Purification and Characterization of Novel Heparan Sulfate Proteoglycans Produced by Murine Erythroleukemia Cells in the Growing Phase

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Murine erythroleukemia cells (Friend erythroleukemia cells of a C-10-6 line) synthesized sulfated glycosaminoglycans consisting mainly of heparan sulfate (more than 95%) with a small amount of chondroitin 4-sulfate. The heparan sulfate occurred as proteoglycans, of which the cell-associated component was separated into urea-insoluble (UI) and urea-soluble (US) fractions. The UI proteoglycan consisted of a single homogeneous molecular species with an estimated $M_r$ of 360,000 (C(UI)PG), whereas the US component was composed of two subfractions: a homogeneous species with an $M_r$ of 280,000 (C(US)PGI) and a mixture of compounds with $M_r$ values of less than 80,000 (C(US)PGII), which were isolated in yields of about 110, 340, and 80 $\mu$g of hexuronate (HexUA), respectively, from 1.37 $g$ of an acetone powder prepared from $5.7 \times 10^8$ cells in the logarithmic phase of growth. The proteoglycan released into the medium (12 liters) was a single homogeneous species with an $M_r$ of 320,000 (MPG) which was purified to a yield of 500 $\mu$g of hexuronate. The major, cell-associated proteoglycan, C(UI)PGI, had very high contents of serine and glycine, accounting for approximately 80% of the total amino acids. This proteoglycan as well as the other two large proteoglycans, C(UI)PG and MPG, were highly resistant to degradation by various proteinases. These three proteoglycans, C(UI)PG, C(US)PGI, and MPG, had heparan sulfates with estimated $M_r$ values of 32,000, 27,000, and 30,000. On the other hand, the $M_r$ of the smaller proteoglycan, C(UI)PGII, was not significantly different before and after $\beta$-elimination, indicating that it contains only a small peptide, if any. The heparan sulfate of this proteoglycan consisted of smaller and heterogeneous molecular species with $M_r$ values of 26,000, 20,000, and 4,000. Digestion of these heparan sulfates with heparitinase I plus II resulted in almost complete depolymerization and gave six unsaturated disaccharides, $\Delta$HexUA-GlcNAc, $\Delta$HexUA-GlcNAc(6-SO$_4$), $\Delta$HexUA-GlcNSO$_4$, $\Delta$HexUA-GlcNSO$_3$ (6-SO$_4$), $\Delta$HexUA(2-SO$_4$)-GlcNSO$_3$, and $\Delta$HexUA(2-SO$_3$)-GlcNSO$_3$(6-SO$_3$). The relative amounts of these disaccharides generated from the individual heparan sulfates showed that an average ratio of sulfate residues to repeating disaccharide units of the C(UI)PGII-derived heparan sulfate (0.97) was significantly higher than those of the other three large proteoglycan-derived glycosaminoglycans (0.54–0.70).

Recent structural studies on proteoglycans of hemopoietic cells have demonstrated some common characteristics of their glycosaminoglycan and core peptide portions. The glycosaminoglycans of hemopoietic cells can be classified into three types on the basis of their degrees of sulfation. The most widespread type is fully sulfated chondroitin 4-sulfate (1–12). The second most common type is oversulfated chondroitin 4-sulfate (or dermatan sulfate) species with an additional sulfate residue at position 6 of their N-acetylgalactosamine residues, named chondroitin sulfate E, or at position 2 of their iduronic acid residues, named chondroitin sulfate Di-B (2, 13–16). The third type is the most highly sulfated glycosaminoglycan, heparin, which is the major glycosaminoglycan component of connective tissue mast cells (17–21). All these glycosaminoglycans are present as proteoglycans, and these proteoglycans have been demonstrated or supposed to exist in storage granules of these hemopoietic cells, forming complexes with various granule proteins, such as with platelet factor 4 (22, 23), basically charged exo- and endopeptidases (24), and lysosomal enzymes (25, 26). Nearly equimolar or excess sulfate groups per repeating disaccharide unit of these glycosaminoglycans are believed to be important for formation of complexes of the proteoglycans with the respective proteins (24).

Other common features of proteoglycans or their core peptides of hemopoietic cells are that they are highly resistant to various proteinases and that the $M_r$ values of their core peptides are relatively small (<20,000) (24). The property of proteinase resistance has been attributed to the highly acidic nature of glycosaminoglycans described above, the high degree of substitution of the glycosaminoglycan chains along the peptide cores, and the existence in these core peptides of clusters of serine-glycine repeats as glycosaminoglycan attachment domains (24). The last possibility is supported by the finding of such a cluster in a core peptide of heparin proteoglycan of connective tissue mast cells (18) and the recent findings of similar clusters in the amino acid sequences deduced from the cDNAs of core peptides of storage granule proteoglycans of other hemopoietic cells such as rat basophilic leukemia cells (27) and human promyelocytic leukemia cells of an HL-60 line (28).

The above findings prompted us to carry out qualitative and quantitative studies on the proteoglycans produced by cells of the erythroid lineage, which have not yet been examined, and to compare their structures with those of proteogly-
cans produced by cells of other hemopoietic lineages. For this purpose we chose murine erythroleukemia cells (Friend erythroleukemia). Friend erythroleukemia cells are virus-transformed cells that are believed to be blocked at a relatively early stage of erythroid differentiation, probably corresponding to the erythroblast colony-forming unit (29). Many cell lines have been established from Friend erythroleukemia cells, and some of them can be differentiated to produce hemoglobin with treatment with dimethyl sulfoxide (30), hexamethylenebisacetamide (31) or other reagents (29). In the present work, we treated cells that are believed to be blocked at a relatively early stage of erythroid differentiation, probably corresponding to the erythroblast colony-forming unit (29). Many cell lines have been established from Friend erythroleukemia cells, and some of them can be differentiated to produce hemoglobin with treatment with dimethyl sulfoxide (32). The results indicated that the proteoglycans synthesized by these cells consist mainly of heparan sulfate proteoglycans with unique biochemical characteristics differing from those of the proteoglycans produced by non-hemopoietic cells.

**EXPERIMENTAL PROCEDURES**

**Material—** Proteins from Chondroitin Sulfate and Hyaluronic Acid

Flavobacterium heparinum was grown on a medium containing complex proteins, such as bovine serum and cultured in suspension in a humidified incubator for 12 liters of culture medium was calculated to contain proteoglycans equivalent to 4.46 mg of hexuronate. For distinguishing proteoglycans produced by the cells from exogenous proteoglycans, a small scale culture (60 ml) was labeled with [35S]sulfate during the period of cell growth (Fig. 1), and then radiolabeled macromolecular fractions were combined with the unlabeled fractions, as shown in Fig. 2, and were used as tracers for purification of these fractions.

**Extraction and Fractionation of Proteoglycans—** As summarized in Table 1, cell cultures were separated into cells and media by centrifugation at 500 x g for 10 min. The 35S-labeled cells (3.0 x 10^7 cells) of these standard proteoglycans were determined by sedimentation-equilibrium centrifugation. Bio-Gel P-6 and Bio-Gel A-0.5m were purchased from Bio-Rad. Sepharose CL-4B and DEAE-Sephaloc were obtained from Pharmacia LKB Biotechnology Inc. The proteinase inhibitors used were N-ethylmaleimide from Nakarai Chemical Co. (Tokyo, Japan), phenylmethylsulfonyl fluoride from Sigma, and pepstatin A from the Protein Research Foundation (Osaka, Japan). [35S] Sulfate (carrier-free) was purchased from the Radiosotope Association (Tokyo, Japan).

