Metabolic properties of a homogeneous proteoglycan of a haemopoietic stem cell line, FDCP-mix

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A biochemical analysis has been carried out of metabolically labelled proteoglycans and glycosaminoglycans synthesized by a haemopoietic multipotential stem cell line, FDCP-mix. The only proteoglycan identified in these multipotential cells was a homogeneous component that contained chondroitin 4-sulphate chains ($M_r \sim 10000$) arranged in close proximity in a proteinase-resistant domain of the protein core. Small quantities of free chondroitin 4-sulphate were also detected. Following a 48 h incubation with $\text{Na}_2^{35}\text{SO}_4$ the majority of the ^{35}S -radiolabelled proteoglycans ($\sim 80\%$) were associated with the cells, mainly in an intracellular compartment, and the remaining 20% were in the culture medium. Pulse-chase studies demonstrated two turnover pathways for the newly synthesized cellular proteoglycans. In the minor pathway, the proteoglycans were secreted rapidly into the medium without any discernable structural modification. In the major pathway the proteoglycans seemed to be transferred into a storage compartment from which the intact macromolecules were not secreted. Eventually, these proteoglycans were degraded to yield free polysaccharide chains and these chains were then released into the medium, but only at a relatively slow rate. There was very little intracellular degradation of chondroitin sulphate chains. The pathway to polysaccharide secretion was a slow stepwise process with a time-lag of about 5 h between proteoglycan synthesis and the appearance of free chondroitin sulphate and a second time-lag, also of about 5 h, before these chains began to be secreted. The existence of separate secretory pathways for proteoglycans and chondroitin sulphate chains is an interesting characteristic that seems to distinguish proteoglycan metabolism in primitive multipotent stem cells from related metabolic processes in mature haemopoietic cells.

INTRODUCTION

In the past few years several interesting studies have been published on the proteoglycans of haemopoietic cells (see Stevens, 1986; Kharchenko, 1982, for reviews). Many of these proteoglycans contain chondroitin 4-sulphate as the major or exclusive polysaccharide in which the repeat disaccharide unit is $\text{GlcA}1\text{--}3\text{GalNAc}4\text{--}\text{SO}_3^-$. This polysaccharide, with its uniform sulphation pattern, is the major glycosaminoglycan in platelets (Okayama *et al.*, 1986), monocytes (Kolset *et al.*, 1983), NK cells (MacDermott *et al.*, 1985), eosinophils (Metcalf *et al.*, 1982), human T-lymphocytes (Levitt & Ho, 1983; Christmas *et al.*, 1988) and the promyelocytic-leukaemia cell line HL-60 (Luikart, 1986). However, more complex polysaccharide chains have been identified in specific cell lineages and their synthesis seems to correlate with cell differentiation. Murine B lymphocytes synthesise chondroitin sulphates composed of 4- or 6-sulphated disaccharides (Rider & Hart, 1987) and the conversion of monocytes to macrophages *in vitro* is accompanied by the formation of chondroitin sulphate E-type chains in which a significant portion of the disaccharides (20% in this particular case) are disulphated: $\text{GlcA}1\text{--}3\text{GalNAc}4,6\text{--}\text{SO}_3$ (Kolset *et al.*, 1983; Uhlin-Hansen & Kolset, 1987).

The most complex types of glycosaminoglycans are found in mast cells in which all the recognizable phenotypes synthesize highly sulphated components. Bone marrow-derived mucosal mast cells produce a species of

chondroitin sulphate E in which about 50% of the disaccharides are disulphated (Razin *et al.*, 1982; Stevens, 1986) and in mucosal mast cells from helminth-infected rats both the E-type (Kusche *et al.*, 1988) and the di-B type ($\text{IdA}2\text{--}\text{SO}_3^- \alpha 1\text{--}3\text{GalNAc}4\text{--}\text{SO}_3^-$) of disaccharide (Stevens *et al.*, 1986) have been identified. In connective tissue mast cells the major glycosaminoglycan is heparin (Yurt *et al.*, 1977; Stevens & Austen, 1982; Lindahl & Kjellén, 1987), the most highly sulphated of all mammalian polysaccharides. It is not clear why the different mast cell subtypes produce distinctive species of glycosaminoglycan.

