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Murine T lymphocytes and T-lymphoma cells produce chondroitin sulphate and heparan sulphate proteoglycans and free heparan sulphate glycosaminoglycan

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SUMMARY

Normal murine splenic T lymphocytes and T-lymphoma cells were incubated with [^{35}S]sulphate in low-sulphate medium for 4 hr. Gel filtration and SDS–PAGE revealed that the radiolabelled macromolecules secreted by these cells were almost exclusively chondroitin sulphate and heparan sulphate proteoglycans of relatively low molecular weight (MW), 100,000–200,000. Triton X-100 extracts of the cells contained similar proteoglycans. Under the conditions employed the incorporation of radiolabel by cells grown *in vivo* was equally distributed between cell-retained and secreted fractions, whereas cells grown *in vitro* retained some 75% of incorporated label. In general heparan sulphate predominated over chondroitin sulphate in both secreted and cell-retained fractions. Cell extracts also contained a minor proportion of free glycosaminoglycan, which is almost exclusively heparan sulphate. These chains, like those incorporated into the proteoglycan, were around 12,000 MW. The T-lymphoma cells RDM-4, whether grown *in vitro* or *in vivo*, also incorporated a substantial proportion of [^{35}S]sulphate into a single, cell-retained protein, 100,000 MW. No such radiolabelled protein was detectable in T cells.

INTRODUCTION

Lymphocytes synthesize the sulphated glycosaminoglycans chondroitin sulphate and heparan sulphate, both of which are secreted as well as retained by the cells.[1] Glycosaminoglycan synthesis is increased by mitogen stimulation in murine thymocytes,[1] human peripheral blood T lymphocytes and murine splenic lymphocytes.[17] In particular, exposure of murine splenic T cells to concanavalin A (Con A) and phorbol 12-myristate 13-acetate increases secretion of glycosaminoglycans 10- to 20-fold within 24 hr.[2] This therefore appears to be a prominent, early biosynthetic response of lymphocytes to mitogen stimulation.

Within the lymphoid population there are also qualitative differences in glycosaminoglycan synthesis. Thus on mitogen stimulation thymocytes switch from the production of only chondroitin 4-sulphate to synthesis of both the 4- and 6-sulphates.[1] Furthermore, there is a striking difference in the chondroitin sulphate produced by B and T lymphocytes. T cells, like resting thymocytes, synthesize almost exclusively the 4-sulphate, whilst B cells produce both the 4- and 6-sulphates, with a preponderance of the latter.[3] This lineage-specific pattern of sulphation is preserved in the murine B- and T-lymphoma cell lines, AKTB-1b and EL-4, respectively.

The intact lymphocyte proteoglycan molecules which carry these sulphated glycosaminoglycans have received little attention. However, chondroitin sulphate proteoglycans have been isolated from the secretory granules of human and rat natural killer (NK) cells (reviewed by Stevens).[4] Moreover, two known lymphocyte glycoproteins have been found to exist partly as chondroitin sulphate proteoglycans: the invariant or γ chain of class II major histocompatibility antigens[5] and the lymphocyte adhesion protein CD44.[6] A single analysis of human peripheral blood T cells has revealed the presence of relatively small chondroitin sulphate proteoglycans.[7] We report here a comparison of the sulphated macromolecules synthesized by murine splenic T lymphocytes with those of T-lymphoma cells, and determine the presence of heparan sulphate both in proteoglycans and as free glycosaminoglycan.

Abbreviations: FCS, foetal calf serum; NEM, N-ethylmaleimide; NK cells, natural killer cells; PMSF, phenylmethylsulphonyl fluoride; SDS-PAGE, SDS–polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Materials

^{35}S-sulphuric acid in HCl-free water was purchased from Dupont Ltd, Stevenage, Herts, U.K., or ICN Biomedicals Ltd, High Wycombe, Bucks, U.K. Ecoscint scintillation fluid was

obtained from National Diagnostics, Aylesbury, Bucks, U.K. RPMI-1640 Dutch Modification, sulphate-free RPMI-1640 Dutch modification, Hanks' balanced salts and foetal calf serum (FCS) were from Gibco BRL, Paisley, Renfrewshire, U.K. Zwittergent 3-08 was from Calbiochem, Nottingham, U.K., and N-butyl nitrate was from Aldrich Chemical Co. Ltd, Gillingham, Dorset, U.K. FITC-conjugated rabbit anti-mouse Ig (A, G and M) was from Serotec Ltd, Bicester, Oxon, U.K. All other chemicals were from Sigma Chemical Co. or BDH, Poole, Dorset, U.K.