**Fig. 1.** Growth of C-10-6 cells and their labeling with [35S] sulfate. Cells were inoculated at an initial density of 3 x 10^6 cells/ml of Ham's F-12 supplemented with 8% fetal bovine serum and cultured in suspension in a humidified incubator for 12 liters of culture medium was calculated to contain proteoglycans equivalent to 4.46 mg of hexuronate. For distinguishing proteoglycans produced by the cells from exogenous proteoglycans, a small scale culture (60 ml) was labeled with [35S]sulfate during the period of cell growth (Fig. 1), and then radiolabeled macromolecular fractions were combined with the unlabeled fractions, as shown in Fig. 2, and were used as tracers for purification of these fractions.

**Fig. 2.** Extraction and fractionation of macromolecules. GnHCl, guanidine HCl.
were washed three times with the medium without fetal bovine serum and the isotope and extracted with 15 ml of 4 M guanidine HCl, 50 mM Tris-HCl, pH 7.3, containing 10 mM EDTA, 10 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, and 0.006 mM pepstatin A as protease inhibitors for 24 h and brought to 7% TCA with continuous stirring. After centrifugation at 50,000 x g for 40 min, the residue was reextracted with 5 ml of the same buffer for another 12 h. Unlabeled cells (5.67 x 10^9 cells) were also washed with medium without fetal bovine serum. The cells were then suspended in 400 ml of chilled (−20 °C) acetone containing the protease inhibitors and stood for 2 h at −20 °C with occasional stirring. Insoluble materials were collected by centrifugation at 7,000 x g for 10 min. This treatment was repeated three times, and the defatted material was then dried at room temperature. The acetone powder (1.37 g) was extracted by suspension in 900 ml of extraction buffer in the same way as described for extraction of radiolabeled materials. The suspension was centrifuged, and the residue was reextracted with 200 ml of the same extraction buffer. The radiolabeled and unlabeled residues were combined, washed with cold water several times, suspended in 50 mM Tris-HCl, pH 8.0, and digested extensively with Pronase. The solubilized material collected by centrifugation is referred to as fraction GI. The radiolabeled and unlabeled extracts were combined and dialyzed against five changes of 4 liters of 4 M urea, 20 mM NaCl, 20 mM Tris-HCl, pH 7.3, on ice. The insoluble material appearing during dialysis was collected by centrifugation at 8,000 x g for 20 min, washed twice with dialyzing buffer, and dissolved in the extraction buffer. This fraction is referred to as fraction UI. The dialyze and the washing medium were combined, washed with cold water, and referred to as fraction US.

The 35S-labeled medium (60 ml) and washing medium (15 ml) were combined and applied to a column (2.6 x 90 cm) of Bio-Gel P-6 in 0.2 M NaCl, 50 mM Tris-HCl, pH 7.3, at 4 °C to remove radiolabeled low molecular materials. The unlabeled medium (12 liters) and the washing medium (500 ml) were combined, dialyzed against water in ice, and lyophilized. The freeze-dried material was dissolved in 2 liters of 0.2 M NaCl, 50 mM Tris-HCl, pH 7.3. This sample was combined with the 35S-labeled macromolecular fraction eluted in the void volume from the Bio-Gel P-6 column and referred to as fraction M.

Chromatographies—Column chromatographies of samples on gels and Sephadex were carried out as shown above. This fractionation was quantitatively repeated even with tracer levels of materials without a large amount of unlabeled materials. Thus, for examination of the overall molecular species of macromolecules incorporating [35S]sulfate, small aliquots of fractions UI, US, and M were analyzed chemically and enzymatically. When samples treated with 0.3 M NaOH in 1 M NaBH₄ were applied to a column of Sephadex G-50, their 35S radioactivity was eluted near the void volume of the column (data not shown). The samples thus obtained were digested with chondroitinase ABC and applied to the Sephadex G-50 column (Fig. 3). Fraction UI contained no chondroitinase-susceptible material (Fig. 3A), and fractions US and M contained materials corresponding to only 2% (Fig. 3C) and 5% (Fig. 3E), respectively, of the total 35S-labeled materials. Fractions containing depolymerized materials were combined, desalted, concentrated, and subjected to paper chromatography. Only one spot co-migrating with authentic ΔΔCOS-4S was detected in both samples (data not shown), indicating that the cells synthesized a small amount of chondroitin 4-sulfate. On specific deamination of the N-sulfate group with nitrous acid, the enzyme-resistant materials eluted near the void volume were completely depolymerized (Fig. 3, B, D, and F). Probing with the 35S-labeled materials were heparan sulfate- and/or heparin-like molecules. The 35S-labeled heparan sulfate-like material associated with cells could be separated into two fractions, UI and US, as shown above. This fractionation was quantitatively repeatable even with tracer levels of materials without a large amount of unlabeled materials. Thus, for examination of the

### RESULTS

**Cell Culture**—When cells of the C-10-6 cell line were inoculated at an initial density of 3 x 10^6 cells/ml and cultured in suspension in Ham’s F-12 medium supplemented with 8% fetal bovine serum, they grew with a doubling time of 16.5 h and reached a saturation density of 6-7 x 10^6 cells/ml on day 4 (Fig. 1).

**Extraction and Fractionation of Proteoglycans**—Macromolecules were extracted and fractionated as shown in Fig. 2. A sample of 5.67 x 10^9 cells yielded 1.37 g of acetonitrile. Almost all the radiolabeled and hexuronate-containing macromolecules associated with the cells could be extracted with extraction buffer. When the extract was dialyzed against 4 M urea, 20 mM NaCl, 20 mM Tris-HCl, pH 7.3, a visible amount of aggregates was precipitated. This material was readily soluble in the extraction buffer. The macromolecules produced by the cells were separated into four fractions, GI, UI, US, and M. The amounts of radioactivity and hexuronate recovered in these four fractions are shown in Table I. Fraction GI did not contain a significant amount of radioactivity or hexuronate and was not studied further. Fraction M contained a large amount of proteoglycans, derived from fetal bovine serum, so the radioactivity of 35S relative to the hexuronate content was low.

For examination of the overall molecular species of macromolecules incorporating [35S]sulfate, small aliquots of fractions UI, US, and M were analyzed chemically and enzymatically. When samples treated with 0.3 M NaOH in 1 M NaBH₄ were applied to a column of Sephadex G-50, their 35S radioactivity was eluted near the void volume of the column (data not shown). The samples thus obtained were digested with chondroitinase ABC and applied to the Sephadex G-50 column (Fig. 3). Fraction UI contained no chondroitinase-susceptible material (Fig. 3A), and fractions US and M contained materials corresponding to only 2% (Fig. 3C) and 5% (Fig. 3E), respectively, of the total 35S-labeled materials. Fractions containing depolymerized materials were combined, desalted, concentrated, and subjected to paper chromatography. Only one spot co-migrating with authentic ΔΔCOS-4S was detected in both samples (data not shown), indicating that the cells synthesized a small amount of chondroitin 4-sulfate. On specific deamination of the N-sulfate group with nitrous acid, the enzyme-resistant materials eluted near the void volume were completely depolymerized (Fig. 3, B, D, and F), indicating that all the 35S-labeled materials were heparan sulfate- and/or heparin-like molecules.