In view of the variations in polysaccharide structure in haemopoietic cells, we considered that it would be of some interest to analyse the glycosaminoglycans and proteoglycans of haemopoietic stem cells, which can differentiate and amplify to produce all the mature cellular elements that are derived from bone marrow. However, marrow tissue contains only very small numbers of stem cells ($< 0.1\%$ of the total cells) and this effectively precludes detailed biochemical investigations of their proteoglycans. Recently, however, a series of multipotential haemopoietic cell lines, FDCP-mix, derived from bone marrow cultures, were established in suspension culture (Spooncer *et al.*, 1986). These cells require the continuous presence of the growth factor interleukin-3 (IL-3), to maintain their viability and to promote their growth in liquid culture. They have a normal karyotype and primitive morphology and are non-leukaemic (Spooncer *et al.*, 1984; Wyke *et al.*, 1986). When deprived

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of IL-3, the cells rapidly die, unless cultured in association with marrow stromal cells (Roberts *et al.*, 1988). In these conditions the cells behave like normal stem cells, i.e. they attach to the stroma, proliferate and differentiate to make erythroid cells, neutrophils, macrophages and megakaryocytes. These cell lines provide a valuable source of material for identifying proteoglycans which may well be characteristic of normal stem cells.

MATERIALS AND METHODS

Materials

[³⁵S]Sulphate (25–40 Ci/mg) and D-[1-³⁵H]glucosamine (2–5 Ci/mol) were obtained from Amersham International. Bovine pancreatic trypsin type III, soyabean trypsin inhibitor, *Streptomyces griseus* protease (Pronase) and *Proteus vulgaris* chondroitin-4-sulphatase were purchased from Sigma. Chondroitinase AC (*Arthrobacter aurescens*) and chondroitinase ABC (*Proteus vulgaris*) were obtained from ICN Biomedicals. DEAE-Sephacel was from Sigma and Sepharose CL-4B and CL-6B were supplied by Pharmacia. Heparin (bovine) and chondroitin sulphate (whale and shark) were from Sigma.

Radiolabelling of cell cultures

FDCP-mix A4, IL-3-dependent, multipotential stem cells, derived from cultures of mouse bone marrow, were grown in suspension culture using medium conditioned by the growth of Wehi-3 cells as a source of interleukin-3 (Dexter *et al.*, 1980; Spooner *et al.*, 1986). Proteoglycans were radiolabelled by the addition of 20 μ Ci of [³⁵S]sulphate/ml. and in the case of materials for high voltage electrophoresis, additionally 10 μ Ci of [³H]glucosamine/ml. Cells were seeded at 1.5×10^5 cells/ml in a total volume of 200 ml and incubated with radioisotopes for 48 h at 37 °C in 5 % CO₂ in air. The culture was harvested by centrifugation at 400 g for 5 min and the cells washed three times with 20 mM-Tris/acetate buffer, pH 7.4, containing 1 mM-calcium acetate and 0.15 M-NaCl. The yield of cells was 1.34×10^8 in total, having a viability of 95 % by Trypan Blue exclusion.

Pulse-labelling of the proteoglycan was carried out using cells in exponential growth. The cells were harvested by centrifugation (400 g for 5 min) and resuspended at high cell concentration (20×10^6 cells/ml) using an aliquot of the original 'conditioned' growth medium, Na₂³⁵SO₄ was added to a final concentration of 250 μ Ci/ml and the cells were incubated for 20 min at 37 °C. The cells were then centrifuged and the supernatant carefully and completely removed. The pellet was washed twice and then resuspended in a large excess of non-radioactive medium to give a cell concentration of 5×10^6 cells/ml. Cells were harvested at zero time and at intervals thereafter as described in Fig. 6. Proteoglycans were extracted and analysed by gel filtration. Full details are given in the legends to figures.