T-lymphocyte isolation

Cell suspensions in sterile Hanks' balanced salts containing 10 mM HEPES, pH 7·2, and 1% (v/v) FCS were obtained from spleens of C3H/He mice (Bantin & Kingman, Hull, U.K.) (8–10 weeks old). Red cells were removed by NH_4Cl lysis.[8] After two washes in Hanks' balanced salts containing 10 mM HEPES, pH 7·2, and 0·5% (v/v) FCS, cell suspensions, 5 ml containing 5–6×10^7 cells, were incubated for 30 min in bacteriological Petri dishes (8·5 cm diameter) previously coated with affinity-purified rabbit anti-rat IgG. Non-adherent cells were pelleted and resuspended in sulphate-free RPMI-1640 medium containing 0·2 mM 2-mercaptoethanol, 2 mM L-glutamine, 1% (v/v) FCS, penicillin-G, 100 U/ml, and streptomycin sulphate, 1·55 μg/ml. Cells were washed once in this medium (labelling medium) and finally resuspended for metabolic labelling. B-cell contamination was determined by fluorescent labelling of cells with FITC-conjugated rabbit anti-mouse IgG and was routinely $< 10\%$; red cell contamination was $< 10\%$.

Lymphoma cell growth

Mycoplasma-free T-lymphoma lines RDM-4[9] and EL-4 (Dr G. W. Hart, Johns Hopkins University, Baltimore, MD) (ATCC TIB 39) were grown *in vitro* at 37° in 5% CO_2/95% air in normal RPMI-1640, with additions as for labelling medium except 10% (v/v) FCS was used. RDM-4 cells were also grown *in vivo* as ascites in AKR mice (Bantin & Kingman) by intraperitoneal injection of $1\text{–}2 \times 10^7$ RDM-4 cells in Hanks' balanced salts containing 10 mM HEPES, pH 7·2. Cells grown *in vivo* were withdrawn aseptically and subjected to NH_4Cl lysis. Red cell contamination was routinely $< 5\%$ and lymphocyte contamination was $< 10\%$. Cell preparations were washed twice in labelling medium.

Metabolic labelling with [^{35}S]sulphate

Cell suspensions, 1 ml containing 1×10^7 cells, were incubated with 150 μCi [^{35}S]sulphate per well of a 24-well plate at 37° in 5% CO_2/95% air routinely for 4 hr. Cell viabilities, as measured by nigrosin dye exclusion, were routinely $> 83\%$ throughout the incubation. Resuspended cells were centrifuged at 500 *g* for 15 min at 4°, washed twice with 1 ml ice-cold 0·01 M sodium phosphate, pH 7·4, containing 0·15 M NaCl, and stored at −20°. Cellular extracts were prepared by shaking cell pellets overnight in 0·75 ml 10 mM Tris/HCl, pH 7·6, containing 25 mM EDTA, 2 M NaCl, 1% (v/v) Triton X-100 and protease inhibitor cocktail (antipain, leupeptin, aprotinin and chymostatin, each 5 μg/ml final concentration), followed by centrifugation at 100,000 *g* for 40 min at 4°. Cellular extracts, and lyophilized incubation supernatants redissolved in column buffer, were desalted on a Bio-Gel P-6 column ($1{\cdot}0 \times 43$ cm) to remove inorganic [^{35}S]sulphate. Column buffer consisted of 50 mM sodium acetate, pH 5·8, containing 4 M guanidine hydrochloride, 0·1% (w/v) Zwittergent 3-08 and protease inhibitors (0·2 mM PMSF, 1 mM benzamidine hydrochloride, 10 mM NEM, and 10 mM 6-aminohexanoic acid). The ^{35}S-labelled material which eluted at the void volume was extensively dialysed against 50 mM ammonium acetate, pH 5·8, and lyophilized.

