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Extraction and separation of proteoglycans

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Abstract Proteoglycans contain a unique carbohydrate component, glycosaminoglycan, which consists of repeating, typically sulfated disaccharides, and is capable of interacting with diverse molecules. Specific, clustered arrangements of sulfate on the glycosaminoglycan backbone form binding sites for many biologically important ligands such as extracellular matrix molecules and growth factors. Core proteins of proteoglycans also show molecular interactions necessary for organizing scaffolds in the extracellular matrix or for anchoring proteoglycans to the plasma membrane. Experimental protocols aiming at extracting maximal amounts of proteoglycans from tissues or cells require disruption of molecular interactions involving proteoglycans by denaturing solvents. Among many of the proteoglycan separation procedures, anion exchange chromatography, which takes advantage of the presence of highly negatively charged glycosaminoglycans in all proteoglycans, serves one of the most convenient general separation techniques.

Keywords Proteoglycan · Glycosaminoglycan · Extraction · Separation

Introduction

Proteoglycans are a class of glycosylated proteins characterized by the presence of glycosaminoglycans as a carbohydrate component, which endows proteoglycans with unique biochemical as well as biological properties. The composition of proteoglycans vary widely, for example, the number of glycosaminoglycan chains per molecule could be as many as over 100 in aggrecan, the major proteoglycan in cartilage, or as few as just one in decorin. There are four general classes of glycosaminoglycans:[1] (1) chondroitin sulfate and dermatan sulfate, (2) heparan sulfate and heparin, (3) keratan sulfate, and (4) hyaluronan (which is not synthesized on a core protein and, therefore, is not classified as a component of a proteoglycan). Glycosaminoglycans consist of linear polymers of repeating (typically 50–100 repeats[2]) disaccharides of defined structure which consists of one hexosamine, which often contains one or more sulfate residues,[3] and either a hexuronic acid or a

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[1] Discussions on biochemical and biological characteristics of each glycosaminoglycan can be found in excellent reviews published elsewhere [1–4].

[2] Except hyaluronan, which is synthesized at the plasma membrane, contains many more repeating disaccharides up to ~25,000 per chain, and does not undergo sulfation.

[3] In many studies on biological functions of proteoglycans, cell culture and tissue explant systems in combination with metabolic radiolabeling techniques are employed. In these experiments, monitoring incoporated radioactivity provides excellent measures to follow proteoglycans through extraction and purification steps and to evaluate recoveries at each experimental step. Due to characteristic sulfation in the glycosaminoglycan moiety, [^{35}S]sulfate is an excellent precursor for metabolic labeling of glycosaminoglycans, thus proteoglycans. In many cell culture systems, more than ~90% of radioactive incorporation of ^{35}S from [^{35}S]sulfate is accounted for by the incorporation into glycosaminoglycans. Concomitant use of other radioactive precursors such as those labeled with ^{3}H, which can be differentiated from ^{35}S by spectral analysis, provides further detailed information about molecular structures and metabolic state of the cell. For example, the use of [^{3}H]glucosamine, as shown in the present manuscript, allows labeling hexosamines ([^{3}H]glucosamine is metabolically converted into both [^{3}H]glucosamine and [^{3}H]galactosamine) in all glycoconjugates synthesized by the cell. Further details on metabolic labeling techniques using cell cultures should be consulted elsewhere [5].

hexose in keratan sulfate. Specific spatial arrangements of clustered sulfate residues on glycosaminoglycans provide binding sites for various biologically active molecules, which regulate cell functions (an aspect of proteoglycan functions extensively studied [1–4]). In many biological circumstances, highly negatively charged glycosaminoglycan structures also attract many positively charged molecules in less specific manners.