Label-chase studies were carried out by incubating the cells for 48 h with 20 μ Ci of Na₂³⁵SO₄/ml. Washed cells were then incubated in non-radioactive chase medium for 30 h. Proteoglycans and glycosaminoglycans in the medium and cell-associated compartments were analysed as a single group of ³⁵S-labelled macromolecules by step-elution from DEAE-Sephacel columns (see below).

Extraction of proteoglycans

Trypsin extract of cell surface. Cells harvested as described above were incubated for 15 min at 37 °C in 5 ml of 20 mM-Tris/acetate, pH 7.4, containing 0.15 M-NaCl and 1 mM-calcium acetate and 50 μ g of trypsin/ml. The reaction was terminated by the addition of 0.25 ml of soyabean trypsin inhibitor (1 mg/ml) in the same buffer and the cells were centrifuged as above. The supernatant trypsin extract was removed and the residual cell pellet was washed once in buffer and then frozen for later detergent extraction (see below).

Extraction of whole cells. Freshly harvested or frozen cell pellets, that on some occasions had been pretreated with trypsin, were solubilized in 5 ml of 50 mM-sodium phosphate buffer, pH 7.4, containing 1 % (v/v) Triton X-100, 6 M-urea, 0.15 M-NaCl, 50 μ g of heparin, 50 μ g of chondroitin sulphate, 100 μ g of bovine serum albumin and 100 μ g of soya bean trypsin inhibitor/ml and freshly added phenylmethanesulphonyl fluoride (1 mM final concentration). The extraction was carried out for 2 h at 4 °C with occasional shaking. Ice-cold trichloroacetic acid (0.1 vol. of 100 %, w/v) was then added and the solution was allowed to stand at 4 °C for 1 h. Trichloroacetic acid precipitated proteins and nucleic acids, but the proteoglycan remained soluble. The precipitate was sedimented by centrifugation (800 g for 5 min) and the supernatant was carefully removed. This was now dialysed against the Triton/urea buffer described above.

Ion-exchange chromatography

Anion-exchange chromatography was performed using a column of DEAE-Sephacel (1 cm $\times$ 5 cm). The trypsin extract was dialysed into 50 mM-sodium phosphate buffer, pH 7.4, containing 0.15 M-NaCl. Trypsin extract or medium (medium was adjusted to 0.25 M-NaCl) were pumped onto the column at 20 ml/h and the column was then washed with 40 ml of the above buffer before eluting the column with a linear gradient of NaCl from 0.15 M to 1.5 M-NaCl in 200 ml of 50 mM-sodium phosphate, pH 7.4. The flow rate was 10 ml/h and 2 ml fractions were collected. The cell pellet material was treated in a similar manner except that chromatography was performed in buffer containing 1 % (v/v) Triton X-100 and 6 M-urea. Peaks of radioactivity were pooled and dialysed against 15 mM-NaCl, concentrated to 2–5 ml with poly(ethylene glycol) and dialysed once more against 15 mM-NaCl. Losses on dialysis and concentration were minimal. On some occasions applied samples were step-eluted from the DEAE-cellulose column with 20 ml of 50 mM-sodium phosphate buffer, pH 7.5, containing 1.0 M-NaCl, 1 % (v/v) Triton and 6 M deionized urea; 1 ml fractions were collected.

Gel filtration chromatography

This was performed on columns of Sepharose CL-6B (1 cm $\times$ 80 cm) eluted with Dulbecco's buffered saline, pH 7.4, containing 6 M deionized urea at a flow rate of 3 ml/h.

