson (1971). When chondroitin sulphate chains of the large bovine nasal-cartilage proteoglycan were re-

Fig. 2. Sepharose CL-6B chromatography of GAG chains of the rat cervical DSPG

Purified DSPG was treated with alkali and chromatographed on a Sepharose CL-6B column (110 cm $\times$ 0.5 cm) as described in the Experimental section. The arrow labelled CS denotes the elution position of the alkali-degraded bovine nasal-cartilage proteoglycan. ▲ Rat cervical DSPG before alkali treatment; ●, rat cervical DSPG after alkali treatment.

leased and chromatographed under the same conditions, M_r 20000 was obtained. This value is in good agreement with the observation made by Wasteson (1971).

The composition of the GAG chains was determined by chondroitinase digestions and subsequent chromatography on Sephadex G-50. Chondroitin AC lyase degraded 25% of the glycosaminoglycans (Fig. 3a); the undegraded material was digested by chondroitin ABC lyase (Fig. 3b). These results indicate that the GAG chains are co-polymeric galactosaminoglycans (dermatan sulphate) composed of 75% iduronic acid- and 25% glucuronic acid-containing disaccharide units.

M_r and amino acid composition of the core protein

The core protein preparation obtained by chondroitin ABC lyase digestion of the DSPG gave two closely spaced bands on SDS/PAGE with an apparent M_r of 45000 (Fig. 1a, lanes 3 and 4). This doublet could not be resolved if too large an amount of core protein was applied to the gel (Fig. 1a, lane 3). HF deglycosylation of the DSPG resulted in a single band of core protein with an apparent M_r of 37000 (Fig. 1b, lane 2). The same treatment did not significantly degrade bovine serum albumin (results not shown). This result indicates that there is only one core protein for the cervical proteoglycan, so that the doublet seen after chondroitin ABC lyase digestion represents this core protein with slightly different amounts of oligosaccharides attached to it. The size of the deglycosylated core of rat cervical DSPG is very similar to M_r 36319 and 36380, deduced from the cDNA sequence of the proteoglycan core protein of human embryonic skin fibroblasts (Krusius & Ruoslahti, 1986) and bovine bone (Day *et al.*, 1987) respectively.

Amino acid analysis of the DSPG showed low serine and threonine content and high aspartic acid, glutamic acid, glycine and leucine content (Table 1). The major hexosamine was galactosamine.

Amino acid sequences

The DSPG was subjected to *N*-terminal amino acid

Fig. 3. Analysis of the chondroitinase-digestion products of GAG chains of the rat cervical DSPG

(a) The GAG chains, obtained by alkali degradation, were digested with chondroitin AC lyase and chromatographed on a Sephadex G-50 column (50 cm $\times$ 0.5 cm) eluted with 0.2 M- NH_4HCO_3 . Fractions (1 ml) were collected and analysed for uronic acid (A_{530}). (b) The chondroitin AC lyase-resistant fractions (8–16 from a) were digested with chondroitin ABC lyase and chromatographed on the same column.

Table 1. Amino acid and amino sugar compositions of small dermatan sulphate proteoglycans

The composition of the rat cervical DSPG is compared with that of the human cervical proteoglycan (Uldbjerg *et al.*, 1983) and calf skin proteoglycan (Fujii & Nagai, 1981). Abbreviation: N.D. not determined.

Amino acid or amino sugar	Composition (residues/1000 residues)		
	Rat cervix	Human cervix	Calf skin
Aspartic acid	123	130	113
Threonine	56	45	37
Serine	87	79	62
Glutamic acid	113	110	109
Proline	65	77	92
Glycine	109	85	108
Alanine	50	53	51
Cysteine	N.D.	17	12
Valine	41	50	56
Methionine	12	3	11
Isoleucine	47	43	56
Leucine	115	122	110
Tyrosine	27	20	19
Phenylalanine	26	33	40
Histidine	29	25	24
Lysine	61	68	74
Arginine	34	41	26
Glucosamine	68	N.D.	18
Galactosamine	301	N.D.	135

sequencing in a gas-phase sequencer. A single sequence was obtained (Fig. 4a) from three different DSPG preparations. The residues at position 4 and 17 could not be identified by sequencing of the intact proteoglycan, but the sequencing of the proteoglycan degraded by HF proved that the fourth residue is serine.

The CNBr degradation of the DSPG resulted in nine protein peaks, clearly separated by h.p.l.c. (results not shown). Amino acid sequencing was attempted only in the case of the six peaks where the amino acid composition showed the presence of more than six amino acid residues. One peak had a sequence corresponding to that of the *N*-terminus of the intact proteoglycan. Two closely spaced peaks were found to have identical sequences for the 22 residues examined. This sequence contained a putative GAG-attachment site (Fig. 4b). Three of the peaks failed to give any sequence information, possibly because of modification of their *N*-termini during the degradation.