Gel electrophoresis and fluorography

SDS–PAGE was performed using the buffer system of Laemmli[10] on 7% gels ($75 \times 100 \times 0{\cdot}75$ mm) at 150 V. After fixing in 45% methanol/5% acetic acid/50% water (v/v/v) gels were incubated in 0·4% (w/v) 2,5-diphenyloxazole (PPO) dissolved in 30% xylene/15% ethanol/55% acetic acid (v/v/v) for 1 hr. Gels were washed in distilled water, dried and flourographed at −70° using pre-flashed Kodak X-Omat film.

Continuous gel electrophoresis was performed using a method adapted from Hampson & Gallagher.[11] Lyophilized samples were solubilized in 0·1 M glycine/1·25 mM EDTA/2·5 mM NaN_3 adjusted to pH 8·9 with solid Tris, containing 1% (w/v) SDS, boiled for 5 min, and applied to 15% acrylamide gels ($75 \times 100 \times 0{\cdot}75$ cm) which had been pre-electrophoresed at 150 V for 2 hr. Gels were run at 150 V and fixed as above for 30 min, then in 10% methanol/5% acetic acid/85% water for 30 min. After incubation with PPO as above for 1 hr, gels were stained for 15 min with 0·1% (w/v) Toluidine Blue in 1% (v/v) acetic acid, destained in 1% (v/v) acetic acid until the standards were just visible, and fluorographed. This rapid preparation of gels before drying minimised loss of radiolabel.

Degradative treatments

Alkaline β-elimination of glycosaminoglycan chains was performed by incubation of lyophilized samples in 0·1 M NaOH with 1 M $NaBH_4$ for 18 hr at 37° with neutralization by drop-wise addition of 25% (v/v) glacial acetic acid.[12] Nitrous acid degradation of heparan sulphate was carried out at room temperature for 2 hr in 0·25 M HCl containing 20% (v/v) N-butyl nitrite in ethanol.[13] Chondroitinase ABC digestion was performed in 1 ml 10 mM Tris-HCl, pH 8·0, with 0·2 units enzyme for 24 hr at room temperature followed by boiling for 20 min. Control degradations of 2 mg heparin or chondroitin sulphate were conducted in parallel, with undegraded glycosaminoglycan detected by precipitation with 100 μl 10% (w/v) cetylpyridinium chloride.

RESULTS

Incorporation of [^{35}S]sulphate

Incubation of cells with [^{35}S]sulphate resulted in macromolecular incorporation of label into both cell-retained and secreted fractions (Table 1). For T cells, and RDM-4 cells grown *in vivo*, there was approximately equal incorporation into both fractions. In contrast, EL-4 and RDM-4 lymphoma cells grown *in vitro* showed a markedly higher incorporation into the cellular fraction, only around 25% being found in the medium. EL-4 cells, and RDM-4 cells grown *in vivo*, reproducibly showed a several-fold higher incorporation of label than normal T cells (Table 1). Use of normal RPMI medium in place of the low-sulphate equivalent produced no change in the distribution of the incorporated label between cellular and secreted fractions, despite the several-fold lower, total incorporation.

Table 1. Incorporation of [^{35}S]sulphate and glycosaminoglycan composition of radiolabelled macromolecules

	Incorporation of [^{35}S]sulphate				Glycosaminoglycan composition			
	(d.p.m./10^7 cells/4 hr)		% total incorporation		Secreted		Cell-retained	
	Secreted	Cell retained	Secreted	Cell retained	% heparan sulphate	% chondroitin sulphate	% heparan sulphate	% chondroitin sulphate
T cells	$1{\cdot}6\times10^5$	$2{\cdot}0\times10^5$ (6)	44	56	52	46	45	39
RDM-4								
in vivo	$5{\cdot}6\times10^5$	$6{\cdot}6\times10^5$ (4)	46	54	61	32	73	14
in vitro	$1{\cdot}2\times10^5$	$3{\cdot}2\times10^5$ (2)	27	73	34	53	59	25
EL-4	$3{\cdot}3\times10^5$	$12{\cdot}4\times10^5$ (4)	21	79	75	20	90	10

T cells were freshly isolated from murine spleens, EL-4 cells were grown *in vitro*, and RDM-4 cells were grown either *in vitro* or obtained from ascitic fluid of AKR mice (RDM-4 *in vivo*). Cells were labelled *in vitro* for 4 hr at 37° in sulphate-free RPMI medium containing 150 μCi [^{35}S]sulphate per 10^7 cells. Bio-Gel P-6 excluded radiolabelled material was treated with either nitrous acid (degrades heparan sulphate) or chondroitinase ABC (digests chondroitin sulphate), as described in the Materials and Methods. The percentage of the recovered activity which eluted at the V_t on Sepharose CL-6B gel filtration was determined. Figures in parenthesis indicate the number of labelling experiments performed.

