Effect of p-Nitrophenyl-β-D-xyloside on Proteoglycan and Glycosaminoglycan Biosynthesis in Rat Serosal Mast Cell Cultures*

Richard L. Stevens$ and K. Frank Austen
From the Department of Medicine, Harvard Medical School and the Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115

(Received for publication, July 13, 1981)

Rat serosal mast cells cultured in the presence of heat-inactivated fetal calf serum incorporated ([35S] sulfate) into heparin proteoglycan of approximately M, = 750,000 after a 3-h pulse and a 2-h chase. β-D-Xyloside (0.1 mM) treatments of cultures of rat mast cells resulted in an insignificant increase in total ([35S] sulfate) incorporation and the appearance of free glycosaminoglycans without a change in proteoglycan size. At higher β-D-xyloside concentrations, total ([35S] sulfate) incorporation was inhibited and an increase in the relative glycosaminoglycan content was observed concomitant with a reduction in proteoglycan amount and size. As assessed by susceptibility to digestion by chondroitinase ABC, hydrolysis by nitrous acid, [3H]hexosamine content, and electrophoretic mobility, only heparin chains were polymerized onto the proteoglycan core in all cultures. In contrast, individual glycosaminoglycans which appeared only after β-D-xyloside treatment were predominantly chondroitin sulfate rather than heparin, indicating that the β-D-xyloside acceptor supported polymerization of chondroitin sulfate but not of heparin glycosaminoglycan. Thus, the peptide core is an important determinant for the synthesis of heparin glycosaminoglycan by rat peritoneal mast cells.

In contrast to the continuously secreted chondrocyte proteoglycan (1, 2), rat mast cell proteoglycan resides intracellularly (3, 4) and is secreted only upon specific activation of the cell (5). Rat mast cell proteoglycan is approximately M, = 750,000 (3) and possesses a peptide core, estimated at M, = 12,000, containing almost exclusively serine and glycine (6). Heparin glycosaminoglycans are attached O-glycosidically to serine, a common linkage site for O-glycosidically-linked glycosaminoglycans with the possible exception of cartilage keratan sulfate (7, 8). The carbohydrate linkage region of heparin, GlcUA → Gal → Gal → Xyl → serine (9), is reported to be identical to that of chondroitin sulfate (10, 11). The addition of the fifth monosaccharide, GlcNAc or GalNAc, is the first biosynthetic difference between heparin and the chondroitin sulfate precursor glycosaminoglycan chains. The requirement of a serine-glycine sequence to signal glycosaminoglycan initiation is consistent with other types of glycosylation of proteins requiring specific peptide sequences, such as the transfer of mannose-type oligosaccharides to glycoproteins via dolichol, which requires the peptide sequence asparagine-X-serine or threonine (12) β-D-Xyloside treatment of chondrocytes in vitro results in the polymerization of large amounts of chondroitin sulfate onto the drug (1, 13-17); whereas β-D-xyloside-treated SV40-transformed Swiss mouse 3T3 fibroblasts (18), mouse mastocytoma tissue (19), and rat hepatocytes (20) polymerize both heparan sulfate-related and chondroitin sulfate-related glycosaminoglycans onto this compound. We now report that β-D-xyloside acts on normal rat serosal mast cells to decrease the size of newly synthesized heparin proteoglycan and to uncover a latent capacity of the cell to polymerize chondroitin sulfate onto the exogenous acceptor. We conclude that more specific initiation requirements than those offered by the β-D-xyloside are needed for heparin biosynthesis, since relatively few, if any, heparin chains are synthesized onto the drug.

EXPERIMENTAL PROCEDURES

Materials—p-Nitrophenyl-β-D-xyloside, N-acetylglucosamine, penicillin, streptomycin, and fetal calf serum; ADi-4S, 2-acetamido-2-deoxy-3-O-(β-D-glucuronic acid)-6-O-sulfate-D-galactose; GdnHCl, guanidine HCl.

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blue staining and incorporation of \((^{35}S)\)sulfate exclusively into heparin. Approximately 0.5 to 1 x 10^6 mast cells were obtained per rat.