Enzyme digestion of glycosaminoglycans

Chondroitinase AC was used at 50 munits in 0.1–0.5 ml of sodium acetate buffer, pH 6.0, containing 0.1 mg of bovine serum albumin/ml and incubated at 37 °C for 16 h. Chondroitinase ABC was used as for the AC enzyme but in 50 mM-Tris/HCl, pH 8.0, containing

60 mM-sodium acetate, 0.15 M-NaCl and 0.1 mg of bovine serum albumin/ml and it was incubated for 3 h at 37 °C. Chondroitin-4-sulphatase was used at 5 munit/ml in chondroitinase ABC buffer and was incubated for 3 h at 37 °C.

Pronase digestion

Samples in 20 mM-Tris/acetate buffer, pH 7.4, containing 0.15 M-NaCl and 1 mM-calcium acetate were digested with Pronase (1 mg/ml) for 24 h at 37 °C under toluene. An additional 0.25 mg of Pronase/ml was then added and the digestion continued for a further 24 h. The digestion was ended by heating to 100 °C for 5 min.

Alkali elimination

To samples in 15 mM-NaCl equal volumes of 0.2 M-NaOH containing 2 M-NaBH₄ were added and this was incubated at 37 °C for 24 h under N₂. Solutions were then neutralized at 4 °C with 1 M-acetic acid using Phenol Red as indicator.

High voltage electrophoresis

Samples were applied in narrow bands to Whatman no. 1 chromatography paper (3 mm × 35 mm × 59 mm) and dried. The paper was then wetted with 1.6 M-formic acid (pH 1.5) and electrophoresed for 1 h at 600 V. The paper was then dried and each sample track cut into 1 cm strips. Each strip was soaked in 0.5 ml of phosphate-buffered saline containing 1 % (v/v) Triton for 30 min prior to scintillation counting.

Paper chromatography

The 4- and 6-sulphate isomers of chondroitin sulphate were identified by paper chromatography, as described by Saito *et al.* (1968), following digestion of the polysaccharide with chondroitinase ABC.

RESULTS

DEAE-Sephacel chromatography

FDCP-mix cells were incubated for 48 h in the presence of Na₂³⁵SO₄ and the trypsin extract, the residual cell-associated material (extracted with Triton X-100) and the culture medium were fractionated on DEAE-Sephacel by elution with a linear NaCl gradient. Two radioactive components were identified in each sample. In the medium the major sharp peak (peak 2) was eluted at 0.7 M-NaCl and was preceded by a minor component (peak 1) which appeared as a small shoulder at the leading edge of the main peak (Fig. 1a). The elution profile for the trypsin extract (not shown) was similar to that of the medium. In the cell pellet major and minor peaks of radioactivity were also observed but they were eluted at lower salt concentrations than their medium counterparts (0.6 M- and 0.5 M-NaCl respectively), an effect attributable to the presence of 6 M-urea in the gradient buffer (Fig. 1b). Data given below show that the major component in each extract (peak 2) is a chondroitin sulphate proteoglycan and the minor species (peak 1) is a free chondroitin sulphate chain. The majority of the ³⁵S-radiolabelled materials (~ 70 %) were in the cell pellet, with minor quantities in the medium (19 %) and trypsin extract (11 %) (see Fig. 2). This trypsin-released material may be derived from the cell surface, although it has been shown that the enzyme can induce a secretory response (Jett & Jamieson, 1973).

Fig. 1. Fractionation of proteoglycans by chromatography on DEAE-Sephacel

FDCP-mix cells were incubated for 48 h with [³⁵S]-sulphate. Cells were separated from the culture medium by centrifugation and treated with trypsin. The cells were then solubilized with 6 M-urea in 1 % (v/v) Triton X-100 (see the Materials and methods section for details). The samples were applied to a DEAE-Sephacel column and eluted with a linear gradient of NaCl in 6 M-urea and 1 % (v/v) Triton X-100; 6 M-urea was excluded from the eluting buffer when the culture medium was fractionated. Fractions (2 ml) were collected at a flow rate of 10 ml/h. Peaks of eluted radioactivity (1 and 2) were pooled as indicated. (a) Culture medium; (b) Triton/urea extract of cells.

