Glycosaminoglycans of Porcine Uteri

HIROSHI MUNAKATA, MAMORU ISEMURA, CHIE KODAMA
and ZENSAKU YOSIZAWA

*Department of Biochemistry, Tohoku University School of
Medicine, Sendai 980*

MUNAKATA, H., ISEMURA, M., KODAMA, C. and YOSIZAWA Z. *Glycosaminoglycans of Porcine Uteri*. Tohoku J. exp. Med., 1985, **147** (1), 73 - 75 — Glycosaminoglycan fractions were obtained from the pronase digests of the endometrium and myometrium of porcine uteri. Their glycosaminoglycan compositions were determined by the use of specific mucopolysaccharide-degrading enzymes and nitrous acid. It was found that the endometrium contained chondroitin sulfates, dermatan sulfate and heparan sulfate and a smaller amount of hyaluronic acid, whereas hyaluronic acid and dermatan sulfate were the major glycosaminoglycans in the myometrium. ——— glycosaminoglycan; endometrium; myometrium; porcine uterus

An important role of proteoglycans in cell adhesion and spreading has been suggested (Bumol et al. 1984). In the previous papers (Isemura et al. 1981; Munakata et al. 1981), we showed that estrogen enhances the sulfation of acidic glycoconjugates in the endometrium of the rabbit uterus and that progesterone has a suppressive effect on the estrogen effect.

There have been many reports on GAGs of uteri. Surprisingly, however, little is known about the GAG composition of the uterine endometrium and myometrium.

In this paper, we report the GAG composition of porcine uteri with special reference to the difference between the endometrium and the myometrium.

MATERIALS AND METHODS

Materials

Pronase was obtained from Kaken Kagaku Co., Tokyo. Chondroitinase AC and chondroitinase ABC were purchased from Seikagaku Kogyo Co., Tokyo. Other materials were those described previously (Munakata et al. 1984).

Preparation of GAG

Porcine uteri were obtained from a local slaughter house. After adhering tissues were removed, uteri were washed with ice-cold saline, and opened by longitudinal cutting. The endometrium was scraped off with a glass plate and the remainder was used as the

Received April 16, 1985; accepted for publication June 12, 1985.

Abbreviations: GAG, glycosaminoglycan; HPLC, high-performance liquid chromatography.

myometrium. The endometrium and the myometrium were homogenized with acetone, delipidated and digested with pronase as described previously to yield glycoconjugate fractions (Munakata et al. 1985a). GAG fractions were separately isolated from the glycoconjugate fractions as described previously (Munakata et al. 1985b).

Quantitation of GAGs

The GAG fraction was dissolved in water (1 mg/ml) and divided into 0.2 ml portions. Each portion was separately incubated with hyaluronidase, chondroitinase AC, chondroitinase ABC and nitrous acid as described previously (Isemura et al. 1982; Munakata et al. 1984). The uronic acid contents in the resistant fractions against these treatments were determined by the modified carbazole-sulfuric acid reaction (Bitter and Muir 1962).

HPLC analysis

The GAG fraction was digested with chondroitinase AC or chondroitinase ABC and the disaccharides released were subjected to reductive amination by the procedure described by Hase et al. (1981). The fluorescence-labeled derivatives were analyzed by HPLC as described previously (Kodama et al. 1984).

RESULTS AND DISCUSSION

GAG fractions were prepared from the endometrium and myometrium of porcine uteri as described in MATERIALS AND METHODS. Their yields expressed as mg per g of dry tissue weight were 1.9 and 2.1 mg from the endometrium and myometrium, respectively. The GAG fractions were separately incubated with *Streptomyces* hyaluronidase, chondroitinase AC, chondroitinase ABC, and nitrous acid. The uronic acid contents in the resistant fractions against these treatments were then determined and the GAG compositions were calculated. The results are summarized in Table 1. The GAG compositions were considerably different between the endometrium and the myometrium. In the endometrium, heparan sulfate, dermatan sulfate and chondroitin sulfate were predominant GAGs, while hyaluronic acid was a minor component. In contrast, hyaluronic acid was the myometrium, respectively. The GAG fractions were separately incubated with myometrium was similar to that of hyaluronic acid. In order to examine further the chondroitin sulfate species, each GAG fraction was digested with chondroitinase AC or chondroitinase ABC. The unsaturated disaccharides released by digestion were derivatized and analyzed by HPLC as described in MATERIALS AND