Most cells (~1 x 10^6) were resuspended in 1 ml of Dulbecco's modified Eagle's medium containing 4 mg/iter of glucose and supplemented with 15 mM 4-(2-hydroxyethyl)-l-piperazineethanesulfonic acid, 10 mM NaCl, 10 mM sodium pyruvate, 2-mM glutamine, 100 mM sodium pyruvate, 0.5 mM 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid (pH 7.2), 100 units/ml of penicillin, 100 units/ml of streptomycin, and 15% (v/v) heat-inactivated fetal calf serum (2, 23). Mast cells were incubated, generally for 3 h, at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air in Medium A containing 2500 μCi of \((^{35}S)\)sulfate/ml, 50-200 μCi of \([^{3}H]\)serine/ml, or 100 μCi of \([^{3}H]\)glycine/ml.

\(\beta\)-Xyloside, dissolved in dimethyl sulfoxide at a concentration of 80 mg/ml, was added to mast cell cultures to make final solutions of 0.1 mM to 10 mM xylose. At the end of the labeling period, cells were centrifuged at 400 x g for 4 min and the culture medium was removed. Cell-associated proteoglycans were liberated at 4 °C by adding 100 μl of 1% (w/v) zwittergent 3-12 containing 0.1 M 6-aminohexanoic acid, 0.01 mM sodium-EDTA, 0.005 M benzamidine HCl, 0.001 M sodium ioodecanic acid, and 0.1 mM sodium acetate, followed 30-60 later by 1 ml of 4 M guanidine HCl containing the same protease inhibitors (24, 25). In some experiments, radiolabeled mast cells were washed with 1 ml of 1 M NaCl in Tyrode's buffer before the zwittergent/GdnHCl disruption step, in order to dissociate any bound pericellular radiolabeled macromolecules (26). Radiolabeled fractions were quantitated directly or were subjected to CsCl2 density gradient centrifugation to characterize the proteoglycans and glycosaminoglycans from \([3H]glucosamine-labeled, \([3H]serine-labeled, and \([3H]glucosamine-labeled mast cells were subjected to cetylpyridinium chloride (CPC) precipitation (29) to remove any carbohydrate-rich glycoproteins also present at the bottom of the dissociative CsCl2 gradient. The fractions were divided into four approximately equal volumes of 70% ethanol containing 1.25 ml of Hydrofluor, were allowed for radioactivity. For a preparative purification, only one-tenth of each fraction was assayed for radioactivity, and the proteoglycan- and glycosaminoglycan-containing fractions were pooled separately, dialyzed exhaustively against water, and lyophilized.

For nitrous acid degradation, lyophilized samples obtained by Sepharose CL-4B gel filtration were suspended in 200 μl of water, 200 μl of dimethoxycetane, and 20 μl of butyl nitrite (30) for 17 h at -40 °C. The samples were neutralized by the addition of 10 μl of saturated sodium acetate, pH 6.5, and were subjected to PD-10 gel filtration with 4 M GdnHCl containing 50 μg/ml of heparin, 0.1 mM sodium sulfate, and 0.1 M Tris-HCl, pH 7.5. The shift in radioactivity from the excluded to the included volume due to nitrous acid treatment was a measure of the amount of total incorporated radioactivity associated with heparin and/or heparan sulfate. For reference, 200 μg of chondroitin sulfate and 200 μg of heparin, incubated with nitrous acid under identical conditions and subjected to gel filtration chromatography, were assessed for changes in size by measurement of uronic acid (31) after the demethoxycetane was removed.

Lyophilized \([^{3}H]\)glucosamine-labeled proteoglycans and glycosaminoglycans were treated with chondroitinase ABC (32) and degradation products were determined by cellulose thin layer chromatography. The relative percentage of the total incorporated radioactivity which localized with \(\Delta D-4 S\) and \(\Delta D-6 S\) was indicated as the percentage of \((^{35}S)\)sulfate incorporated into chondroitin sulfates. A 5-fold (w/w) excess of heparin glycosaminoglycan over chondrosarcoma proteoglycan did not alter chondroitinase ABC digestion of macromolecular chondroitin sulfate.

\([^{3}H]\)Glycosamine-labeled proteoglycans and glycosaminoglycans were acid hydrolyzed in 8 M HCl for 3 h at 90 °C, and analyzed by automated ion exchange chromatography for distribution of radioactivity into glucosamine and galactosamine (33).