Proteoglycans widely distribute as major molecular components in most extracellular matrices (representative molecules include aggrecan, versican, decorin and perlecan among many), as integral plasma membrane proteins (including syndecan and glypican families), and as an intracellular molecule (serglycin) associated with storage granules in mast cells. Relatively limited number, over 50 separate gene products, of core proteins have been identified, and they show specific spatial as well as temporal expression patterns. The core proteins fundamentally define biochemical compositions of proteoglycans under genetic control and direct cell biological behavior of the molecules, *e.g.,* posttranslational modification, trafficking through intracellular compartments, and targeting to their destinations *etc*. In extracellular matrices, proteoglycans form highly ordered scaffolds through specific molecular interactions with other major matrix molecules (binding of core proteins of aggrecan and versican to hyaluronan, and binding of decorin to collagens are good examples). On the plasma membrane, core proteins of syndecan family proteoglycans are intercalated into the plasma membrane, while those of glypicans are linked to glycosylphosphatidylinositol (GPI)-anchors. Understanding molecular interactions involving proteoglycans, especially those with major extracellular molecules, is key in studying biological functions of proteoglycans and in designing experiments extracting proteoglycans from extracellular matrices.

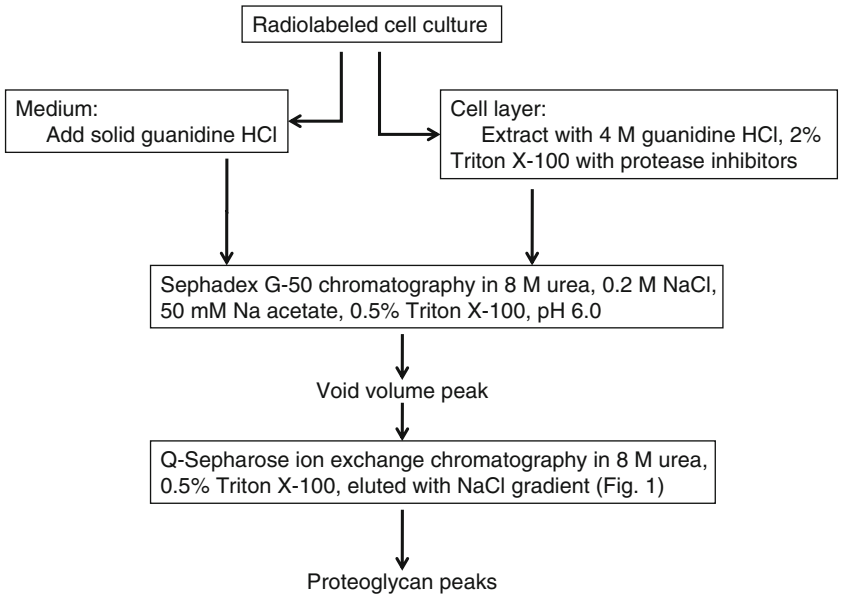
General considerations on extraction and separation of proteoglycans Proteoglycans have been recognized as a crucial hub in organizing supramolecular scaffolds in the extracellular matrices by their binding to many extracellular matrix molecules through multiple binding domains in glycosaminoglycans and core protein. Thus, intact proteoglycans generally resist extraction in associative solvent conditions, which preserve molecular interactions, and require the use of dissociative solvent conditions for efficient extraction from the tissue [5, 6]. Dissociative conditions of high guanidine HCl concentrations (ordinarily at 4 M) have been most widely employed. For the extraction of cell-associated proteoglycans, the concomitant use of a detergent is necessary. Most non-ionic detergents (such as Triton X-100 and NP-40 with low critical micellar concentration, CMC) or zwitter-ionic detergents (such as 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate, CHAPS, with high CMC) are compatible with 4 M guanidine HCl. In addition, the use of detergent, not only at the time of proteoglycan solubilization from the cell membrane, but also throughout purification procedures, has been shown to dramatically improve recoveries of proteoglycans in most experimental procedures.

Because of their exceptional diversity in molecular constructions and in properties of molecular interactions, there is no single set of extraction or separation procedure which is usable for every proteoglycan. Therefore, the techniques described below illustrate general principles that can be successfully modified in different combination for characterizing a particular proteoglycan. This manuscript focuses on experimental procedures, which are particularly useful obtaining total proteoglycan and glycosaminoglycan species non-selectively and in high yield from various biological sources. Other reviews on similar subjects should be consulted for more detailed discussions on specific subjects [5–8].