The 35S-labeled heparan sulfate-like material associated with cells could be separated into two fractions, UI and US, as shown above. This fractionation was quantitatively repeatable even with tracer levels of materials without a large amount of unlabeled materials. Thus, for examination of the

**TABLE I**

| Fraction | 35S | Hexuronate | 35S/hexuronate |
|----------|-----|------------|---------------|
| GI       | 3670| 53.0       | 1406          | 18.1 | 2610 |
| UI       | 468 | 6.8        | 1188          | 15.5 | 3970 |
| US       | 3192| 46.1       | 1288          | 16.6 | 2480 |
| Medium M | 3251| 47.0       | 6370          | 81.9 | 510  |
| Total    | 3810| 53.0       | 1288          | 18.1 | 2610 |

**ND**, not detected.
Proteoglycans of Murine Erythroleukemia Cells

Fig. 3. Treatments with chondroitinase ABC and nitrous acid of radiolabeled glycosaminoglycans of fractions UI, US, and M. A small aliquot (1 × 10⁶ cpm) of each fraction was treated with 0.3 M NaOH in 1 M NaBH₄, overnight at room temperature, neutralized with acetic acid, and applied to a column (1 × 50 cm) of Sephadex G-50 in 1 M ammonium acetate, pH 5.5. Released glycosaminoglycans eluted near the void volume were digested with chondroitinase ABC. The digests of samples derived from fractions UI, US, and M were applied to the same column; their elution profiles are shown in panels A, C, and E, respectively. Chondroitinase-resistant glycosaminoglycans, indicated by bars, were then treated with nitrous acid. The treated samples from fraction UI, US, and M were applied to the same column; their elution profiles are shown in panels B, D, and F, respectively. Arrows show the void volume and the total volume of the column.

Fig. 4. Digestion with heparitinase, heparinase, and a combination of both the enzymes of the chondroitinase-resistant radiolabeled glycosaminoglycans of the three fractions. Aliquots (1 × 10⁶ cpm) of the chondroitinase-resistant glycosaminoglycans of fractions UI (panels A, B, and C), US (panels D, E, and F), and M (panels G, H, and I) were digested with heparitinase (panels A, D, and G), heparinase (panels B, E, and H), and heparitinase plus heparinase (panels C, F, and I) and applied to a column of Sephadex G-50 under the same conditions for Fig. 3. Arrows show the void volume and the total volume of the column.

Fig. 5. Ion-exchange chromatography on DEAE-Sephacel of fractions US and M. Fraction US (533 mg of protein/1.2 liters) was applied to a column (2.5 × 27.5 cm) of DEAE-Sephacel equilibrated with 4 M urea, 20 mM NaCl, 50 mM Tris-HCl, pH 7.5. The column was washed with 5 column volumes of the equilibrating buffer. Bound materials were then eluted with 2 liters of a linear gradient of 20 mM−1 M NaCl in the urea solution. The flow rate was 44 ml/h, and fractions of 10 ml were collected (A). Fraction M (54 g of protein/2 liters) was applied to a column (2.5 × 44 cm) of DEAE-Sephacel equilibrated with 0.2 M NaCl, 50 mM Tris-HCl, pH 7.3. The column was washed with 5 column volumes of the equilibrating buffer, and then bound materials were eluted with 2 liters of a linear gradient of 0.2−1 M NaCl in 50 mM Tris-HCl, pH 7.3. The flow rate was 80 ml/h, and fractions of 13.4 ml were collected (B). The absorbance at 280 nm (○—○), ³⁵S radioactivity (□—□), and hexuronate content (□—□) were monitored. The fractions shown by bars were pooled for further purification.
proteoglycans were heterogeneous not only in charge density being eluted in fraction M-e. (iii) A relatively larger amount eluted in fraction M-a (Fig. 4-sulfated repeating disaccharide, 1.76), proteoglycan being eluted in fraction M-b, and fully sulfated chondroitin sulfate an intermediate extent of sulfate (ratio of nonsulfated to 4-sulfated chondroitin sulfate as a minor one, proteoglycan summarized. Notable features of these proteoglycans (38) of 35S-labeled- and hexuronate-containing materials from culture in fetal bovine serum (38), as seen from the elution patterns of the proteoglycans with low sulfated chondroitin sulfate was developed with guanidine HCl solution. The flow rate was 18.3 ml/h, and fractions of 6.0 ml were collected. The absorbance at 280 nm (O–O), 35S radioactivity (O–O), and hexuronate content (O–O) were measured. The elution profiles of samples derived from fractions U1, C(US)-b, and M-b are shown in panels A, B, and C, respectively. The fractions shown by bars were pooled for further purification. Large arrows show the void volume and the total volume of the column. The inset in panel A shows the calibration curves obtained by plotting KD values (abscissa) versus log M, (ordinate) of standard molecules, pullulans (P-400, P-200, P-100, and P-50 with M, values of 350,000, 196,000, 100,200, and 48,000, respectively, which were used for confirming the linearity of a calibration curve in the high molecular weight region), and proteoglycans isolated from rat mesangial cells (1), human platelets (2) and rabbit bone marrow (3) with M, values of 230,000, 136,000, and 46,000, respectively. The positions of the radioactive peaks of the respective samples are shown by small arrows a, b, and c.

Large amounts of proteoglycans were introduced into the culture in fetal bovine serum (38), as seen from the elution profiles of hexuronate-containing materials in Fig. 5B, so the characteristics of these exogenous proteoglycans should be summarized. Notable features of these proteoglycans (38) were as follows. (i) Their glycosaminoglycan consisted mainly of chondroitin sulfate with no detectable amount of heparan sulfate. (ii) The chondroitin sulfate consisted of three molecular species that differed in their extents of sulfation, i.e. low sulfated chondroitin 4-sulfate (average ratio of nonsulfated to 4-sulfated repeating disaccharide, 1.76), proteoglycan being eluted in fraction M-a (Fig. 5B); chondroitin 4-sulfate with an intermediate extent of sulfation (ratio of nonsulfated to 4-sulfated repeating disaccharide, 1.32), proteoglycan being eluted in fraction M-b, and fully sulfated chondroitin sulfate consisting of chondroitin 4-sulfate as a major glycosaminoglycan and chondroitin 6-sulfate as a minor one, proteoglycan being eluted in fraction M-c. (iii) A relatively larger amount of proteoglycan with low sulfated chondroitin sulfate was recovered from cultured cells, as seen in Table II. (iv) These proteoglycans were heterogeneous not only in charge density but also in their M, values and molecular densities, as shown during the respective purification steps.