Glycosaminoglycan attachment site

Cathepsin C digestion of the cervical DSPG for 15 min resulted in a decrease in the M_r from 91 000 to 42 000 on SDS/PAGE (results not shown); this corresponds to the result obtained with bovine skin proteoglycan (Chopra *et al.*, 1985). Analysis of the dipeptides released by the action of cathepsin C demonstrated the presence of the first and third dipeptides from the *N*-terminus (Asp-Glu and Gly-Ile respectively), but the second and fourth dipeptides were not detected (Ala-Ser and Ile-Pro respectively) (results not shown). The absence of the second dipeptide can be explained by the presence of the attached GAG chain, since, because of its large size, the dipeptide bearing the GAG chain would fall through the ion-exchange column of the amino acid analyser (Callahan *et al.*, 1971). These findings also indicate that the proline at residue 8 in the *N*-terminus of the cleaved core prevented the splitting of the fourth dipeptide; such an inhibitory effect of proline has been demonstrated by McDonald *et al.* (1971).

Reactivity of the anti-DSPG antibody

The anti-DSPG antibody did not show cross-reactivity with collagen types I, III or IV, with fibronectin or with the core preparation of the large proteoglycan from the bovine nasal cartilage, as determined by inhibition e.l.i.s.a. (results not shown). On the Western blots this antibody reacted with a single band from extracts of cervixes of rat, human, mouse, cat, dog, cow and sheep (Fig. 5). The M_r of these immunoreactive molecules was in the range 90 000–115 000. These macromolecules were each shown to be sensitive to digestion with chondroitin ABC lyase, and the immunoreactive digestion product in every species was found to have an M_r in the range 42 000–46 000. These immunoreactive molecules, therefore, are small proteoglycans by definition.

Changes in the amount of the cervical proteoglycan during pregnancy

Quantification of the proteoglycans from cervixes of non-gravid, parturient and post-partum rats revealed that the amount of the DSPG per cervix increased from 43 μg /cervix ($n = 13$) in non-gravid animals to 196 μg /cervix ($n = 14$) by the time of parturition, parallel to the softening of the cervix (R. Kokenyesi & J. F. Woessner, Jr., unpublished work). The amount of small

Fig. 4. Amino acid sequences of core proteins of small proteoglycans

(a) *N*-Terminal sequences: a, rat cervical DSPG; b, bovine skin proteoglycan (Pearson *et al.*, 1983); c, bovine bone proteoglycan (Day *et al.*, 1987); d, human fetal membrane proteoglycan (Brennan *et al.*, 1984); e, human embryonic skin proteoglycan (Krusius & Ruoslahti, 1986). The identical residues are enclosed in boxes. (b) Sequences: a, CNBr-cleavage peptide of rat cervical DSPG; b, human embryonic skin proteoglycan (Krusius & Ruoslahti, 1986); c, bovine bone proteoglycan (Day *et al.*, 1987). The identical residues are enclosed in boxes. * indicates the putative GAG-attachment site. The numbering of the residues is based on that of b and c.

Fig. 5. Immunoreactive cervical proteoglycans in different species

For details of the extraction and detection on Western blots see the Experimental section. (a) Rat cervix extract; (b) human cervix extract; (c) mouse cervix extract; (d) dog cervix extract; (e) cow cervix extract; (f) sheep cervix extract. Extracts were studied before (–) and after (+) chondroitinase ABC lyase digestion.

proteoglycan falls to 126 $\mu\text{g}/\text{cervix}$ at 4 h *post partum* ($n = 7$) and to 79 $\mu\text{g}/\text{cervix}$ by 1 day *post partum* ($n = 12$), in parallel with the increasing rigidity of the cervix. The concentration of DSPG also fell from 0.80 $\mu\text{g}/\text{mg}$ wet wt. at term to 0.42 $\mu\text{g}/\text{ml}$ by 4 h *post partum*.

DISCUSSION

The major proteoglycan of the rat uterine cervix has been isolated and characterized. On the basis of the size of its core protein and its amino acid composition, the rat cervical DSPG is a member of the family of small

interstitial proteoglycans (Hassell *et al.*, 1986), but it has uniquely large and iduronic acid-rich GAG chains. The M_r values of the proteoglycan, the core protein and the GAG chains indicate that the core protein carries a single GAG chain.