Materials and methods

Proteoglycan material Rat ovarian granulosa cell cultures radiolabeled with [^{35}S]sulfate and [^{3}H]glucosamine are used as a starting material containing proteoglycans [9]. See footnote 3 for detailed discussion on metabolic labeling experiments using cell cultures.

Extraction At the conclusion of cell culture, medium is removed from the cell culture dish and analyzed separately (Scheme 1). Approximately 2 ml (per 35 mm diameter cell culture dish) of extraction buffer (4 M guanidine HCl, 0.05 M Na acetate, pH 6.0, containing 2% (*w*/*v*) Triton X-100 and a cocktail of protease inhibitors [*i.e.,* 1 mM 4-(2-aminoethyl)benzenesulfonylfluoride (AEBSF, from Calbiochem), 10 mM *N*-ethylmaleimide (NEM), 10 mM disodium ethylenediaminetetraacetic acid (EDTA)] [10] is then added to a culture plate. AEBSF and a stock solution of NEM (100 × concentrated in ethanol) are added to the buffer just prior to use (as they are relatively unstable in aqueous solutions). Proteoglycans are extracted within 2–3 h of constant shaking at 4°C. When the extraction is complete, vigorous vortexing of the sample for 1–2 min allows fragmentation of macromolecular DNA by shear forces, making samples amenable for easy handling in the subsequent chromatographic procedures. As used originally in the extraction of proteoglycans from the cartilage tissue [6], guanidine HCl at 4 M concentration generally provides



Scheme 1 A flow diagram of proteoglycan separation using radiolabeled cell culture

excellent solubilization of proteoglycans from most tissues. When solid tissues are to be extracted, approximately ten times volume of 4 M guanidine HCl buffer is used to solubilize proteoglycans after finely mincing the tissue and incubating for 12 h at 4°C. Ordinarily, only gentle agitation is required for efficient extraction. Extraction of proteoglycans from calcified tissues often requires additional decalcification solvents, which can be prepared by including a high concentration of EDTA (*e.g.,* at 0.5 M) [11]. Although secreted proteoglycans in cell culture media are generally already soluble, in order to minimize interactions between highly charged proteoglycans and other molecules, solid guanidine HCl (0.53 g solid guanidine HCl per ml of media makes 4 M guanidine HCl solution) and a protease inhibitor cocktail at concentrations described above are directly added to the medium. Usually, omission of detergents for the extraction of proteoglycans in the medium compartment is not critical at this stage, as, perhaps, samples contain only limited amounts of hydrophobic elements (as long as detergents are included in subsequent chromatographic steps, see below).

The cells or tissues usually contain several different species of proteoglycans, some of which are integrated in the extracellular matrix by noncovalent interactions, associated with the plasma membrane through hydrophobic interactions either by intercalation of core protein or by phosphatidylinositol anchors, and as a content of intracellular granules. The extraction of proteoglycans usually aims at solubilizing maximum amounts of proteoglycan without degradation, which requires the use of denaturing solvents. For this purpose, 4 M guanidine HCl containing detergent is the solvent employed most often.[4] Inclusion of an appropriate detergent is not only crucial when extracting cell-associated proteoglycans, but also is generally beneficial achieving good recovery in many experimental procedures. Since proteoglycans can be easily degraded, especially by proteases, inclusion of a protease inhibitor cocktail is desirable. Inhibitors, which introduce covalent modification of amino acids, have been shown to be most effective [10]. For solid tissues such as tissue explants, efficiency of extraction can be drastically improved by fine mincing [6].