Fraction U1 and the radiolabeled main fractions, C(US)-b and M-b, from DEAE-Sephadex were applied to a column of Sepharose CL-4B in 4 M guanidine HCl, 50 mM Tris-HCl, pH 7.3 (Fig. 6). The 35S-labeled material in fraction U1 was eluted from the column as a single peak at KD 0.35 (referred to as C(US)PG) (Fig. 6). The hexuronate content was not monitored during this chromatography to save material. The radiolabeled material as well as that containing hexuronate in the C(US)-b sample was separated into two fractions, C(US)PG1, eluted as a sharp, symmetrical peak at KD 0.40, and C(US)PGII eluted as a broad peak between KD 0.60 and 0.86 (Fig. 6B). The main radioactive peak (KD 0.64) of C(US)PGII was shifted significantly from the main hexuronate peak (KD 0.72), indicating that this fraction was composed of heterogeneous molecules differing in their ratios of radioactivity of 35S to hexuronate. About 68% of the radioactivity and 81% of the hexuronate in the C(US)-b sample were recovered in fraction C(US)PGI and the rest in fraction C(US)PGII. The radiolabeled material in fraction M was eluted as a single sharp peak at KD 0.37 (referred to as MPG) whereas the material containing hexuronate was eluted as two peaks, one

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**TABLE II**

Yields and recoveries of 35S radioactivity and hexuronate in fractions eluted from a DEAE-Sephaloc column and their specific radioactivities

| Fraction | cpmp × 10^6 | % | ug | % | cpmp/ug |
|----------|--------------|---|----|---|--------|
| US^a     | 3192         | 100| 1288| 100| 2480   |
| C(US)-a  | 289          | 9  | 318 | 25 | 910    |
| C(US)-b  | 1840         | 58 | 528 | 41 | 3480   |
| C(US)-c  | 200          | 6  | 95  | 7  | 2110   |
| Sum      | 2232         | 73 | 941 | 73 |    |
| Medium^* | 3251         | 100| 6370| 100| 510    |
| M-a      | 44           | 1  | 1145| 18 | 40     |
| M-b      | 2290         | 70 | 1409| 23 | 1440   |
| M-c      | 286          | 9  | 2421| 38 | 120    |
| Sum      | 2620         | 81 | 5155| 81 |    |

^a Amounts of radioactivity and hexuronate applied to the column.
of which, accounting for 62% of the applied hexuronate, was eluted as a sharp peak in a slightly different position from the radioactive peak, and with a hexuronate-positive shoulder (Fig. 6C), suggesting some contamination with a fetal bovine serum-derived proteoglycan. The other material without radioactivity, which was thus derived from fetal bovine serum, was eluted as a somewhat broad peak in retarded fractions (tubes 64–77). From a calibration curve with standard proteoglycans (Fig. 6A, inset), the $M_r$ values of the radiolabeled proteoglycans with $K_D$ values of 0.35 (C(UI)PG), 0.40 (C(US)PGI), and 0.60–0.86 (C(US)PGII) from the cells and the proteoglycan from the medium with a $K_D$ of 0.37 (MPG) were estimated to be 360,000, 280,000, less than 90,000, and 320,000, respectively. The smallest proteoglycan was found only in cells but not in the medium.

Since the four fractions from Sepharose CL-4B were contaminated with a considerable amount of protein as indicated by the elution profiles of absorbance at 280 nm, they were subjected to CsCl density gradient centrifugation in 4 M guanidine HCl solution to remove protein (Fig. 7). The $^{35}$S-labeled proteoglycans in the three samples co-sedimented with hexuronate-containing materials to the bottom three fractions with densities of more than 1.53 g/ml, free from most contaminating proteins, which were recovered in the top three fractions (Fig. 7, A–C). Nucleic acid in the samples, with a buoyant density of 1.38–1.50 g/ml, was also separated from the bottom fractions. Most of the radiolabeled materials in the MPG sample were collected in the bottom three fractions and completely separated from protein (Fig. 7D). The hexuronate-containing materials in this sample were, however, distributed in the bottom, middle, and top fractions, the latter two being serum-derived chondroitin sulfate proteoglycans. As the bottom three fractions of this sample were considered still to be contaminated with a significant amount of serum-derived proteoglycans, they were purified further. For this, these bottom fractions of the MPG sample were pooled and digested with proteinase-free chondroitinase ABC. On Sepharose CL-4B (Fig. 8A), 97% of the radioactivity and 87% of the hexuronate of the digest were eluted from the main proteoglycan fraction. The radioactivity, hexuronate, and protein in the sample were monitored. The fractions shown by a bar in panel A were dialyzed against water and lyophilized. The residue was dissolved in 20 mM NaCl, 50 mM Tris-HCl, pH 7.3, and applied to a column (1.1 x 116 cm) of Sepharose CL-4B (Fig. 8B). The column was washed with 7 column volumes of the equilibrating buffer, and materials were eluted with 300 ml of a linear gradient of 20 mM–1 M NaCl in 50 mM Tris-HCl, pH 7.3. The flow rate was 3.5 ml/h, and fractions of 1 ml were collected. The absorbance at 280 nm ( ), hexuronate ( ), and absorbance at 235 nm ( ) were monitored. The fractions shown by a bar in panel B were pooled for further study.

$^{35}$S-labeled chondroitin sulfate proteoglycans (3%) and serum-derived chondroitin sulfates, and proteoglycans (about 10% of the hexuronate-containing materials in this sample) were removed from the main proteoglycan fraction. The main fraction was then subjected to DEAE-Sephacel chromatography (Fig. 8B). The radioactivity, hexuronate, and protein in the sample were co-eluted, indicating that the proteoglycan was reasonably purified. Thus we concluded that these four molecular species of heparan sulfate proteoglycans, C(UI)PG, C(US)PGI, C(US)PGII, and MPG, were purified to almost homogeneity.

Characterization of the Purified Heparan Sulfate Proteoglycans—The recoveries and chemical compositions of the heparan sulfate proteoglycans, C(UI)PG, C(US)PGI, C(US)PGII, and MPG, were determined (Table III). About 80% of the radioactivity in fraction UI was recovered in fraction C(UI)PG whereas 36 and 14% of those in fraction US were recovered in fractions C(US)PGI and C(US)PGII, respectively, and 57% of that in fraction M was recovered in fraction MPG. Moreover, 94, 26, 6, and 8% of the hexuronate in individual original fractions were recovered in fractions C(UI)PG, C(US)PGI, C(US)PGII, and MPG, respectively. The ratios of radioactivity of $^{35}$S to hexuronate content were significantly different in these four proteoglycans. Glucosamine was the only hexosamine in all the cell-derived proteoglycan samples. On the other hand, the MPG sample contained a significant amount of galactosamine (about 6% of the total hexosamine). In all these samples, the molar ratio of glucosamine to hexuronate was approximately 1, indicating
that these glucosamines were derived from glycosaminoglycan chains of these purified proteoglycans. This result also indicated that the glycosaminoglycan side chains of all the purified proteoglycans were exclusively heparan sulfates. The amino acid composition of the C(US)PGI proteoglycan, which could be obtained in relatively large amount, was analyzed after hydrolysis of an aliquot with 6 M HCl (Table IV). Serine and glycine accounted for 80% of the total amino acids.

The C(US)PGI proteoglycan had a very unbalanced amino acid composition and its susceptibilities to four proteinases, Pronase, trypsin, papain, and pepsin, which are often used to test the susceptibilities of various proteoglycans to proteinases, were examined and compared with those of the two other large proteoglycans, C(UI)PG and MPG. Small aliquots of the purified samples were digested with these proteoglycans, the digests were chromatographed on Sepharose CL-4B, and the radioactivities of the eluates were monitored (Fig. 9). Chondroitin sulfate proteoglycan H from chick embryo cartilage (50) was run as a positive control that is susceptible to these proteinases. The elution profiles of 35S-labeled C(US)PGII proteoglycan containing chondroitin sulfate Di-B and heparin from rat basophilic leukemia cells (18) were released by p-elimination, and aliquots (1 pg of hexuronate) of the released samples were subjected to two-dimensional gel electrophoresis (Fig. 10), indicating that this proteoglycan has only a small peptide, if any.