 Autoradiography of Labeled Glycosaminoglycans—The \((^{3}S)\)sulfate-labeled intracellular proteoglycans and glycosaminoglycans formed in the presence and absence of \(\beta\)-xylose were purified by density gradient centrifugation. Dialyzed and lyophilized bottom fractions were dissolved in 0.5 ml of 0.05 M NaOH, 1.0 M NaBH₄, and heated at 45 °C for 48 h (34). After neutralization with 2 M acetic acid, the labeled \(\Delta D-4 S\)-glycosidically linked glycosaminoglycans were dialyzed for 6 h against 0.001 M sodium acetate. A 10 μl sample was applied to a thin layer chromatography plate containing 75 μl of isobutyric acid containing 0.46 M formic acid, pH 3.0 (35). The dried electrophoresis sheet was then sprayed with EN3HANCE and exposed to Kodak XR-5 film for 48 h at -80 °C.

**RESULTS**

The in vitro conditions developed by Handley et al. (23) and Kimura et al. (2) for cultured chondrocytes were found to be suitable for short term primary cultures of rat serumal mast cells. \((^{3}S)\)Sulfate incorporation into mast cell proteoglycan was dependent on the presence in the culture medium of fetal calf serum (Fig. 1), and its effect was heat-stable at 60 °C for 6 h. As assessed by quantitative PD-10 chromatography after 3 h in Medium A, approximately 10-15% of the total macromolecular proteoglycans were released into the culture medium and another 10-20% were displaced from the cell pellet by a 1 M NaCl wash. Treatment of the cell pellet with 4 M GdnHCl solubilized less than 15% of the cell-associated \((^{3}S)\)sulfate macromolecules, whereas a 60-s exposure to zwittergent before 4 M GdnHCl extraction liberated the intracellular mast cell radiolabeled macromolecules, and was routinely used rather than sonication in 1 M NaCl (3) or digestion with pronase (6).

The zwittergent/GdnHCl extraction procedure also permitted immediate density gradient centrifugation under dissociative conditions so as to remove endogenous mast cell proteases and glycosidases. More than 90% of the \((^{3}S)\)sulfate-labeled macromolecules and all of the chemically determined heparin
Mast Cell Proteoglycan Synthesis

Fig. 1. Incorporation of \( ^{35} \text{S} \) sulfate into macromolecules by rat serosal mast cells cultured in Medium A with (○) and without (□) heat-inactivated fetal calf serum; heat-inactivated fetal calf serum was routinely included in all subsequent cultures.

**TABLE I**

| Equilibrium density gradient purification of heparin proteoglycan, \( ^{35} \text{S} \) sulfate- and \( ^{3} \text{H} \) serine-labeled macromolecules in the mast cell extract fraction |
|---|
| Cells (10^6) were exposed to 200 µCi/ml of either \( ^{35} \text{S} \) sulfate (n = 3) or \( ^{3} \text{H} \) serine (n = 5). |
| Density gradient fraction | Heparin (carbazole) | \( ^{35} \text{S} \) sulfate macromolecules | \( ^{3} \text{H} \) serine macromolecules |
| D4 (top) | —" | 2,700 (±50) | 68,300 (±5500) |
| D3 | 0.7 (±0.7) | 1,400 (±40) | 21,000 (±1000) |
| D2 | 0.7 (±0.7) | 2,600 (±130) | 11,300 (±800) |
| D1 (bottom) | 18.4 (±1.4) | 85,000 (±3400) | 3,400 (±20) |

* The carbazole reaction of fraction D4 produced a nonspecific brown color, and only fraction D1 was positive for heparin by the Azure A test.

Fig. 2. Incorporation of \( ^{35} \text{S} \) sulfate into macromolecules of rat serosal mast cells cultured in Medium A without fetal calf serum (A), in Medium A (B), and in Medium A with increasing concentrations of \( \beta \)-D-xyloside (C-E). The peak proteoglycan-enriched fractions (fractions 25-37) were pooled separately from the peak glycosaminoglycan-enriched fractions (fractions 42-50).

When cultures were pulsed for 3-h time intervals, the rate of \( ^{35} \text{S} \) sulfate incorporation was linear up to approximately 20 h. The age and the size of rats within the range used in this study, 150-300 g, did not influence overall proteoglycan synthesis. Studies on mast cell proteoglycan biosynthesis were routinely conducted for less than 24 h and in the presence of heat-inactivated fetal calf serum; \( ^{35} \text{S} \) macromolecules were extracted with zwittergent and GdnHCl and were isolated by density gradient centrifugation.