Solvent exchange In order to prepare the extracted proteoglycans in 4 M guanidine HCl for anion exchange chromatography procedure in the next step, guanidine

[4] Additional consideration should be made when one is attempting to extract cell-associated proteoglycans; *i.e.,* inclusion of sufficient amounts of detergent may be necessary (such as 2% Triton X-100, a non-ionic detergent, or 1% CHAPS, a zwitter-ionic detergent) for the solubilization of proteoglycans [5]. It should be noted that critical micellar concentration of detergents could change drastically in chaotropic solvents such as guanidine HCl or urea [12], and some of the detergents are not soluble in such solvents. Adding the detergent from the beginning of extraction is highly preferred in order to completely disrupt hydrophobic interactions, since irreversible, artificial molecular complexes may be formed between newly exposed hydrophobic sites in proteins during the process of denaturation by 4 M guanidine HCl.

HCl has to be replaced with a solvent compatible with the procedure. A preferred solvent is a urea buffer since it disrupts molecular interactions by interfering with the formation of hydrogen bonds. A convenient buffer exchange procedure can be done by gel filtration (such as Sephadex G-50 chromatography) using a small disposable pipette.[5] This process is also convenient to remove unincorporated radioactive precursors, when radiolabeled cell cultures were extracted with 4 M guanidine HCl solvent.

Separation of proteoglycans Separation of proteoglycan species can be achieved by taking advantage of their biochemical characteristics, especially those of glycosaminoglycans, including high negative charge, large hydrodynamic size, high buoyant density, *etc*. Hydrophobicity or biological recognition by various ligands can also be utilized.

A. Charge—Anion exchange chromatography described here is an excellent single step procedure to separate proteoglycans from other molecules[6]; all proteoglycan species can be collected since they all possess highly negatively charged glycosaminoglycan components by definition. In some experiments, this separation technique alone provides samples of sufficient purity in proteoglycan content. In addition, since different kinds of glycosaminoglycans tend to have distinct charge properties based on the extent of sulfation, separation of proteoglycan species based on their glycosaminoglycan composition may, at least partially, be achieved. For example, chondroitin sulfate is generally more negatively charged than most heparan sulfate (in Fig. 1, two distinct ^{35}S-labeled proteoglycan peaks are observed in the NaCl gradient; a peak eluting at lower NaCl concentrations (indicated by a horizontal

[5] Solvent exchange by Sephadex G-50 chromatography in a disposable pipette—Preswell SephadexG-50, fine (obtained from GE Healthcare Life Science) in hot water off the heater, which achieves sterilization, degassing and shortening of swelling time. An extreme caution should be exercised when adding Sephadex powder to boiling water to avoid flushing. A convenient concentration of gel (50% slurry) can be made by mixing 5 g of Sephadex G-50 with 100 ml water. Bacteriostatic agents (*e.g.,* 0.02% Na azide) should be added for a long-term storage. Pour preswollen Sephadex G-50 into a 10 ml plastic disposable pipette (Falcon), which was cut at the top with a file and plugged with glass wool (no. 3950, Corning) at the bottom, to make 8 ml bed volume. Remove excess water and equilibrate the column with a buffer (8 M urea, 0.20 M NaCl, 0.05 M Na acetate, 0.5% Triton X-100, pH 6.0, a total of 9 ml is sufficient to equilibrate the column). Carefully prepare a flat gel surface with a glass Pasteur pipette and remove excess urea buffer. Apply 2 ml sample and discard the eluent. After the entire sample is in the column, carefully overlay 3 ml of buffer and collect eluent until the entire buffer is in the column (3 ml of V_0 fraction collected). This fraction contains proteoglycans and other macromolecules in 8 M urea buffer, while leaving small molecules in the original extract (guanidine HCl, isotope precursors *etc*.) behind in the column. At this point, column can be safely disposed as a radioactive waste. Dimension of the column may be changed proportionately when sample size varies.