For characterization of glycosaminoglycan moieties of the purified proteoglycans, the glycosaminoglycan side chains were released by β-elimination, and aliquots (1 μg of hexuronate) of the released samples were subjected to two-dimensional gel electrophoresis (Fig. 10), indicating that this proteoglycan has only a small peptide, if any.

![Fig. 9. Susceptibilities of the purified proteoglycans to various proteinases.](image)

**Table III**

| Fraction | 35S | Hexuronic | Glucosamine | Galactosamine | Protein | 35S/hexuronic |
|----------|-----|-----------|-------------|---------------|---------|--------------|
| C(UI)PG  | 455 | 111       | 107         | ND*           | 17.2    | 4100         |
| C(US)PGI | 1122| 336       | 305         | ND            | 11.3    | 3340         |
| C(US)PGII| 449 | 80        | 71          | ND            | 17.2    | 5610         |
| MPG      | 1824| 497       | 445         | 354           | 53.2    | 3670         |

* Determined by the method of Lowry et al. (43).
* Not detected.

**Table IV**

Comparison of amino acid composition of the purified proteoglycans C(UI)PGI with those of heparin proteoglycan of rat serosal mast cells (18), chondroitin sulfate E proteoglycan of mast cells from mouse bone marrow (49), and hybrid proteoglycan containing chondroitin sulfate Di-B and heparin from rat basophilic leukemia cells (16)

| Residue | C(UI)PGI | Heparin proteoglycan | Chondroitin sulfate E proteoglycan | Hybrid proteoglycan |
|---------|----------|----------------------|-------------------------------------|---------------------|
| Asx     | —        | 35                   | 50                                  | 48                  |
| Thr     | 10.9     | (0)                  | 12                                  | 27                  |
| Ser     | 607.1    | (115)                | 394                                 | 175                 | 225 |
| Glx     | —        | (0)                  | 24                                  | 105                 | 66  |
| Pro     | 10.2     | (2)                  | 20                                  | 14                  | —   |
| Gly     | 193.8    | (37)                 | 433                                 | 446                 | 354 |
| Ala     | 12.1     | (2)                  | 16                                  | 62                  | 95  |
| Val     | 13.1     | (2)                  | 20                                  | 22                  | 24  |
| Met     | 9.7      | (2)                  | —                                   | —                   | —   |
| Ile     | 28.2     | (6)                  | —                                   | 14                  | 13  |
| Leu     | 14.8     | (3)                  | —                                   | 44                  | 33  |
| Tyr     | 26.3     | (5)                  | —                                   | —                   | —   |
| Phe     | 9.5      | (2)                  | —                                   | —                   | —   |
| Lys     | 8.9      | (2)                  | 12                                  | 10                  | 25  |
| His     | 55.4     | (10)                 | 20                                  | 20                  | 18  |
| Arg     | —        | (0)                  | 20                                  | 10                  | 18  |

* No correction was made for the destruction of amino acids during HCl hydrolysis. Values in parentheses are nearest integers. Numbers of amino acid residues are calculated on the basis of the Mr, (280,000) of the C(UI)PGI proteoglycan.
* Not detected.
electrophoresis on a cellulose-acetate membrane (Fig. 11). Alcian blue-reactive materials in all the samples co-migrated with $^{35}$S-labeled materials. The glycosaminoglycans derived from the three proteoglycans, C(UI)PG, C(US)PGI, and MPG, gave single compact spots without any tailing in a position coinciding with that of bovine kidney heparan sulfate used as an external reference, indicating that these glycosaminoglycans were heparan sulfates with very homogeneous molecular sizes and charge densities. On the contrary, the heparan sulfate of the C(US)PGII proteoglycan migrated as a single but rather broad spot with tailing. The glycosaminoglycans in the tailing portion seemed to have higher specific radioactivity than that of the portion in the head of the spot, staining strongly with Alcian blue, suggesting that the heparan sulfate was heterogeneous not only in molecular size but also in charge density. These results were consistent with those obtained by gel filtration of the heparan sulfates on Bio-Gel A-0.5m and by HPLC analysis of their disaccharide units as described below.

The heparan sulfates from proteoglycans C(UI)PG, C(US)PGI, and MPG were eluted from a Bio-Gel A-0.5m column as sharp symmetrical peaks of both radioactivity and hexuronic at $K_D$ values of 0.22, 0.26, and 0.23, respectively (Fig. 12, A, B, and D, respectively), indicating that the chain sizes of these heparan sulfates were all very homogeneous. From a calibration curve (inset in Fig. 12A), their apparent $M_r$ values were estimated to be 32,000, 27,000, and 30,000, respectively. On the contrary, the heparan sulfate of the small proteoglycan C(US)PGII was distributed over the wide $M_r$ range and separated into three peaks with $K_D$ values of 0.27, 0.42, and 0.63 (estimated $M_r$ values of 26,000, 11,000, and 4,000, respectively; Fig. 12C). Moreover, the ratios of radioactivity of $^{35}$S to the hexuronic content of these three glycosaminoglycans appeared to differ as suggested by the results of electrophoresis. These results showed that the heparan sulfate of the C(US)PGII proteoglycan was composed of components with heterogeneous sizes and charge densities.

For characterization of repeating disaccharide units consisting of heparan sulfate chains, the heparan sulfate samples were digested with a combination of heparitinases I and II, and the degrees of depolymerization of the four heparan sulfates in the digests were estimated by HPLC on a TSK-gel column with monitoring of the refractive index (33). Results showed that all the heparan sulfates were almost completely converted to unsaturated disaccharides (data not shown). The digests were then subjected to HPLC on an amine-bound silica column with a linear gradient of 16 mM to 800 mM NaH$_2$PO$_4$ (Fig. 13). Almost all the disaccharides (86–94% of the individual samples) were eluted with the same retention times as those of the six authentic disaccharides (Fig. 13E). The remaining minor unidentified compounds, probably with unsaturated hexuronic acid at their nonreducing ends, which were released significantly from the C(US)PGII sample, were eluted as four unidentified peaks, $X_1$–$X_4$ (Fig. 13C), and some of these compounds were also released from the other samples (Fig. 13, A, B, and D). The molar amounts of the six disaccharides of the individual samples identified were calculated to be shown in Table V. A nonsulfated disaccharide was the major component (43–64%) of all four samples, and $\Delta$DiHS-NS was the second largest component (13–17%). The content of $\Delta$DiHS-triS in the C(US)PGII-derived sample was significantly higher than those of the other three samples, as indicated by the HPLC profiles. The separation of unsaturated disaccharides with the same number of sulfate groups was not complete, and fractions containing these disaccharides were pooled as shown in
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Fig. 12. Gel chromatography on Bio-Gel A-0.5m of heparan sulfates released from the purified proteoglycans. The purified heparan sulfate proteoglycans were treated with 0.3 M NaOH in 1 M sodium borohydride overnight at room temperature, and the reaction mixtures were neutralized with acetic acid. The samples were applied to a column (1 x 116 cm) of Bio-Gel A-0.5m and eluted with 1 M NaCl, 50 mM Tris-HCl, pH 7.3. The flow rate was 3.5 ml/h, and fractions of 1 ml were collected. The 35S radioactivity (○) and hexurionate content (●) were monitored. Elution profiles A, B, C, and D are for the heparan sulfates released from the proteoglycans C(UI)PG, C(US)PGI, C(US)PGII, and MPG, respectively. Arrows show the void volume and the total volume of the column. The inset in A shows a calibration curve obtained by plotting Kd values versus log M, values of standard proteoglycans and glycosaminoglycans. Arrows 1, 2, and 3 show the positions of chondroitin 6-sulfate proteoglycans of rabbit bone marrow with M, values of 46,000, 16,000, and 8,300, respectively, and arrows 1'-3' show those of chondroitin 6-sulfates with M, values of 14,400, 10,900, and 7,700, respectively.