**Macromolecular \( ^{35} \text{S} \) Sulfate Incorporation in Response to \( \beta \)-D-Xyloside Treatment**—Three sets of six mast cell cultures each were prepared in Medium A containing \( ^{35} \text{S} \) sulfate. Five members of each set received xyloside at a concentration ranging from 0.1 mM to 10 mM; the sets were then incubated for 3, 5, or 20 h. The presence of dimethyl sulfoxide in Medium A, at the concentrations used in \( \beta \)-D-xyloside-treated rat mast cell cultures, had no effect on \( ^{35} \text{S} \) sulfate incorporation. At a \( \beta \)-D-xyloside concentration of 0.1 mM, there was an insignificant increase (p > 0.1) of total \( ^{35} \text{S} \) sulfate incorporation (Fig. 2), whereas a progressive inhibition of incorporation occurred at higher \( \beta \)-D-xyloside concentrations. Inhibition was significant at 3 mM (p < 0.005) and at 10 mM \( \beta \)-D-xyloside, \( ^{35} \text{S} \) sulfate incorporation was suppressed by 90% (Fig. 2).
Mast Cell Proteoglycan Synthesis

In size to the \( M_t = 750,000-1,000,000 \) rat proteoglycan obtained by pronase treatment of rat skin (6) or by freeze-thawing and 1 M NaCl salt extraction of isolated rat mast cells (3) and less than 4% of the macromolecules filtered in the range of individual glycosaminoglycan chains. At a \( \beta\)-d-xylos ide concentration of 0.1 mM, the intracellular \( ^{35}S \) macromolecules were comparable in size to those produced by control cultures (Fig. 3C), and some radioactivity was shifted to a low molecular weight glycosaminoglycan fraction. In the presence of \( \beta\)-d-xylos ide concentrations which decrease total \( ^{35}S \) sulfate incorporation, mast cell proteoglycan was progressively smaller in size and the relative percentage of incorporated radioactivity in free glycosaminoglycans increased (Fig. 3, D and E).

Sepharose CL-4B chromatography was performed on the macromolecular \( ^{35}S \)-radioactivity in the culture medium, in the 1 M NaCl wash of cells, and in the detergent/GdnHCl extract of cells to compare the relative quantities of proteoglycan and free glycosaminoglycans released and retained by rat mast cell cultures when exposed to 1 mM \( \beta\)-d-xyloside. In the culture medium, the ratio of \( ^{35}S \) glycosaminoglycans to \( ^{35}S \) proteoglycans was approximately 4:1 as compared to the ratios of 1:4 and 1:2, respectively, for the pericellular and intracellular compartments (Fig. 4). However, about 85% of the total \( ^{35}S \) glycosaminoglycans after a 3-h pulse with radioactivity still remained cell-associated.

Mast cell cultures that were exposed 3 h to 3 mM \( \beta\)-d-xyloside and \( ^{35}S \) sulfate were chased for varying periods of time with Medium A to observe the relative loss of the intracellular pool of \( ^{35}S \) proteoglycan and the \( ^{35}S \) glycosa-
minoglycan polymerized onto the exogenous β-D-xyloside (Fig. 5). With increasing chase times of up to 2 h, the [35S]heparin proteoglycan synthesized by the cells not treated with xyloside did not change in molecular weight (Fig. 5, A–D). In β-D-xyloside-treated cultures there was a similarity of behavior of intracellular glycosaminoglycan and proteoglycan (Fig. 5, E–H), which contrasts to the short retention time of glycosaminoglycans in cell types such as chondrocytes (16, 17) and transformed fibroblasts (18), and suggests that the [35S]glycosaminoglycan did not originate in a cell contaminant which continuously secretes proteoglycan.

Characterization of Radiolabeled Proteoglycan and Glycosaminoglycan Fractions in Control and β-D-Xyloside-Treated Cultures—[35S]Sulfate-labeled intracellular macromolecules from mast cells cultured without and with 0.3 mM β-D-xyloside or 3 mM β-D-xyloside were separated into proteoglycan-enriched and glycosaminoglycan-enriched fractions by Sepharose CL-4B gel filtration and subjected to nitrous acid degradation. The [35S]proteoglycan fraction obtained from the cultures of control rat mast cells were degraded >96% (Fig. 6A) as indicated by conversion of macromolecular [35S]-radioactivity to oligosaccharides which eluted in the included volume from Sephadex G-25 columns. With the addition of 0.3 mM β-D-xyloside to Medium A, almost the entire [35S]proteoglycan fraction was again hydrolyzed by nitrous acid to smaller oligosaccharides (Fig. 6B). At a 3 mM β-D-xyloside concentration which further reduced the size and amount of proteoglycan produced, approximately 82% of the [35S]-macromolecular proteoglycan fraction was degraded (Fig. 6C) indicating that the cultures continued to incorporate [35S]sulfate into heparin chains which were polymerized onto the mast cell proteoglycan core. In contrast, the [35S]sulfate-labeled glycosaminoglycans appearing in the β-D-xyloside-treated cultures were more than 85% resistant to nitrous acid hydrolysis (Figs. 6, D and E). Under the conditions employed, approximately 90% of standard pig mucosa heparin carrier was hydrolyzed to oligosaccharides, while commercial chondroitin sulfate carrier was not susceptible to nitrous acid degradation.