[6] Q-Sepharose chromatography—Q-Sepharose, fast flow (GE Healthcare Life Science) has to be pre-equilibrated with the low salt buffer (8 M urea, 0.20 M NaCl, 0.05 M Na acetate, 0.5% Triton X-100, pH 6.0) used in the NaCl gradient. Two ml of preequilibrated Q-Sepharose (1 ml of Q-Sepharose can bind up to 3–5 mg of proteoglycans) is packed into a small column (10 ml plastic pipette is cut by a file and plugged with glass wool at the bottom). Alternatively, 2 ml of preequilibrated Q-Sepharose is mixed with the sample in 8 M urea buffer (of any volume) and gently shaken for 1 h, then packed into the column; this latter method gives uniform binding of proteoglycans to Q-Sepharose gel resulting in a better flow property, especially when a large quantity of materials (proteoglycan, protein, nucleic acid, *etc*.) is used. After sample application, the column is washed with 10 ml of the low salt buffer. Then the column is connected to a gradient former (using, for an example, a high salt buffer: 8 M urea, 1.5 M NaCl, 0.05 M Na acetate, 0.5% Triton X-100, pH 6.0) and eluted with approximately a total 40 ml of buffer with a flow rate of 10–15 ml/h. Every 1–2 ml fraction is collected and monitored for NaCl concentration by conductivity measurement, Fig. 2. Eluent fractions are monitored for proteoglycans by radioactivity detection or colorimetric procedures, such as a convenient and safe colorimetric procedure using Safranin O [6, 13] or a classic procedure measuring uronic acid by m-phenylphenol reactants [14], which can be modified to use a microtiter plate for easier handling. Typically, heparan sulfate proteoglycans are eluted in a peak at approximately 0.5 M NaCl and chondroitin sulfate proteoglycans at 0.65 M NaCl. The use of step elution for the purpose of differentially eluting proteoglycan species is not recommended unless salt concentrations in which individual proteoglycans elute are widely separate. Determination of the exact salt concentration which enables clear separation of proteoglycan species may be rather delicate. One of the major technical problems associated with anion exchange chromatography of proteoglycans, especially when purifying molecules are present in small quantities (*e.g.,* isolation of proteoglycans from cell cultures), is poor recovery. This can be, in most cases, overcome by the use of detergents (either non-ionic or zwitter-ionic) in elution buffer. Routinely, the use of 0.5% (*w*/*v*) Triton X-100 dramatically improves recovery of proteoglycans (even glycosaminoglycans) from ion exchange columns. Most non-ionic detergents (such as Triton X-100 and NP-40) possess strong absorbance in the UV range, thus making the UV tracing for protein detection difficult. If this causes problems in the analysis, non-UV absorbing, non-ionic detergents such as Genapol X-100® (Calbiochem) can be used with virtually the same chromatographic result. Also, when the removal of detergents in later experimental steps is required, the use of ones with high CMC (such as CHAPS, Calbiochem) in place of Triton X-100 is beneficial.

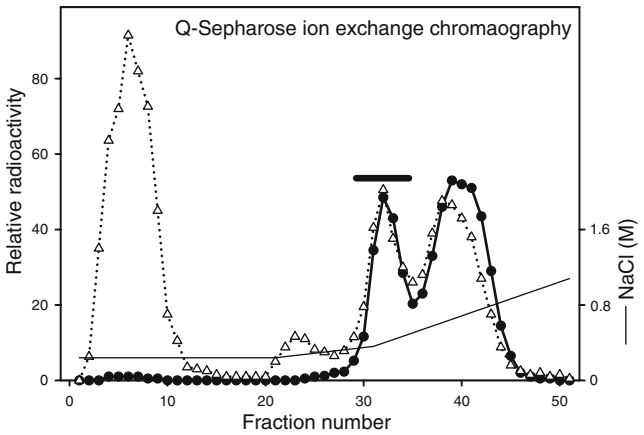


Fig. 1 Q-Sepharose anion exchange chromatography. Extract of a cell culture metabolically labeled with [^{35}S]sulfate and [^{3}H]glucosamine [9] is analyzed. A large peak at the breakthrough containing ^{3}H-labeled (*unfilled triangle* and *dot line*) macromolecules, which mostly represented glycoproteins, is efficiently separated from ^{35}S-labeled (*filled circle* with *solid line*) proteoglycan peaks eluting later in high salt fractions. The earlier ^{35}S-labeled peak (indicated by a *horizontal bar* and used for gel filtration analysis shown in Fig. 2) contains mostly heparan sulfate proteoglycans and the latter mostly chondroitin/dermatan sulfate proteoglycans