Fig. 13, and the radioactivities of the pooled fractions were determined (Table VI). The recoveries of the radioactivities of the C(UI)PG, C(US)PGI, C(US)PGII, and MPG-derived samples from the column were 88, 90, 83, and 91%, respectively. The radioactivities of 35S/sulfate residue calculated for the contents of sulfated disaccharides were not greatly different, the mean value being 1,100 ± 110 cpm/nmol of sulfate.

Fig. 13. HPLC of digests of heparan sulfates with a combination of heparitinases I and II. The heparan sulfates of the purified proteoglycans were digested with a combination of heparitinases I and II, and the digests were analyzed by HPLC. The elution profiles of digests of the heparan sulfates derived from the proteoglycans C(UI)PG, C(US)PGI, C(US)PGII, and MPG are shown in panels A, B, C, and D, respectively. The retention times of the respective disaccharides were determined previously using authentic disaccharides (E). Letters 1-6 represent elution of ADHS-OS, ADHS-S, ADHS-N, ADHS-diS0, ADHS-diS1, and ADHS-triS, respectively. Fractions indicated by bars were pooled for determination of the radioactivities.

Discussion

The present study demonstrated that Friend erythroleukemia cells of a C-10-6 cell line, which can be differentiated to produce hemoglobin by dimethyl sulfoxide treatment (32), synthesized sulfated glycosaminoglycans consisting mainly of heparan sulfate (more than 95%) with a small amount of chondroitin 4-sulfate. Examination of other cell lines of Friend erythroleukemia cells including the dimethyl sulfoxide- and hexamethylenebisacetamide-sensitive cell lines, TSFAT-3 (32) and DS19SC4(51), and the insensitive cell lines, C-9-9 (32) and DR10 (52), showed that all these cell lines synthesized these two types of sulfated glycosaminoglycans although the relative ratios of the two types varied significantly in different lines of cells (data not shown). Thus, regardless of their difference in sensitivity to chemical inducers, Friend erythroleukemia cells clearly synthesized heparan sulfate as a major sulfated glycosaminoglycan and chondroitin sulfate as a minor component.

The heparan sulfate produced by C-10-6 cells occurred as proteoglycans. During the process of isolation of the proteoglycans, the cell-associated component could be separated into
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Analyses of unsaturated disaccharides produced by digestion of heparan sulfates derived from the four proteoglycans with a combination of heparitinases I and II

Values were determined from the areas recorded.

| Sample | C(U1)PG | C(US)PGI | C(US)PGII | MPG |
|--------|---------|----------|-----------|-----|
|        | nmol    | %        | nmol      | %   |
| Applied|         |          |           |     |
| Recovered|       |          |           |     |
| ΔDiHS-Os| 20.6    | 53.9     | 30.0      | 63.4|
| ΔDiHS-BS| 0.5     | 1.3      | 0.7       | 1.5 |
| ΔDiHS-NS| 6.4     | 16.8     | 7.3       | 15.4|
| ΔDiHS-disS| 0.5     | 1.3      | 0.6       | 1.3 |
| ΔDiHS-disS| 2.4     | 6.3      | 2.4       | 5.1 |
| ΔDiHS-tris| 3.7     | 9.7      | 3.5       | 7.4 |
| Sum    | 34.0    | 89.5     | 44.5      | 94.0|

TABLE VI

Recovery of {superscript}35S radioactivity co-eluted with the identified unsaturated disaccharides generated from the heparan sulfates by digestion with a combination of heparitinases I and II and ratio of radioactivity of {superscript}35S to the disaccharide content

| Sample | C(U1)PG-HS | C(US)PGI-HS | C(US)PGII-HS | MPG-HS |
|--------|------------|-------------|--------------|--------|
|        | cpm        | %           | cpm/nmol     | cpm    |
| Applied| 30,400     | 100         | 800          | 30,700 |
| ΔDiHS-nonS| 300     | 1.0         | 1.0          | 10     |
| ΔDiHS-monoS| 8,270  | 27.2        | 1,200        | 9,550  |
| ΔDiHS-diS| 6,200     | 20.4        | 2,140        | 6,060  |
| ΔDiHS-triS| 11,980 | 39.4        | 3,240        | 11,410 |
| Sum    | 26,750    | 88.0        | 790          | 27,430 |

TABLE VII

Chemical composition of the major cell-associated proteoglycan C(US)PGI

| Component | % |
|-----------|---|
| Hexurionate| 39 |
| Glucosamine| 35 |
| Galactosamin| ND* |
| Acetate | 8 |
| Sulfate | 11 |
| Protein | 7 |

*Not detected.

urea-insoluble (UI) and urea-soluble (US) fractions. This separation was quantitatively repeatable even with tracer levels of material without addition of a large amount of unlabeled material. The heparan sulfate of the UI proteoglycan differed from that of the US component in susceptibilities to heparitinase I, heparitinase II, and a combination of the two enzymes. This finding, together with the facts that the C(U1)PG proteoglycan in the crude fraction readily redissolved in 4 M guanidine HCl and also after purification readily dissolved in 4 M urea solution, suggests strongly that this proteoglycan is associated, probably by specific interaction of its heparan sulfate chains, with some material(s) in low salt solution and forms an aggregate(s).

On the basis of the ratios of the radioactivity of {superscript}35S to hexurionate content of the purified proteoglycans, the amounts of the four heparan sulfate proteoglycans, C(U1)PG, C(US)PGI, C(US)PGII, and MPG, present in cultures of 5.7 x 10⁶ cells/12 liter were calculated to be 114, 597, 191, and 841 μg of hexurionate, respectively. The three former values represent the pool sizes of the respective proteoglycans in cells in the logarithmic growth phase whereas the fourth represents the amount of the proteoglycan secreted and accumulated in the medium during the period of cell growth (80 h) because no further metabolism of MPG proteoglycan was observed in the present culture system. The content of total cell-associated heparan sulfates in C-10-6 cells (16 μg of hexurionate/10⁶ cells) is comparable to those of sulfated glycosaminoglycans in other hemopoietic cells such as rat basophilic leukemia cells (21.7 μg of hexurionate/10⁶ cells) (53) and mouse bone marrow-derived mast cells (32-48 μg of hexurionate/10⁶ cells) (15) but much lower than that of connective tissue mast cells (1,800 μg of hexurionate/10⁶ cells) (17). In comparison with the amount of protein in other glycoconjugates of cells of the erythroid lineage, the content of the C(US)PGI proteoglycan in C-10-6 cells (1.9 μg of core protein/10⁶ cells, calculated from the data shown in Table VII), is comparable to that of glycophorin A in human erythrocytes (3.5 μg of glycophorin A protein/10⁶ cells) or K-562 cells (3.9 μg of glycophorin A protein/10⁶ cells) (54).