When the proteoglycans isolated by density gradient centrifugation and Sepharose CL-4B filtration were incubated with chondroitinase ABC and the liberated chondroitin sulfate disaccharides analyzed by thin layer chromatography, there was insignificant degradation of proteoglycan from control or from 0.3 mM β-D-xyloside-treated cultures and only 10% degradation of the proteoglycan from 3 mM β-D-xyloside-treated cultures (Table II). On the other hand, the individual glycosaminoglycans induced by β-D-xyloside treatment were more than 70% digested, and the radioactivity comigrated with the ADi-6S and ADi-4S carriers. In three consecutive experiments with mast cells exposed to 0.1 or 0.3 mM β-D-xyloside, but not to 3 mM, the chondroitinase ABC-treated glycosaminoglycans contained hydrolysate products which chromatographed in the position of disulfated disaccharides. This material was not further characterized in terms of the number of sulfate residues per disaccharide or the presence of oligosaccharides resistant to chondroitinase ABC.

After a 3-h pulse with [3H]glucosamine and subsequent density gradient centrifugation, 62%, 49%, and 42% of the total radioactivity in the cell extract fractions of non-β-D-xyloside, 0.3 mM β-D-xyloside, and 3 mM β-D-xyloside-treated mast cells, respectively, were found in the high buoyant density macromolecules. As analyzed by Sepharose CL-4B chromatography, the only substantial difference of β-D-xyloside-treated mast cells exposed to [3H]glucosamine (Fig. 7) versus [35S]sulfate (Fig. 3) was a higher relative predominance of [3H]-radioactivity incorporated into the glycosaminoglycan fraction. Cetylpyridinium chloride precipitation of the density gradient fraction before Sepharose CL-4B chromatography did not change the chromatographic profile, indicating that the [3H]-radioactivity was not in glycoproteins. After 8 M HCl hydrolysis, approximately 85% of the total radioactivity in the proteoglycan fraction from the control cultures, or from those maintained in 0.3 mM β-D-xyloside, eluted from ion exchange chromatography with glucosamine carrier, whereas about 85% of the total radioactivity in the glycosaminoglycan fraction from 0.3 mM β-D-xyloside-treated mast cells chromatographed as [3H]galactosamine. Thus, the resistance to degradation by

![Fig. 7. Sepharose CL-4B gel filtration chromatography of [3H]glucosamine-labeled rat mast cell proteoglycan and glycosaminoglycans isolated by density gradient centrifugation of the cultures in Medium A alone (A) or with xyloside, 0.3 mM and 3 mM, respectively (B and C).](http://www.jbc.org/)

**Table II**

| Culture | Sepharose CL-4B Fraction | Distribution of [35S]Sulfate * | % |
|---------|--------------------------|-----------------------------|---|
| Medium A | Proteoglycan | 97.6 | 0.6 | 0.6 | 1.0 |
| Medium A with 0.3 mM xyloside | Proteoglycan | 99.2 | 0 | 0.3 | 0.6 |
| Medium A with 3 mM xyloside | Proteoglycan | 89.8 | 1.4 | 4.5 | 4.3 |
| Medium A | Glycosaminoglycan | 28.7 | 14.9 | 36.0 | 19.6 |
| Medium A with 0.3 mM xyloside | Glycosaminoglycan | 28.7 | 14.9 | 36.0 | 19.6 |
| Medium A with 3 mM xyloside | Glycosaminoglycan | 28.7 | 14.9 | 36.0 | 19.6 |

* A change of ±5% in the distribution of radioactivity was considered to be within the error of the procedure because there was some overlapping of proteoglycans and glycosaminoglycans on gel filtration (Figs. 3 and 7).