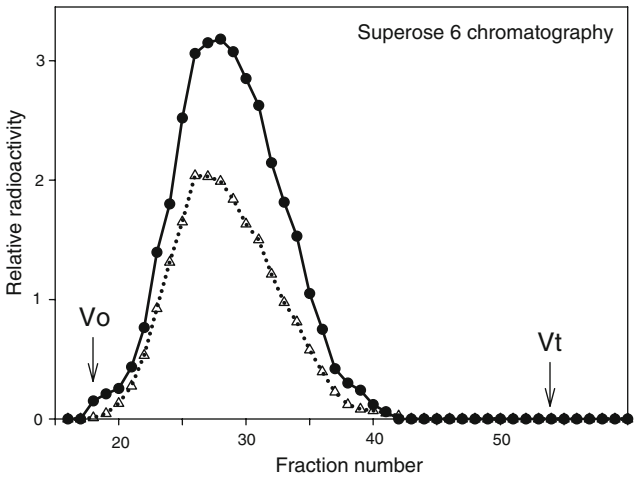


Fig. 2 Superose 6 chromatography of the first ^{35}S-labeled proteoglycan peak (as indicated by a *bar* in Fig. 1, representing heparan sulfate proteoglycan) is analyzed in 4 M guanidine HCl, 0.5% Triton X-100. A cell culture is metabolically labeled using [^{3}H]glucosamine (*unfilled triangle* and *dot line*) and [^{35}S]sulfate (*filled circle* with *solid line*). Void volume (V_0) and the column total volume (V_t) are indicated by *arrows*

bar) contains mostly heparan sulfate proteoglycans, while that eluting in higher NaCl concentrations contains mostly chondroitin/dermatan sulfate proteoglycans). Heparin is more highly sulfated than chondroitin sulfate and is eluted at even higher concentrations of NaCl. Ion exchange chromatography is generally a step allowing both purification and concentration at the same time, a useful tip to remember, when performing multiple sequential procedures.

B. Molecular size—Hydrodynamic sizes of proteoglycans are generally large because of the contribution by glycosaminoglycans, but vary greatly by species. In addition, proteoglycans typically show considerable molecular size microheterogeneity because of variation in glycosaminoglycan number or composition in a single proteoglycan molecule. Because of these molecular characteristics, many frequently used procedures such as gel electrophoresis do not provide desirable, clear separation of proteoglycans (see later section on analytical methods). Gel filtration chromatography has been used more successfully in many experiments. It also has additional advantages of providing quantitative information about the recovery of materials in the procedure, and if appropriate combinations of elution buffer and gel material are used, excellent recovery may be expected. For the analysis of proteoglycan species with large hydrodynamic sizes such as aggrecan and perlecan, Sephacryl S-500 or S-1000, Sepharose CL-2B (gel filtration media all from GE Healthcare Life Science) are appropriate, while proteoglycans with smaller hydrodynamic sizes such as decorin, biglycan, cell surface heparan sulfate proteoglycans or partially degraded proteoglycans can be analyzed by Superose 6, HR 10/30 (GE Healthcare Life Science). In all cases, the use of 4 M guanidine HCl, 0.05 M Na acetate, 0.5% (*w*/*v*) Triton X-100, pH 6.0 allows analysis of molecules in dissociated, monomeric forms. The use of associative solvents, such as guanidine HCl buffer below 0.5 M concentrations, on the other hand, permits some specific molecular interactions involving proteoglycans to occur, for example, association of aggrecan or versican with hyaluronan. As an illustration, gel filtration analysis of cell surface heparan sulfate proteoglycan purified by Q-Sepharose chromatography[7] is shown in Fig. 2.