The C(US)PGI proteoglycan was demonstrated to have a characteristic amino acid composition with very high contents of serine and glycine and to be highly resistant to treatments with various proteases. The core peptide was calculated to be composed of 189 amino acids with an M₉ of about 17,000. Core peptides having such unbalanced amino acid compositions and small M₉ values have been found in heparin proteoglycan of rat serosal mast cells (17-19), chondroitin sulfate E proteoglycan of mouse bone marrow-derived mast cells (49), a hybrid type proteoglycan with chondroitin sulfate Di-B and heparin-like glycosaminoglycan of rat basophilic leukemia cells (16), and other proteoglycans in several peripheral blood cells (55-57). These proteoglycans are present in storage granules of these hemopoietic cells and are highly resistant to degradation by a number of proteases. The latter property is attributed to a high degree of sulfation (or an acidic nature) of their glycosaminoglycans, a high degree of substitution of these glycosaminoglycans along the core peptides, and the existence in their core peptides of clusters of serine-glycine

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repeats as glycosaminoglycan attachment domains (8, 18, 19, 24). Similar unique clusters have been found recently in the amino acid sequences deduced from the cDNAs of core peptides of storage granule proteoglycans of hemopoietic cells such as rat basophilic leukemia cells (27) and human promyelocytic leukemia cells of an HL-60 cell line (28). Taken together, the results obtained in the present study suggest strongly that the core peptide of the C(US)PGI proteoglycan contains a cluster of serine-glycine repeats. Heparan sulfate proteoglycan containing this kind of core peptide has not been reported previously. Moreover, the present study demonstrated that the core peptides of two other large heparan sulfate proteoglycans, C(UI)PG and MPG, were very similar, if not identical, to that of the C(US)PGI proteoglycan.

The properties of core peptides described above appear to be common to storage granule proteoglycans of hemopoietic cells (24). However, it should be noted that such core peptides are not limited to the storage granule proteoglycans but are also found in the proteoglycans present in the extracellular matrices of a rat yolk sac tumor (58) and rabbit bone marrow, a hemopoietic organ (37). Proteoglycans in the extracellular matrix of hemopoietic organs are considered to be produced and secreted into the matrix by nonhemopoietic cells, i.e., hemopoiesis-supporting stromal cells (37, 59–61). In this regard, it is noteworthy that the yolk sac is the first hemopoietic environment in embryonic development, where the blood islands are formed. Thus, it is more likely that the above structural characteristics of core peptides, regardless of the molecular species of glycosaminoglycans linked to them, are common to hemopoiesis-related proteoglycans rather than to the storage granule proteoglycans of hemopoietic cells. This concept may be extended to cell surface proteoglycans because our preliminary results indicated that some heparan sulfate proteoglycans produced by C-10-6 cells are located on their cell surface (62).

The small C(US)PGII proteoglycan, which had a very small, degraded peptide, was found only in the cellular fraction. The heparan sulfate released by β-elimination of this proteoglycan consisted of smaller, heterogeneous molecular species, which were degraded peptide, was found only in the cellular fraction. The heparan sulfate released by β-elimination of this proteoglycan consisted of smaller, heterogeneous molecular species, which were degraded by a combination of heparitinases I and II, as seen in fully sulfated chondroitin 4-sulfate, chondroitin sulfate E, chondroitin sulfate Di-B, and heparin.

These high degrees of sulfation are considered to be important for the interactions of these proteoglycans with other glycan proteins to form complexes (24). The sulfation degree of the degraded smaller C(US)PGII-heparan sulfate was significantly higher than those of three other heparan sulfates. This finding leads us to speculate about the occurrence of two possible degradation ways to produce such highly sulfated heparan sulfate fragments: one is a degradation coupled with a further sulfation reaction, and the other is a degradation coupled with a specific cleavage or elimination reaction of nonsulfated and/or lower sulfated portions of the heparan sulfate chains. In regard to the former possibility, it is noteworthy to refer to the internalization of the cell surface heparan sulfate proteoglycan observed in the cultured rat hepatocytes, which proceeds in association with a degradation of the heparan sulfate, coupling with a further sulfation specific to a position 2 of glucuronic acid residues (65). This processing is implicated in the control of cell growth (66, 67).

Our previous studies indicated that some cell-associated heparan sulfates are located on the cell surface and disappear during differentiation of C-10-6 cells into erythroblasts induced by treatment with dimethyl sulfoxide (62). This finding is consistent with a histochemical observation that early proliferating cells of the erythroid lineage were enveloped by sulfated glycosaminoglycans, whereas cells in later stages are not (68) because Friend erythroblasts are virus-transformed cells whose differentiation is believed to be blocked in an early stage of the erythroid lineage (29). These findings are also consistent with a fact that no sulfated proteoglycans have yet been found in erythrocytes (69). Taken together, the above results suggest that all heparan sulfate proteoglycans described in the present study are expressed only transiently in an early stage(s) of erythroid differentiation and are involved in regulation of cellular activities such as differentiation, proliferation, and adhesion of cells, as shown for other types of heparan sulfate proteoglycans produced by nonhemopoietic cells (48).

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REFERENCES

1. Okayama, M., Oguri, K., Fujisawa, Y., Nakaniishi, H., Yonekura, H., Kondo, T., and Ui, N. (1986) Biochem. J. 233, 73–81
2. Kobayashi, S. O., Bielen, L., Seljeld, R., and Lindahl, U. (1983) Biochim. Biophys. Acta 715, 196–204
3. Dvorak, A. M., Galli, S. J., Marcum, J. A., Nabel, G., Der Simonian, H., Goldin, J., Monahan, R. A., Pyke, K., Cantor, H., Rosenberg, R. D., and Dvorak, H. F. (1983) J. Exp. Med. 157, 843–86
4. Bland, C. E., Rosenthal, K. L., Pluznik, D. H., Dennert, G., Hengartner, H., Bienenstock, J., and Metcalfe, D. D. (1984) J. Immunol. 132, 1937–1942
5. Parmley, R. T., Rahemtulla, F., Cooper, M. D., and Roden, L. (1985) Blood 66, 20–25
6. Levitt, D., and Ho, P. L. (1983) J. Cell Biol. 97, 351–358
7. Metcalfe, D. D., Litvin, J., and Wasserman, S. I. (1982) Biochim. Biophys. Acta 715, 196–204
8. McQuillan, D. J., Yangashita, M., Haskell, V. C., and Bickel, M. (1989) J. Biol. Chem. 264, 19246–19251
9. Luikart, S. D. (1986) Exp. Hematol. (N. Y.) 14, 672–675
10. Lohman, L. S., Arnljots, K., and Yanagishita, M. (1990) J. Biol. Chem. 265, 5802–5808
11. Minguzzo, J. J., and Tavassoli, M. (1989) Blood 73, 1821–1827
12. Morris, A. J., Dexter, T. M., and Gallagher, J. T. (1989) Biochem. J. 260, 479–495
13. Rider, C. C., and Hart, G. W. (1987) Mol. Immunol. 24, 963–968
14. Uhlin-Hansen, L., and Kolset, S. O. (1987) Cell Differ. 21, 189–197
15. Razin, E., Stevens, R. L., Akiyama, F., Schmid, K., and Austen, K. F. (1982) J. Biol. Chem. 257, 7229–7236
Proteoglycans of Murine Erythroleukemia Cells