Sepharose CL-6B gel filtration

The macromolecular radiolabelled material was analysed by gel filtration chromatography on Sepharose CL-6B. For T cells, and RDM-4 cells grown *in vivo*, the secreted fraction was eluted essentially as a single peak, K_{av} 0·33, containing >90% of the recovered activity (Figs 1a and 2a). The major proportion of the cellular material (65–85%) also eluted as a single peak, K_{av} 0·31. The remaining activity was eluted as two minor components, one at the void volume and the second following the major peak, K_{av} 0·62 (Figs 1b and 2b). Essentially similar elution profiles were obtained for cellular and secreted fractions from EL-4 and RDM-4 cells grown *in vitro*.[14,15]

To establish that the radioactivity was incorporated into proteoglycan, samples were subjected to alkaline β-elimination. After such treatment, gel filtration revealed a shift of the major peak of radioactivity to a higher elution volume for both cellular and secreted material from T cells (Fig. 1a,b). Similar shifts were seen for RDM-4 and EL-4 lymphoma cells (data not shown). This decrease in size showed that the major proportion of [^{35}S]sulphate had indeed labelled proteoglycan. Calibration of the column with globular protein standards indicated that the apparent MW of the major peak of radiolabelled proteoglycan from T cells and lymphoma cells was approximately 100,000. The fragments produced after alkaline β-elimination corresponded to a MW of around 13,000 by comparison with the calibration of Sepharose 6B for chondroitin sulphate by Wasteson.[16]

Characterization of glycosaminoglycan side chains

Specific degradation of heparan sulphate or chondroitin sulphate was performed using nitrous acid or chondroitinase ABC, respectively, followed by gel filtration. Such treatments resulted in a shift of a proportion of the activity to the total bed volume for T-cell secreted and cellular samples (Fig. 1 c–f). The sum of the incorporated label sensitive to nitrous acid and chondroitinase digestion was 84–100%, indicating that the [^{35}S]sulphate had been almost exclusively incorporated into glycosaminoglycan. As shown in Table 1, heparan sulphate and chondroitin sulphate were found in both the cellular and secreted fractions of all the cell types. T-cell secreted and cell-retained fractions possessed similar proportions of both glycosaminoglycans. For lymphoma cells heparan sulphate was found to be the major glycosaminoglycan of the cell-retained proteoglycans. Secreted proteoglycans from lymphoma cells were enriched two-fold in chondroitin sulphate compared to cellular preparations. However, heparan sulphate remained the major class of glycosaminoglycan of the secreted proteoglycans, with the one exception of RDM-4 cells grown *in vitro*.

SDS–PAGE

Cellular and secreted proteoglycans were further analysed by SDS–PAGE and subsequent fluorography. Figure 3, lanes 1 and 2, show the proteoglycan profiles for T cells. The major proportion of the incorporated label appears as a diffuse zone typical of proteoglycans, which are highly disperse in size. For cellular samples a significant amount of the radiolabel, 20% of the total, was present at the gel dye front (Fig. 3, lane 1). A much smaller proportion of this activity was seen in secreted fractions (Fig. 3, lane 2). As for T cells, the major proportion of radioactivity incorporated by lymphoma cells was observed as a diffuse band (Fig. 3, lanes 3–8). However, a marked difference was that the major diffuse zone of radiolabel appeared over a higher MW range, >180,000 MW and 84–180,000 MW for lymphoma cells and T cells, respectively. This was the case for both cellular and secreted fractions. It was also apparent that for RDM-4 cells grown *in vivo*, unlike all the other cell types, the cellular proteoglycans appeared to be of larger MW than those of the secreted fraction.