The DSPG of the rat cervix is significantly larger than the major proteoglycan of the human cervix (Uldbjerg *et al.*, 1983) (M_w 95000 versus 72000). The size difference is due chiefly to the larger GAG chain of the rat proteoglycan (M_r 50000 versus 25000). The GAG chain of the rat proteoglycan is also richer in iduronic acid (75% versus 50%). The amino acid composition revealed that the rat cervical DSPG is richer in glycine and methionine

than its human counterpart (Table 1), and most closely resembles the composition of the calf skin proteoglycan (Fujii & Nagai, 1981).

Comparison of the *N*-terminal amino acid sequence of the rat cervical DSPG with sequences of the other small interstitial proteoglycans reveals that the first six residues are identical in all cases (Fig. 4a). Beyond the eighth residue the sequence of the rat cervical DSPG is different from that of small proteoglycans of human and bovine origin. This divergence supports the notion of the 'hyper-variable' region in the *N*-terminal sequence (Day *et al.*, 1987). The 'hypervariable' region seems to reflect a species-related polymorphism rather than a tissue-related polymorphism of the core proteins, since the 'hyper-variable' regions are identical in proteoglycans isolated from different tissues within the same species (Fig. 4).

The cathepsin C digestion of the rat cervical DSPG, similarly to the results obtained with the bovine skin DSPG (Chopra *et al.*, 1985), indicated that the enzyme is able to split off only the first three dipeptides from the *N*-terminus. This digestion also resulted in a large decrease (49000) in the M_r of the proteoglycan, which corresponds to the size decrease after the removal of the GAG chain M_r (50000), indicating that the GAG chain is attached to one of the released dipeptides. The amino acid sequence of the core protein after HF degradation showed that the second dipeptide is Ala-Ser. The failure to detect Ala-Ser at the expected elution time upon the analysis of the digestion mixture may be attributed to the presence of a GAG chain attached to this dipeptide. From these findings it is concluded that the serine residue at position 4 in the *N*-terminal amino acid sequence is the GAG-attachment site. This position was shown to be the GAG-attachment site in bovine skin proteoglycan (Pearson *et al.*, 1983).

The *N*-terminus of the rat cervical DSPG has a flanking sequence (Ser-Gly-Ile-Ile) around the GAG-attachment site that is different from the consensus sequence for GAG attachment (Ser-Gly-Xaa-Gly) found in other small proteoglycans (Bourdon *et al.*, 1987), although the results of cathepsin C digestion and the sequence of the HF-degraded core protein indicate that the GAG chain is attached to the fourth amino acid residue in the *N*-terminus. Attachment of a chondroitin/dermatan chain has been shown to take place in the absence of the consensus sequence in the case of type IX collagen (McCormick *et al.*, 1987). The transfer of a xylose residue to the serine residue of the proteoglycan core by xylosyltransferase is the initial step in the synthesis of the chondroitin/dermatan chains (Rodén *et al.*, 1985). The diversity of the amino acid sequences around the xylosylation site suggests that the conformation and accessibility of that site might also be important in determining the point of GAG attachment.

The amino acid sequence of a CNBr-cleavage fragment (Fig. 4b) corresponded to the middle portion (residues 147–168) of the core proteins from human skin fibroblast and bovine bone proteoglycans (Krusius & Ruoslahti, 1986; Day *et al.*, 1987). These sequences contain a putative GAG-attachment site, but it is not known if it is ever substituted with a GAG chain.

Polyclonal antibody against the rat cervical DSPG showed the presence of small proteoglycans for the first time in the cervixes of mouse, cat, dog and cow. The small proteoglycan of the sheep cervix has been described (Fosang *et al.*, 1984), but its relationship to other

proteoglycans has not been studied. These data point to the ubiquitous distribution of small interstitial proteoglycans in the cervixes of different orders of mammals.

Our studies indicate that the amount of DSPG and the DSPG/collagen ratio increase with cervical softening during pregnancy and decrease with the increased rigidity of the tissue *post partum*. The mechanism by which the small proteoglycans might alter the resistance of the tissue to mechanical stress is not known, but via its ability to bind to collagen fibres (Scott, 1980) the increased amount of DSPG might be able to interrupt the formation of large bundles from newly synthesized collagen fibres. The disorganized collagen network may lead to increased tissue extensibility, thereby facilitating cervical dilatation.

The disintegration of collagen bundles and the increased amount of 'ground substance' are common light-microscopic findings at term in the cervixes of rats (Williams *et al.*, 1982), humans (Danforth *et al.*, 1960), sheep (Ellwood, 1980), mice (Leppi, 1964) and guinea pigs (Williams *et al.*, 1984). X-ray-diffraction studies have shown also that the collagen fibres in the rat cervix are more disorganized at term than in the non-gravid stage (Aspden, 1988).

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