Gel chromatography on Sepharose CL-6B

The major ³⁵S-labelled proteoglycan in the Triton X-100 extract of the cell pellet (peak 2 from Fig. 1b) was analysed by gel filtration on Sepharose CL-6B (Fig. 3a). This material yielded a homogeneous component (*K*_{av} 0.33) which was slightly decreased in molecular size by Pronase digestion but which was degraded by alkali treatment to give a symmetrical glycosaminoglycan peak with a *K*_{av} of 0.60 (Fig. 3a). Similar gel filtration profiles were obtained with the proteoglycan in the medium (peak 2 in Fig. 1a) and trypsin extract. These proteoglycans were also largely resistant to Pronase digestion but were degraded by alkali to yield a glycosaminoglycan peak identical in size to that produced by alkali treatment

Fig. 2. Relative proportions of proteoglycan and free chain chondroitin sulphate in each compartment

The histograms show the distribution of ^{35}S radioactivity in the medium (M), trypsin extract (T) and cell pellet (C). Chondroitin sulphate chains (peak 1) and proteoglycans (peak 2) were separated by DEAE-Sephacel chromatography (Fig. 1).

of the intracellular proteoglycan (results not shown). ^{35}S -labelled proteoglycans in all the extracts were degraded by chondroitinase AC to give a single ^{35}S -labelled peak eluting at the total volume on Sepharose CL-6B (Fig. 3a).

The minor ^{35}S -labelled component in the Triton X-100 extract (peak 2 in Fig. 1b) gave a symmetrical peak when chromatographed on Sepharose CL-6B (Fig. 3b). This component was similar in size to the chondroitin sulphate component of the intracellular proteoglycan. It was identified as a free chondroitin sulphate chain (or chondroitin sulphate with a small amount of residual peptide) on the basis of its degradation by chondroitinase AC and resistance to treatment by Pronase or alkali (Fig. 3b). Chondroitin sulphate chains were also detected as the minor components in the medium and trypsin extracts (see Fig. 2). By reference to the calibration of Sepharose CL-6B by Wasteson (1971) we estimate a molecular size of about 10000 for the chondroitin sulphate chains.

Identification of chondroitin 4-sulphate using enzyme digestion and high voltage electrophoresis

For these analyses proteoglycans and glycosaminoglycans were metabolically labelled with both $\text{Na}_2^{35}\text{SO}_4$ and $[^3\text{H}]\text{glucosamine}$ so that both sulphated and non-sulphated disaccharides could be detected. The ^{35}S -

Fig. 3. Gel filtration of proteoglycans and glycosaminoglycans on Sepharose CL-6B

(a) Proteoglycans isolated from the culture medium by DEAE-Sephacel chromatography (peak 2 in Fig. 1) were analysed by gel filtration on Sepharose CL-6B. Samples were applied and eluted in phosphate-buffered saline, pH 7.4, containing 6 M-urea at a flow rate of 3 ml/h. Fraction volumes were 0.6 ml. Profiles of untreated samples (-----) were compared with samples treated with Pronase (---), alkaline borohydride (—) or chondroitinase AC (Δ — Δ). (b) Glycosaminoglycans in the culture medium (peak 1 in Fig. 1) were fractionated under the same conditions as described for (a). The elution profile for the untreated material (—) was not affected by Pronase treatment (not shown), marginally affected by alkali treatment (-----) and degraded by chondroitinase AC (Δ — Δ). Proteoglycans and glycosaminoglycans in the trypsin extract and in the intracellular compartment gave similar profiles.

labelled macromolecules in the cell-associated proteoglycans were degraded to disaccharides by treatment with chondroitinase AC or chondroitinase ABC. Analysis of the degradation products by high voltage electrophoresis showed that the disaccharides were almost entirely monosulphated structures which were converted to unsulphated disaccharides and inorganic $[^{35}\text{S}]\text{sulphate}$