A striking feature of the cellular fraction from RDM-4 lymphoma cells was the presence of a well-defined, narrow band more characteristic of protein than proteoglycan, with an apparent MW 100,000 (Fig. 3, lanes 3 and 5). RDM-4 cells,

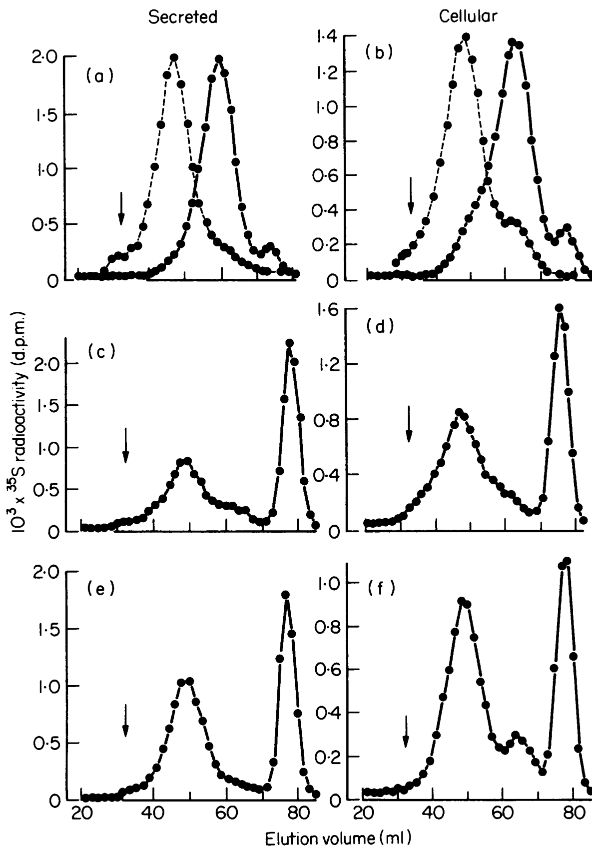

Figure 1. Sepharose CL-6B gel filtration of cell-retained and secreted radiolabelled material from normal T cells. Bio-Gel P-6 excluded material from the secreted fraction, (a) (---), or Triton X-100 extracted normal T cells, (b) (---), was applied to the column (1·0 × 100 cm; V_t 78·5 ml) and eluted with 50 mM sodium acetate, pH 5·8, containing 4 M guanidine hydrochloride, 0·1% (w/v) Zwittergent 3-08 and protease inhibitors (see the Materials and Methods). The remaining profiles show the change in elution after various degradative treatments (see the Materials and Methods): alkaline β-elimination (a,b); nitrous acid degradation (c, d); chondroitinase ABC digestion (e, f); (a, c, e) secreted samples; (b, d, f) cell-retained samples; 400-μl aliquots of fractions (1·8 ml) were counted. The arrows indicate the position of the void volume.

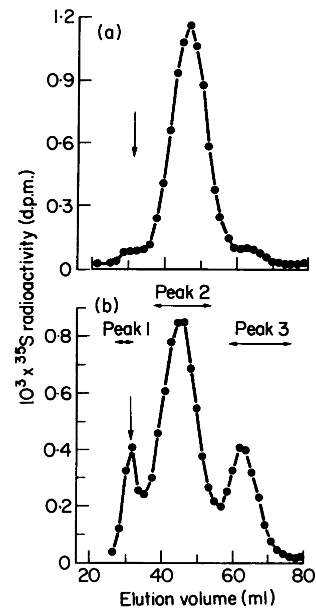

Figure 2. Sepharose CL-6B gel filtration of the cell-retained and secreted radiolabelled material from RDM-4 cells grown *in vivo*. Bio-Gel P-6 excluded radiolabelled material from the secreted fraction (a) or Triton X-100 extracted cells (b) was applied to the column as described for Fig. 1. Lines in (b) define peaks used in subsequent experiments.