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16. Seldin, D. C., Austen, K. F., and Stevens, R. L. (1985) J. Biol. Chem. 260, 11131-11139
17. Yurt, R. W., Leid, R. W., Austen, K. F., and Silbert, J. E. (1977) J. Biol. Chem. 252, 518-521
18. Robinson, H. C., Horner, A. A., Höök, M., Ögren, S., and Lindahl, U. (1978) J. Biol. Chem. 253, 6687-6693
19. Metcalfe, D. D., Smith, J. A., Austen, K. F., and Silbert, J. E. (1980) J. Biol. Chem. 255, 11753-11758
20. Bland, C. E., Ginsberg, H., Silbert, J. E., and Metcalfe, D. D. (1982) J. Biol. Chem. 257, 8661-8666
21. Lindahl, U., and Kjellen, L. (1987) in The Biology of Extracellular Matrix Proteoglycans (Wight, T. N., and Mecham, R., eds) pp. 59-104, Academic Press, New York
22. Huang, S. S., Huang, J. S., and Deuel, T. F. (1982) J. Biol. Chem. 257, 11546-11550
23. Levine, S. F., Knieriemen, L. K., and Rager, M. A. (1990) Blood 75, 902-910
24. Stevens, R. L. (1986) CIBA Found. Symp. 124, 272-285
25. Parmley, R. T., Hurst, R. E., Takagi, M., Spicer, S. S., and Austin, R. L. (1983) Blood 61, 257-266
26. Avila, J. L. (1978) Biochem. J. 171, 489-491
27. Avraham, S., Stevens, R. L., Gartner, M. C., Austen, K. F., Lalley, P. A., and Weis, J. H. (1988) J. Biol. Chem. 263, 7292-7296
28. Nicodemus, C. F., Avraham, S., Austen, K. F., Purdy, S., Jablonski, J., and Stevens, R. L. (1990) J. Biol. Chem. 265, 5888-5896
29. Marks, P. A., and Rifkind, R. A. (1978) Annu. Rev. Biochem. 47, 431-448
30. Friend, C., Scher, W., Holland, J. G., and Sato, T. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 378-382
31. Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A., and Marks, P. A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 862-866
32. Ikawa, T., Inoue, Y., Aida, M., Kameji, R., Shibata, C., and Sugano, H. (1976) Bibl. Haematol. 43, 37-47
33. Yoshida, K., Miyauchi, S., Kikuchi, H., Tawada, A., and Tokuyasu, K. (1989) Annu. Rev. Biochem. 58, 327-332
34. Kato, T., Okamoto, T., Tokuya, T., and Takahashi, A. (1982) Biopolymers 21, 1623-1633
35. Kawahara, K., Ohita, K., Miyamoto, H., and Nakamura, S. (1984) Carbohydr. Polymers 4, 335-336
36. Yaita, E., Oguri, K., Okayama, E., Kawasaki, K., Kobayashi, S., Kihara, I., and Okayama, M. (1990) J. Biol. Chem. 265, 522-531
37. Okayama, E., Oguri, K., Kondo, T., and Okayama, M. (1988) Blood 72, 745-755
38. Nakashima, H., Oguri, K., Sugahara, K., and Okayama, M. (1986) Seibugaku 58, 882
39. Bitter, T., and Muir, H. M. (1962) Anal. Biochem. 4, 330-334
40. Elison, L. A., and Morgan, W. T. J. (1933) Biochem. J. 27, 1824-1828
41. Boas, N. F. (1953) J. Biol. Chem. 204, 553-563
42. Oguri, K., Okayama, E., Caterson, B., and Okayama, M. (1987) Blood 70, 501-510
43. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
44. Spackman, D. H., Stein, W. H., and Moore, S. (1958) Anal. Chem. 30, 1190-1206
45. Saito, H., Yamagata, T., and Suzuki, S. (1968) J. Biol. Chem. 243, 1586-1592
46. Kobayashi, S., Oguri, K., Kobayashi, K., and Okayama, M. (1983) J. Biol. Chem. 258, 12051-12057
47. Lindahl, U., Backström, G., Jansson, L., and Hallén, A. (1973) J. Biol. Chem. 248, 7234-7241
48. Gallagher, J. T., Lyon, M., and Steward, W. P. (1986) Biochem. J. 236, 313-325
49. Stevens, R. L., Otzu, K., and Austen, K. F. (1985) J. Biol. Chem. 260, 14194-14200
50. Kimmata, K., Okayama, M., Ochira, A., and Suzuki, S. (1974) J. Biol. Chem. 249, 1646-1653
51. Murate, T., and Kaneda, T. (1989) Leuk. Res. 13, 227-231
52. Terada, M., Egner, E., Nadeu, U., Salmon, J., Fibach, E., Rifkind, R., and Marks, P. A. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2789-2799
53. Metcalfe, D. D., Wasserman, S. I., and Austen, K. F. (1980) Biochem. J. 185, 367-372
54. Gahmberg, C. G., Jokinen, M., and Andersson, L. C. (1979) J. Biol. Chem. 254, 7442-7448
55. Perin, J.-P., Bonnet, F., Maillet, P., and Jolles, P. (1988) Biochem. J. 255, 1007-1013
56. MacDermott, R. P., Schmidt, R. E., Caufield, J. P., Hein, A., Bartley, G. T., Ritz, J., Schlossman, S. F., Austen, K. F., and Stevens, R. L. (1985) J. Exp. Med. 162, 1717-1787
57. Kihara, I., and Okayama, M. (1990) in Glycoconjugates (Davidson, E. A., Williams, J. C., and Di Ferrante, N. M., eds) Vol. 1, pp. 323-324, Praeger Publishers, New York
58. Wight, T. N., Kinsella, M. G., Keating, A., and Singer, J. W. (1986) Blood 67, 1333-1343
59. Oguri, K., Okayama, E., Caterson, B., and Okayama, M. (1987) Keio J. Med. 36, 67-70
60. Okayama, M., Oguri, K., Sato, C., and Kimura, K. (1981) in Glycoconjugates (Yamakawa, T., Osawa, T., and Handa, S., eds) pp. 371-372, Japan Scientific Societies Press, Tokyo
61. Oguri, K., Sato, C., Nakahashi, H., and Okayama, M. (1985) Seibagaku 57, 1178
62. Jacobson, K.-G., and Lindahl, U. (1987) Biochem. J. 246, 409-414
63. Fedarko, N. S., and Conrad, H. E. (1986) J. Cell Biol. 102, 587-599
64. Ishihara, M., Fedarko, N. S., and Conrad, H. E. (1986) J. Biol. Chem. 261, 13575-13580
65. Ishihara, M., Fedarko, N. S., and Conrad, H. E. (1987) J. Biol. Chem. 262, 4708-4716
66. McCuskey, R. S., Meineke, H. A., and Townsend, S. F. (1972) Blood 39, 697-712
67. Fukuda, M., and Fukuda, M. N. (1984) in The Biology of Glycoproteins (Ivatt, K. J., ed) pp. 181-204, Plenum Publishing Corp., New York