Fig. 4. Analysis of secretion and turnover of proteoglycans and glycosaminoglycans

Cells were labelled for 48 h with [^{35}S]sulphate and then chased in radiolabel-free medium for 30 h. At each time point shown samples were removed and centrifuged to separate cells and growth medium. The cells were extracted with a buffered solution of 6 M-urea and 1% (v/v) Triton X-100 as described in the Materials and methods section. The cell extract and growth medium were applied separately to a DEAE-Sephacel column and step-eluted with 1.0 M-NaCl for combined determination of proteoglycans and glycosaminoglycans. Symbols designate ^{35}S -labelled macromolecules in the cells (●) the culture medium (△) and the sum of radiolabel in these two fractions (○).

by treatment with chondroitin-4-sulphatase (results not shown). Analysis by paper chromatography confirmed that the disaccharide was the 4-sulphate isomer (see the Materials and methods section). Similar results were obtained for proteoglycans and glycosaminoglycans from the medium fraction. These findings show that the polysaccharide component of the stem-cell proteoglycan is chondroitin 4-sulphate.

Label-chase experiments

Since the majority of the ^{35}S -labelled proteoglycans (70%) were located inside the cells after a 48 h incubation with $\text{Na}_2^{35}\text{SO}_4$ and only 20% were found in the culture medium, it seemed likely that proteoglycans might be stored for prolonged periods by FDCP-mix stem cells before being secreted. To investigate proteoglycan metabolism in more detail, the macromolecules were radiolabelled by incubating proliferating cells for 48 h with $\text{Na}_2^{35}\text{SO}_4$. The cells were then separated from the labelling medium and re-incubated for various times up to 24 h in non-radioactive 'chase' medium. Loss of cellular proteoglycans and glycosaminoglycans by secretion into the culture medium or intracellular degradation was monitored by applying the culture medium

Fig. 5. Pulse-chase analysis of proteoglycans and glycosaminoglycans in the cells (a) and culture medium (b)

Cells were incubated for 20 min in [^{35}S]sulphate and then transferred to unlabelled medium for up to 24 h. Proteoglycans and glycosaminoglycans in the cells and the culture medium were isolated at the time intervals shown and separated by chromatography on Sepharose CL-6B (Fig. 3). ^{35}S -labelled macromolecules in the cells and the culture medium are given in panels (a) and (b) respectively. The vertical axes of ^{35}S radioactivity have been corrected for cell number (panel a) or medium volume (panel b) sampled. Peak areas of radioactivity within each panel are thereafter directly comparable. Material in peak I is proteoglycan and glycosaminoglycans are in peak II. Note that only proteoglycan is present in the cells at time zero (i.e. immediately after pulse-labelling). The scales of the vertical axes in panels (a) and (b) are different.

and Triton X-100/6 M-urea extracts of the cells directly to a DEAE-Sephacel column and step-eluting the ^{35}S -labelled macromolecules with 1.0 M-NaCl. Proteoglycans and glycosaminoglycans are co-eluted by this method. Because only small amounts of proteoglycan were found in trypsin extracts of the cells, trypsin treatment was not carried out and the cells were extracted directly with Triton X-100 in 6 M-urea.

The results of this experiment (Fig. 4) showed that there was a slow secretion of proteoglycans and glycosaminoglycans into the culture medium and even after 30 h only about 15% of the ^{35}S -labelled materials had

Fig. 6. Time course of secretion and degradation of pulse-labelled proteoglycan

Data were taken from the pulse-label experiment described in Fig. 5. Radioactivity in proteoglycans was taken from fractions 37–58 and in glycosaminoglycans from fractions 59–80. Panel (a) shows the rate of loss of proteoglycan from the cells and the time-course of the formation of free chondroitin sulphate chains; panel (b) shows the time-course of secretion of these macromolecules into the culture medium. Note that the scales of the vertical axes are different in the two panels. $\circ$, Proteoglycan; $\triangle$, free chondroitin sulphate chains.

been secreted. The majority of the ${}^{35}\text{S}$ -labelled macromolecules remained in the cell-associated fraction (Fig. 4). It was significant that the net loss of ${}^{35}\text{S}$ -labelled macromolecules present at the start of the chase period was very small. About 85% of the proteoglycans and glycosaminoglycans were recovered in the cell and medium fractions after the 30 h chase incubation (Fig. 4).