C. Buoyant density—Glycosaminoglycans have high buoyant densities in CsCl equilibrium density gradient because of their intrinsically high buoyant density and the presence of a large number of anionic groups, which bind to dense cesium ion. Thus, this procedure allows efficient separation of proteoglycans with high glycosaminoglycan-to-protein ratios (their densities often reach almost as high as those of nucleic acids) from bulk proteins. This procedure is particularly

[7] Superose 6 chromatography—A Superose 6 column (HR 10/30, 1× 30 cm) is preequilibrated with 4 M guanidine HCl, 50 mM Na acetate, pH 6.0 containing 0.5% Triton X-100 and eluted with the same buffer at a flow rate of 0.4 ml/min and fractions of 0.4 ml are collected for measurement of proteoglycan content (by radioactivity or colorimetric analysis [6, 13, 14]). Inclusion of detergent in the elution buffer is critical to obtain quantitative recovery. Most efficient detergents in this respect have been non-ionic detergent such as Triton X-100.

suited for the purification of proteoglycans with many glycosaminoglycan chains (*i.e.,* the procedure has been extensively used to separate and characterize proteoglycans in cartilage tissues [6]). On the other hand, the procedure is not suitable for isolating proteoglycans with only few glycosaminoglycans or with high protein contents (*e.g.,* 'small, leucine-rich proteoglycans' and cell surface heparan sulfate proteoglycans). One advantage of the procedure is that experiments can be performed in associative solvent conditions retaining specific molecular interactions involving proteoglycans, thus enabling to isolate molecules which show specific interactions with proteoglycans [6].

Separation of selective proteoglycans Above described separation techniques, namely, anion exchange chromatography, gel filtration and buoyant density ultracentrifugation, separate proteoglycans non-selectively as long as their physical characteristics are similar. However, in some cases when individual proteoglycan species are analyzed, particular cells/tissues are used or experimental design demands special requirements, *etc*., additional separation techniques are required. Many such example cases have been compiled elsewhere [8], and can be consulted. Here, two additional relatively general procedures are discussed. They add further dimensions to the principles of proteoglycan separation and could provide means to separate hard-to-separate proteoglycans or hard-to-remove contaminants (such as nucleic acid, highly negatively charged glycoproteins, *etc*). Again, the procedures have been optimized mainly for separation and quantitative recovery.

Hydrophobic chromatography Hydrophobic [5] nature of core proteins could be used to separate proteoglycan species otherwise difficult to separate. Excellent separation of two similar proteoglycan species was achieved by the use of Octyl-Sepharose chromatography in the presence of 4 M guanidine HCl eluted with a detergent gradient [15].

Lectin affinity chromatography There have been reports of lectins, such as annexins and galectins, which specifically bind to glycosaminoglycans. As an example, an annexin, which specifically binds to heparan sulfate, has been used to purify heparan sulfate proteoglycans by affinity chromatography[8] [16], Fig. 3. One of the major contaminants

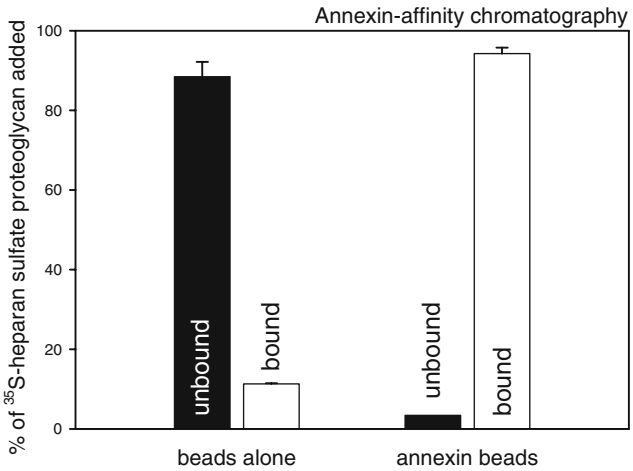


Fig. 3 Affinity chromatography using a lectin, annexin, which specifically binds to heparan sulfate [14]. Binding of radiolabeled heparan sulfate proteoglycan to unconjugated beads (*left columns*) and annexin-conjugated beads (*right columns*) is compared