**ADi-DS** (disulfated disaccharide) refers to the position of the marker and does not necessarily mean that the hydrolysate product is a disaccharide.
nitrous acid and the susceptibility to chondroitinase ABC of the glycosaminoglycan-enriched fraction appearing after β-D-xyloside treatment was not due to an incomplete processing of heparin precursor chains, but to polymerization of chondroitin sulfate glycosaminoglycan onto the exogenous drug.

The total (35S)sulfate-labeled pools of macromolecules from drug- and nondonor-treated mast cell cultures were each purified by density gradient centrifugation and subjected to β-elimination. The glycosaminoglycans were separated by cellulose acetate electrophoresis and analyzed by autoradiography. Glycosaminoglycans from mast cells maintained in Medium A were polydispersed in negative charge and co-migrated with cosaminoglycans from mast cells maintained in 'Medium A drug- and nondrug-treated rat mast cells were each purified by density gradient centrifugation and subjected to β-elimination. The ratio was 0.1-1 mm concentration, with total suppression at 10 mM.

DISCUSSION

β-D-Xyloside, an acceptor for glycosaminoglycan polymerization, has previously been shown to uncouple glycosaminoglycan synthesis from proteoglycan core synthesis in chondrocyte and fibroblast cultures (1, 13-18) which normally continuously secrete proteoglycan. β-D-Xyloside at 0.1-1 mm concentrations reportedly augmented total (35S) sulfate incorporation by polymerizing chondroitin sulfate onto the drug acceptor without altering the size of the proteoglycan molecule. At higher concentrations (3-10 mM β-D-xyloside) (35S)sulfate incorporation was suppressed, proteoglycans were smaller in size due to the addition of fewer and smaller glycosaminoglycans to the core, and the relative amount of chondroitin sulfate polymerized onto the β-D-xyloside acceptor was increased.

Rat mast cells, which secrete stored proteoglycan only in response to specific activation (5), can be concentrated to >97% purity and contain more than 90% heparin proteoglycan of defined hydrodynamic size and peptide core composition (3, 6). The introduction of heat-inactivated fetal calf serum to cultures of rat mast cells accelerated the sulfation and formation of macromolecular heparin proteoglycan such that 3-h cultures (Figs. 1 and 3B), rather than 14-h cultures (3), were sufficient to yield labeled completed macromolecular products. Zwittergent detergent, GdnHCl, and protease inhibitors were used to liberate mast cell proteoglycan in order to avoid the necessity of adding exogenous proteases and to minimize the effects of endogenous proteases and glycosidases during cell disruption. In addition, liberated macromolecules were generally subjected to immediate density gradient centrifugation to remove proteases, glycosidases, and glycoproteins (Table 1).

β-D-Xyloside produced a dose-related suppression of total (35S)sulfate incorporation (medium and cell pellet) from 0.3 mM to 3 mM concentration, with total suppression at 10 mM (Fig. 3). There was no net release in cytoplasmic lactate dehydrogenase or in secretory granule β-hexosaminidase into the culture medium or apparent alteration in granule density as assessed by phase microscopy or metachromatic staining with up to 3 mM β-D-xyloside. Mast cell cultures exposed to 0.1 and 0.3 mM β-D-xyloside contained a cell-associatated proteoglycan with a hydrodynamic size and polydispersity comparable to that of the control cultures, but in addition formed radiolabeled macromolecules which filtered with the size of glycosaminoglycans (Fig. 3). With 1 and 3 mM β-D-xyloside there was a decrease in quantity and size of cell-associated radiolabeled proteoglycan and a relative increase in the quantity of radiolabeled glycosaminoglycan. When the effect of 1 mM β-D-xyloside was examined for the distribution of proteoglycan relative to glycosaminoglycan, the ratio was approximately 4:1 for cell-associated macromolecules (Fig. 4). Approximately 75% of the total macromolecules were intracellular and 15% were cell surface-associated as indicated by 1 mM NaCl salt elution before cell disruption. Under conditions of β-D-xyloside treatment, the mast cell glycosaminoglycans had an apparent one-half intracellular retention time of >2 h (Fig. 5), which is substantially longer than chondroitin sulfate polymerized onto proteoglycan or β-D-xyloside by chondrocytes. The lack of extracellular proteoglycan in β-D-xyloside-treated cultures suggests that the glycosaminoglycans attached to the drug are released because either they are not bound to cationic protein in a manner similar to heparin, or because they are not in the same subcellular compartment. Thus, β-D-xyloside treatment of normal rat mast cell cultures decreased (35S)sulfate incorporation into macromolecules, uncoupled glycosaminoglycan synthesis from proteoglycan formation, and caused the preferential and spontaneous release of glycosaminoglycans into the extracellular fluid.