Pulse-chase experiments

These experiments were carried out in order to provide direct information on the primary products of proteoglycan biosynthesis and the subsequent distribution and metabolism of these molecules into secretion, storage and intracellular degradation pathways. ${}^{35}\text{S}$ -labelled macromolecules were examined by gel filtration on Sepharose CL-6B (Fig. 5) in order to distinguish between

Fig. 7. Schematic representation of the metabolism of proteoglycans in haemopoietic stem cells

Times shown are the approximate time lags between proteoglycan synthesis and the onset of a particular metabolic process. Newly synthesized proteoglycans (PG_N) are either secreted rapidly into the culture medium (PG_S) or transferred into a storage compartment inside the cell (PG_I). The majority of PG_N (90%) follow the intracellular pathway to PG_I . PG_I are not secreted in their intact form. Metabolic turnover of PG_I , which begins about 5 h after proteoglycan synthesis, is a slow, stepwise process beginning with degradation to glycosaminoglycans (GAG_I) and these molecules are then secreted from the cell (GAG_S) after a further delay of up to 5 h. We assume that GAG_I are stored in a different compartment (b) from their metabolic precursors PG_I (a) to account for the specific and progressive secretion of glycosaminoglycans that begins between 5 and 10 h into the chase incubation (Fig. 6).

proteoglycans and glycosaminoglycans. Rates of proteoglycan secretion and degradation, determined from the gel filtration analyses, are plotted in Fig. 6.

Cells were pulse-labelled for 20 min with a high concentration of $\text{Na}_2{}^{35}\text{SO}_4$ and then transferred to non-radioactive medium for 24 h. The pulse-labelled macromolecules were shown by gel filtration to be exclusively proteoglycans (Fig. 5a). The $t_{1/2}$ of these proteoglycans was about 30 h, turnover being achieved either by secretion or degradation to chondroitin sulphate chains (Figs. 5 and 6). The secretory fraction was small (approx. 10%) and secretion was largely complete within 2 h (Fig. 6b). The remaining proteoglycans were retained inside the cells, presumably in a storage granule, and after a delay of about 5 h they began to be slowly degraded to yield free chondroitin sulphate chains (Figs. 5a and 6a). After 24 h, 30% of the intracellular proteoglycan had been degraded in this way. Secretion of chondroitin sulphate chains was a slow process, first

detected after 10 h, and by 24 h only 8% of these molecules had been secreted (Figs. 5b and 6b).

Of the pulse-labelled proteoglycan, 95% was recovered from the cells and medium as intact macromolecules or free polysaccharides, confirming the data in Fig. 4 that proliferating haemopoietic stem cells display only a very limited capacity to degrade chondroitin sulphate chains.

DISCUSSION

The results presented in this paper demonstrate that a chondroitin 4-sulphate proteoglycan, which is homogeneous when examined by ion-exchange chromatography or gel filtration (Figs. 1 and 3 respectively) is the only proteoglycan produced by the multipotential haemopoietic cell line FDCP-mix. The basic design of the proteoglycan, in which the glycosaminoglycan chains are located in a protease-resistant domain of the protein core (Fig. 3) is comparable to that of the main proteoglycan components isolated from mast cells, NK cells (Stevens *et al.*, 1986; Stevens, 1986) leukaemic basophils (Seldin *et al.*, 1985) and human T-cells (Christmas *et al.*, 1988). Heparin proteoglycan isolated from rat skin, which was assumed to be produced by mast cells, was also resistant to proteases (Robinson *et al.*, 1978).