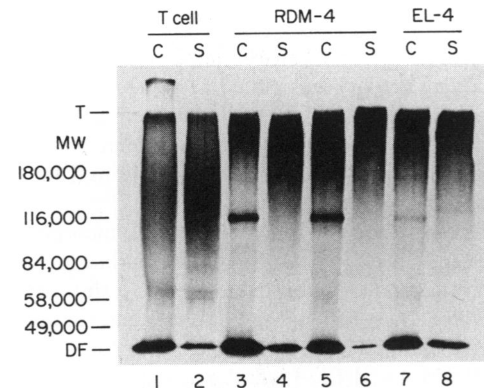

Figure 3. SDS–PAGE of cell-retained and secreted ^{35}S-labelled material from normal T cells, and RDM-4 and EL-4 lymphoma cells. Bio-Gel P-6 excluded radiolabelled material was analysed using a 5% stacking gel and 7% separating gel. Lanes show normal T cells, cell-retained (1) and secreted (2); RDM-4 cells grown *in vivo*, cell-retained (3) and secreted (4); RDM-4 cells grown *in vitro*, cell-retained (5) and secreted (6); EL-4 cells grown *in vitro*, cell-retained (7) and, secreted (8). Each lane was loaded with 3000 d.p.m. The positions of the globular protein markers are indicated with their size in MW. T, the top of the separating gel; DF, the dye front.

whether grown *in vivo* or *in vitro*, contained the greatest proportion (15%), but a similar band was faintly observed for EL-4 cells (lane 7). No 100,000 MW band was seen in the cellular fraction from T cells, or in any of the secreted fractions.

The relationships between the various components identified by gel filtration and those separated by SDS–PAGE were determined using the cellular fraction from RDM-4 cells grown *in vivo*. Pooled fractions of the three peaks from gel filtration (Fig. 2b) were run on 7% gels. As shown in Fig. 4, the major proteoglycan fraction from gel filtration, peak 2, corresponds to the major diffuse band on the gel. The excluded radiolabel, peak 1, is comprised almost entirely of the 100,000 MW band seen on the gels. The smaller included peak, peak 3, appears solely at the dye front.

The presence of cell-retained free heparan sulphate chains

The major gel filtration peak of proteoglycan, peak 2, eluted, after alkaline β-elimination, at the same volume as peak 3, suggesting that peak 3 may consist of free glycosaminoglycan chains. Since peak 3 eluted at the dye front on SDS–PAGE, further analysis was performed using a continuous gel system. The cellular fraction from RDM-4 cells grown *in vivo* may be separated into three components of activity (Fig. 5a, lane 1). An intense band of activity at the top of the gel is the major proteoglycan species, peak 2 (lane 3). Peak 3 runs as a diffuse

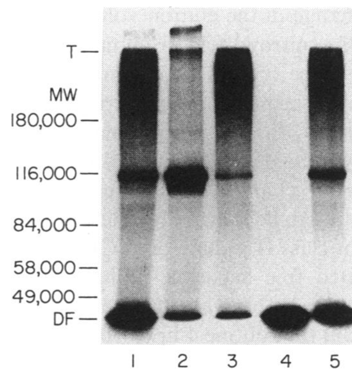

Figure 4. SDS–PAGE of Sepharose CL-6B gel filtration peaks from the cell-retained fraction of RDM-4 cells grown *in vivo*. Pooled peak fractions from gel filtration (see Fig. 2b) were dialysed against 50 mM ammonium acetate, pH5·8, and lyophilized before analysing by SDS–PAGE (5% stacking gel, 7% separating gel). Lane 1, complete cell-retained sample, 3000 d.p.m.; lane 2, peak 1, 1500 d.p.m.; lane 3, peak 2, 1500 d.p.m.; lane 4, peak 3, 1500 d.p.m.; lane 5, complete cell-retained sample, 1500 d.p.m. The positions of the globular protein markers are indicated. T, the top of the separating gel; DF, the dye front.