That the glycosaminoglycan polymerized onto the β-D-xyloside acceptor is chondroitin sulfate, whereas that forming on proteoglycan is heparin, was established by chemical and biochemical analyses of separated macromolecules. The (35S) proteoglycan obtained from control cultures by extraction and gel filtration chromatography was more than 95% degraded by nitrous acid (Fig. 6), indicating a glycosaminoglycan composition containing the characteristic N-sulfated glucosamine monosaccharide of heparin and heparan sulfate (30). The proteoglycan synthesized in the presence of 0.3 and 3 mM β-D-xyloside was also heparin by this criterion. The small amount of proteoglycan apparently resistant to degradation by nitrous acid is attributed to a reduced hydrodynamic size, which results in incomplete chromatographic resolution from glycosaminoglycans. The glycosaminoglycans formed in the presence of either 0.3 mM or 3 mM β-D-xyloside were more than 85% resistant to nitrous acid hydrolysis, indicating that they were not heparin or heparan sulfate. Further evidence that the proteoglycan formed in the presence of 0.3 or even 3 mM β-D-xyloside contained heparin was derived from the electrophoretic mobility of its liberated glycosaminoglycans and from its resistance to degradation by chondroitinase ABC (Table II). In contrast, the glycosaminoglycans derived from the same β-D-xyloside-treated cultures were 70% degraded by chondroitinase ABC to form chondroitin-6-sulfate and chondroitin-4-sulfate disaccharides. That so much radioactivity was recovered in these individual disaccharide fractions indicated a predominant "all or none" polymerization of chondroitin sulfate onto the exogenous drug. When intracellular [3H]proteoglycans and [3H]glycosaminoglycans were purified by density gradient centrifugation, cetylpyridinium chloride precipitation, and Sepharose CL-4B chromatography (Fig. 7), 85% of the total radioactivity in the glycosaminoglycan fraction from 0.3 and 3.0 mM β-D-xyloside-treated mast cell cultures were identified as [3H]lactosamine. In contrast, 85% of the [3H]hexosamine in the proteoglycan fractions from all cultures was [3H]glucosamine; thus, heparin was polymerized onto proteoglycan, and chondroitin sulfate predominantly onto the β-D-xyloside acceptor.

The peptide sequence of serine-glycine at glycosaminoglycan initiation sites and the carbohydrate sequence of GlcUA → Gal(1,3) → Gal(1,3) → Gal(1,4) → Xyl(1,3) → serine have been reported to be identical for chondroitin sulfate and heparin-rich proteoglycans (10). That the proteoglycan core affects in some manner the choice for addition of the fifth monosaccharide in glycosaminoglycan biosynthesis, GlcNAc or GalNAc, is indicated by the present study in which rat serosal mast cells make heparin proteoglycan but polym-
erize chondroitin sulfate rather than heparin onto the competing β-D-xyloside acceptor. The dual capacity to generate both chondroitin sulfate and heparin glycosaminoglycan has long been recognized in mouse mastocytoma cells (36), and the balance is shifted in favor of chondroitin sulfate by β-D-xyloside (19). The finding that the normal rat mast cell consistently synthesizes heparin proteoglycan, despite a steric inhibition for the chondroitin sulfate polymerizing enzymes by the dense number of heparin initiation sites on the complex core than the serine-glycine copolymer, that cores is consistently synthesized heparin proteoglycan, despite a steric inhibition for the chondroitin sulfate polymerizing enzymes by the dense number of heparin initiation sites on the complex core than the serine-glycine copolymer, that cores is steric inhibition for the chondroitin sulfate polymerizing enzymes by the dense number of heparin initiation sites on the complex core than the serine-glycine copolymer, that cores.

**Acknowledgments**—We thank Alex Zabik and Judith Litvin for technical assistance, and Dr. David Swann, of Shriners Burns Institute, Boston, for hexosamine analyses.

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R L Stevens and K F Austen

*J. Biol. Chem.* 1982, 257:253-259.

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