The identification of chondroitin 4-sulphate as the only polysaccharide constituent of the haemopoietic stem cell proteoglycan is notable. Polysaccharides of similar structure, though with some variations in chain length, are found in lymphocytes, monocytes, neutrophils, platelets and NK cells; amongst the wide variety of haemopoietic cells, highly sulphated glycosaminoglycans of variable structure are found only in mast cells, basophils (Seldin *et al.*, 1985), and to a lesser extent in macrophages (see the Introduction). In general, therefore, glycosaminoglycan modifications that accompany cellular diversification in haemopoiesis are relatively conservative in nature. However, this conclusion is largely based on studies of cells at opposite ends of the developmental pathways (i.e. stem cells and mature end cells) and it is possible that cells at intermediate stages of development, including leukaemic cells, might synthesize unique patterns of glycosaminoglycans (Kharchenko, 1982; Seldin *et al.*, 1985).

Metabolic studies demonstrated an unexpected complexity of proteoglycan metabolism in haemopoietic stem cells (Figs. 5 and 6). Label-chase and in particular pulse-chase experiments indicated two turnover pathways for newly synthesized proteoglycans, these being either rapid secretion (minor pathway) or intracellular degradation to free chondroitin sulphate chains (Figs. 5 and 6). These chains were then slowly secreted into the medium. The various metabolic fates of proteoglycans and glycosaminoglycans are summarized in Fig. 7.

Proteoglycan metabolism in haemopoietic stem cells may be distinguished in a number of ways from the metabolism of corresponding macromolecules in other types of haemopoietic cell. For example, cultured monocytes and macrophages secrete the majority of their proteoglycans into the culture medium (Kolset, 1987a). In a monocytic leukaemic cell line the proteoglycans were also mainly secretory products but some proteoglycan was rapidly degraded to yield non-secretory chondroitin sulphate chains (Kolset, 1987b). Similar non-

secretory pools of glycosaminoglycans have been identified in human T-lymphocytes (Christmas *et al.*, 1988). The highly sulphated proteoglycans of bone marrow and mucosal mast cells are stored in secretory granules in the undegraded form (Razin *et al.*, 1982; Stevens *et al.*, 1986) and heparin isolated from rat skin was isolated mainly as a proteoglycan rather than free chains or large oligosaccharides (Robinson *et al.*, 1978; Horner, 1986). However, in cultures of malignant mast cells (mastocytomas), a two-stage degradation process has been identified in which heparin chains are released by proteolytic attack on the core protein of the proteoglycans and the released chains are then degraded to oligosaccharides of variable size by an endoglucuronidase (Jacobsson & Lindahl, 1987). Activity of this endoglucuronidase seems to require co-cultivation with macrophages. Interactions of mast cells with macrophages could also account for the degradation of heparin proteoglycan that has been observed in some rat tissues (Horner, 1986). In our studies we could find no evidence for the degradation of chondroitin sulphate to large oligosaccharides, nor could we identify partially degraded proteoglycan intermediates during the formation of glycosaminoglycan chains (the K_{av} of the intracellular proteoglycan was unchanged during the 24 h chase period in the experiment illustrated in Fig. 5). It seems likely therefore that once degradation of proteoglycan is initiated, it proceeds extremely rapidly.

The present studies give no direct indication of the function of stem cell proteoglycans. It is also not known how the balance between secretion and intracellular storage of the proteoglycan is regulated. The degree of homogeneity of the proteoglycan was impressive (Figs. 1 and 3); comparable molecules in haemopoietic and other cells are often both polydisperse and heterogeneous. The results indicate a biosynthetic system for proteoglycans that operates with great precision and it is tempting to speculate that the predominantly intracellular proteoglycan described in the present study may have an important role to play in maintaining the unique biochemical properties of haemopoietic stem cells.

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