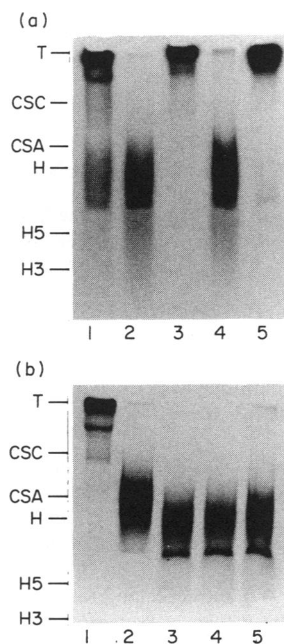

Figure 5. Continuous SDS–PAGE of Sepharose CL-6B gel filtration peaks from RDM-4 cells grown *in vivo*. Pooled peak fractions of cell-retained material (see Fig. 2b) were either subjected to alkaline β-elimination or left untreated and loaded onto a 15% acrylamide continuous gel. (a) Lane 1, complete cell-retained sample; lane 2, peak 3; lane 3, peak 2; lane 4, peak 3; lane 5, complete secreted sample; lanes 1, 4 and 5, 6000 d.p.m., lanes 2 and 3, 3000 d.p.m. (b) Lane 1, peak 2; lane 2, peak 2 alkaline β-eliminated; lane 3, peak 3 alkaline β-eliminated; lane 4, peak 3 subjected to mock alkaline β-elimination; lane 5, peak 3 untreated; 2000 d.p.m./lane. The glycosaminoglycan markers used are CSC, chondroitin sulphate C, 40,000–80,000 MW; CSA, chondroitin sulphate A, 45,000–50,000 MW; H, heparin, 6000—20,000 MW; H5, heparin, 5000 MW; H3, heparin, 3000 MW; 15 μg/lane. The midpoints of the marker bands are as indicated.

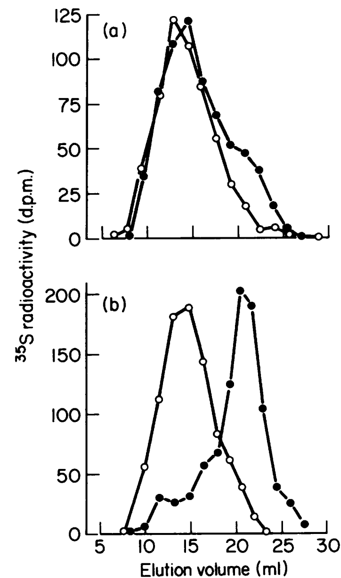

Figure 6. Bio-Gel P-6 gel filtration of peak 3 activity after chondroitinase ABC digestion or nitrous acid degradation. Pooled peak 3 fractions from Sepharose CL-6B gel filtration of the cell-retained fraction from RDM-4 cells grown *in vivo* were dialysed against 50 mM ammonium acetate, pH 5·8, and lyophilized. After nitrous acid degradation or chondroitinase ABC digestion as described in the Materials and Methods, samples were applied to a Bio-Gel P-6 column (1·6 × 10 cm) and eluted with 50 mM sodium acetate, pH 5·8, containing 4 M guanidine hydrochloride. (a) (●) chondroitinase ABC digested sample; (○), undegraded sample. (b) (●), nitrous acid degraded sample; (○), control degradation.

band of radiolabelled material with a MW of around 11,000 (lanes 2 and 4). The discrete band running ahead of the peak 2 activity is the 100,000 MW protein identified in Fig. 3 (data not shown). The secreted fraction (lane 5) was found again to contain only proteoglycan.

To confirm the glycosaminoglycan nature of peak 3, it was subjected to alkaline β-elimination. After such treatment no change in the size of the radiolabelled material was observed (Fig. 5b, lanes 3–5). Alkaline β-elimination of peak 2 obtained from the same gel filtration experiment showed the expected shift to a smaller size comparable to that for peak 3 (Fig. 5b, lanes 1 and 2). Peak 3 was subjected to chondroitinase ABC digestion or nitrous acid degradation and subsequent Bio-Gel P-6 chromatography. These experiments indicated that this free glycosaminoglycan contains exclusively heparan sulphate chains (Fig. 6a,b).

DISCUSSION

Previous studies have shown that lymphoid cells incorporate sulphate into glycosaminoglycan.[1,17,18] However, in only one report, on human T and NK cells, has it been shown that these glycosaminoglycans occur in the form of intact proteoglycans.[7] Our more detailed analyses demonstrate that the ^{35}S-sulphated macromolecules synthesized by murine T lymphoid cells are predominantly proteoglycan. The secreted macromolecules consist almost entirely of heparan sulphate and chondroitin sulphate proteoglycans. The cell-retained sulphated material, however, is more complex. The major component, comprising

65–85%, is proteoglycan, which is of similar size to the secreted proteoglycan and also possesses both heparan sulphate and chondroitin sulphate glycosaminoglycans. In addition, the cellular material contains a labelled component (20%), which runs at the dye front on discontinuous gel electrophoresis. The elution of this fraction from Sepharose CL-6B coincides with that of the proteoglycan after alkaline β-elimination. The same observation for human cells by Christmas *et al.*[7] led them to suggest that this peak might be free glycosaminoglycan. Here we show unequivocally that this material is free heparan sulphate glycosaminoglycan. Estimates of size by gel filtration and continuous gel electrophoresis are consistent at around 12,000 MW.