encountered in proteoglycan specimens obtained by anion exchange chromatography, gel filtration and equilibrium density gradient centrifugation includes nucleic acid, and it could be efficiently removed by this procedure. Applicability of this method in other situations, however, needs to be further examined. Specific hyaluronan-binding properties of some proteoglycans (*i.e.,* hyaluronan-binding proteoglycans, such as aggrecan and versican) have been used extensively [6] for the purification and characterization of proteoglycans. In this case, lectin-like properties of proteoglycan are used.

Other analytical methods For non-radiolabeled samples, chemical or immunological detection of proteoglycans is required during separation procedures. It is not the objective of this manuscript to detail a comprehensive list of analytical procedures for proteoglycans, which can be consulted elsewhere [8]. Here, some of the selected analytical methods, which are recently developed and/or convenient to use, are discussed briefly. The use of polyacrylamide gel electrophoresis for the molecular size analysis of intact proteoglycans has been hampered because of extremely large hydrodynamic sizes of some proteoglycans (especially aggrecan, versican and perlecan, *etc*.) However, the use of polyacrylamide/agarose composite gel allows electrophoretic analysis of these large proteoglycans [17]. However, the poor transfer of proteoglycans from gel to membrane often precludes some useful procedures such as immunological detection with Western blot analysis. Identification of core proteins, on the other hand, is most often done with polyacrylamide gel electrophoresis, using core protein preparations generated by the treatment of proteoglycans with glycosaminoglycan degrading enzymes (*i.e.,* bacterial eliminases such as chondroiti-

[8] Annexin affinity chromatography—SH-derivatized Magnetic beads® (obtained from Promega) is conjugated with annexin. Binding of radiolabeled heparan sulfate proteoglycan to the beads is performed in 200 µl of buffer consisting of 10 mM Hepes, 50 mM NaCl, pH 7.0, containing 0.5%Triton X-100 and 1% BSA. Supernatant is removed after incubation at 4°C for 1 h. Washed beads are then eluted with 10 mM Hepes, 500 mM NaCl, 5 mM EDTA, pH 7.0, containing 0.5% Triton X-100, and the eluant is counted for radioactivity.

nase and heparitinase). Core proteins prepared by such a method form tight bands suitable for accurate molecular weight estimation as ordinary proteins, and they are also efficiently transferred to membranes used for immunological identification by specific antibodies raised against individual core proteins [18]. Some of the excellent analytical methods for glycosaminoglycans and their constituent disaccharides developed recently are also worth mentioning. They include capillary electrophoresis of fluorescently labeled glycosaminoglycans [19], and fluorophore-assisted carbohydrate electrophoresis [20, 21] for the analysis of constituent disaccharides of glycosaminoglycans. The former provides high speed and sensitivity analysis suitable for studying molecular interactions, such as those between glycosaminoglycans and growth factors.

Conclusion and future directions

Explosive expansion of genetic information and molecular biological techniques, together with rapidly accumulating knowledge on molecules using structural biological techniques greatly demands renewed efforts and refinements in biochemical analyses of proteins. Proteoglycans are no exception with this argument. The general techniques for proteoglycan separation described in this article only fulfill minimum necessities of proteoglycan research in the new generation, but hopefully they would aid us to catch up with the developments in other field of life sciences. Some of the already available high resolution analytical techniques, such as mass spectroscopy, for example, would provide extremely powerful biochemical analytical tool, when it becomes more accessible and is combined with other newer separation techniques. We hope that the days are not too far when the studies of life sciences are further accelerated by the concerted developments in all fields of life sciences to answer many more fundamental questions on life.

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