TABLE 1. *GAG composition* of porcine uteri*

GAG	Endometrium	Myometrium
Hyaluronic acid	8.2	36.1
Chondroitin sulfate A/C	24.7	11.5
Dermatan sulfate	31.8	35.9
Heparan sulfate	35.3	16.4

* Expressed as percent in total GAG calculated from the results of three experiments.

METHODS. Molar ratios of chondroitin 4-sulfate and chondroitin 6-sulfate were 0.6 and 1.0 for the GAG fractions from endometrium and myometrium, respectively. These data indicate that GAGs in the uterine endometrium and myometrium are different not only in the GAG composition but also in the isomer pattern of chondroitin sulfate. These differences may be important in different functions between the endometrium and the myometrium, but their significance in biological roles remains to be elucidated.

Acknowledgments

This work was supported in part by a Grant-in Aid for Scientific Research from Ministry of Education, Science and Culture of Japan.

References

- 1) Bitter, T. & Muir, H. (1962) A modified uronic acid carbazole reaction. *Analyt. Biochem.*, **4**, 330-334.
 - 2) Bumol, T.F., Walker, L.E. & Reisfeld, R.A. (1984) Biosynthetic studies of proteoglycans in human melanoma cells with a monoclonal antibody to a core glycoprotein of chondroitin proteoglycans. *J. biol. Chem.*, **259**, 12733-12741.
 - 3) Hase, S., Ikenaka, T. & Matsushima, Y. (1981) A highly sensitive method for analyses of sugar moieties of glycoproteins by fluorescence labeling. *J. Biochem.*, **90**, 407-414.
 - 4) Isemura, M., Munakata, H. & Yosizawa, Z. (1981) Hormonal effects on the sulfation of sulfated glycoprotein in a particulate fraction of the endometrium of rabbit uterus. *J. Biochem.*, **89**, 1815-1819.
 - 5) Isemura, M., Munakata, H., Ototani, N., Goto, K. & Yosizawa, Z. (1982) Glycosaminoglycans of rat colorectal adenocarcinoma. *Gann*, **73**, 721-727.
 - 6) Kodama, C., Ototani, N., Isemura, M. & Yosizawa, Z. (1984) High performance liquid chromatography of pyridylamino derivatives of unsaturated disaccharides produced from chondroitin sulfate isomers by chondroitinases. *J. Biochem.*, **96**, 1283-1287.
 - 7) Munakata, H., Isemura, M. & Yosizawa, Z. (1981) Hormonal effects on the sulfation of sulfated glycoconjugates in the uterine endometrium of non-ovariectomized rabbit. *Tohoku J. exp. Med.*, **135**, 275-279.
 - 8) Munakata, H., Isemura, M. & Yosizawa, Z. (1984) Sulfated glycosaminoglycans synthesized in rabbit uterus. *Tohoku J. exp. Med.*, **143**, 205-212.
 - 9) Munakata, H., Isemura, M., Sato, N., Kikuchi, M. & Yoshizawa, Z. (1985a) Characterization of blood group active glycopeptides derived from porcine kidneys. *Int. J. Biochem.*, **17**, 301-308.
 - 10) Munakata, H., Isemura, M. & Yosizawa, Z. (1985b) Effects of female hormones on the activity of 3'-phosphoadenylylsulphate: desulphated heparan sulphate sulphotransferase in the endometrium of rabbit uterus. *Int. J. Biochem.*, **17**, 1077-1083.
-