An unexpected observation on SDS–PAGE was the presence of an intense, discrete band of sulphate-labelled material from RDM-4 lymphoma cells. This band has an apparent MW of 100,000, as determined by gel electrophoresis, but is excluded on Sepharose CL-6B chromatography ($MW > 1 \times 10^6$). We interpret this to be a highly sulphated protein which can exist as an aggregate even under the dissociating conditions used for gel filtration, namely 4 M guanidine hydrochloride. Given the large number of acceptor sites for [^{35}S]sulphate available in proteoglycans, it is remarkable that in RDM-4 cells such a high percentage of radiolabel is found in a single discrete band. We are attempting to characterize and identify this sulphated protein.

Our data showing that T lymphocytes and lymphoma cells synthesize both heparan sulphate and chondroitin sulphate proteoglycans differ from the only previous characterization of lymphoid cell proteoglycans in which no heparan sulphate was detected.[7,19] However, in that work human peripheral blood T lymphocytes and NK cells were studied and these had been expanded by culture for 10–14 days with IL-2 and mitogen prior to labelling. The synthesis and secretion of both heparan and chondroitin sulphate glycosaminoglycans from freshly isolated lymphocytes has, however, been reported elsewhere.[1,3,17,18] We show that proteoglycans with glycosaminoglycans of both classes are abundant in each fraction analysed, whether cell-retained or secreted. The lymphoma cells in particular tend to retain heparan sulphate proteoglycans and secrete chondroitin sulphate proteoglycans.

Previous studies of proteoglycan biosynthesis in lymphoid cells have used incubations of 24 hr or longer and shown 85–90% incorporation into the secreted fraction.[7,17–19] The 4 hr labelling period used here results in a more equal distribution of label between secreted and cell-retained pools. Our findings show that both growth conditions and proliferative transformation markedly influence proteoglycan synthesis and secretion. This observation, together with the documented effect of mitogen stimulation on glycosaminoglycan synthesis, supports the view that proteoglycan synthesis and secretion in T lymphoid cells are highly regulated processes. Overall our present findings indicate no major qualitative change on T-cell transformation despite the apparent increased rate of synthesis.

The existence of proteoglycans containing heparan sulphate and chondroitin sulphate may indicate the presence of either multiple proteoglycans, each bearing one class of glycosaminoglycan, or hybrid proteoglycans, in which both glycosaminoglycans are attached to the same core proteins. One such hybrid is syndecan, an epithelial proteoglycan[20] which is also expressed on pre-B cells but not on splenic lymphocytes.[21] However, since there was no change in the elution volume of the undegraded material following nitrous acid or chondroitinase ABC degradation, the existence of a high proportion of hybrid proteoglycan is unlikely. The proteoglycans are of relatively low MW, 100,000–200,000, with relatively small glycosaminoglycan chains. Thus lymphoid proteoglycans are likely to have few glycosaminoglycan chains on each core protein. This has already been found to be the case for the lymphocyte proteoglycans, variants of class II γ chain[5] and CD44.[6]

Cell-associated free heparan sulphate glycosaminoglycan chains were not detectable when the metabolic labelling period was reduced from the routine 4 hr to 30 min (data not shown). This finding indicates that the free heparan sulphate is not an artefact of extraction and suggests that it arises from the physiological degradation of heparan sulphate proteoglycan. Free glycosaminoglycan chains have been detected in various cells and it has been suggested that their synthesis and function may be independent of proteoglycans in general.[22]

Lymphocytes possess cell-surface receptors for heparin,[23] one such receptor being CD45.[24] CD4 also binds heparin and furthermore this interaction blocks its binding to the gp120 antigen of the HIV-1 virus.[25] Lymphocytes can also bind specifically heparan sulphate but not chondroitin sulphate isolated from murine spleens.[26] Our demonstration of the retention of free heparan sulphate raises the possibility that lymphocyte glycosaminoglycan receptors may be occupied by their own heparan sulphate. Thus cell-surface heparan sulphate may modulate the activity of CD4, and other lymphocyte surface glycoproteins.

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