Proteoglycans from Bovine Proximal Humeral Articular Cartilage

STRUCTURAL BASIS FOR THE POLYDISPERSY OF PROTEOGLYCAN SUBUNIT

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Polydisperse proteoglycan subunit from bovine proximal humeral articular cartilage has been separated into a series of relatively monodisperse fractions which have been chemically and physically characterized. The proteoglycan subunit species of the lowest molecular weight contains the least chondroitin sulfate and had an amino acid composition relatively low in serine and glycine and relatively high in cysteine, methionine, and aspartic acid, almost identical to that of the hyaluronic acid-binding region of proteoglycan subunit isolated by Heinegard and Hascall (Heinegard, D., and Hascall, V. C. (1974) J. Biol. Chem. 249, 4250-4256).

The molecular weight of proteoglycan subunit increases in proportion to its chondroitin sulfate content. As the molecular weight and chondroitin sulfate content of proteoglycan subunit increase, there is a parallel increase in the serine and glycine contents, and a decrease in the cysteine, methionine, and aspartic acid contents of proteoglycan subunit core protein. The pattern of polydispersity observed strongly supports the concept that proteoglycan subunit core protein contains a hyaluronic acid-binding region of constant size and composition and a polysaccharide attachment region of variable length and composition, composed of repeating peptide sequences containing serine and glycine in equimolar amounts.

In native cartilage, most of the proteoglycan exists as aggregates (1-4) formed by the noncovalent association of proteoglycan subunit molecules with hyaluronic acid and link protein (5-12). Proteoglycan subunit is polydisperse in length (12, 13), molecular weight, and composition (14-19), the molecular weights of individual members of the polydisperse population increasing in proportion to their chondroitin sulfate contents and chondroitin sulfate to protein ratios. One fundamental question of current interest is the structural basis for the observed polydispersity of proteoglycan subunit.

In the work reported here, polydisperse proteoglycan subunit from bovine proximal humeral articular cartilage has been separated into a series of relatively monodisperse fractions. These fractions have been characterized in terms of chemical composition and sedimentation coefficients. The composition and size of the proteoglycan subunit fraction of lowest molecular weight closely resembles that of the hyaluronic acid-binding region of proteoglycan core protein (11). As the fractions increase in molecular weight, there is an increase in their chondroitin sulfate content and a parallel progressive increase in amino acid residues (Ser, Gly) found predominantly in the polysaccharide attachment region of proteoglycan subunit core protein. The results reported here support the concept (11) that proteoglycan subunit core protein contains a polysaccharide attachment region of variable length, composed of repeating sequences of peptides containing serine and glycine in equal amounts, which provide the attachment sites for chondroitin sulfate chains. The polydispersity of proteoglycan subunit appears related to the variable length of the polysaccharide attachment region of proteoglycan subunit core protein.

EXPERIMENTAL PROCEDURES

Materials—Pepstatin was provided by Dr. Alan Barrett, Strangeways Research Laboratories, Cambridge, England. Iodoacetic acid was from Schwarz/Mann, GdmCl "absolute grade" and CsCl "O.D. grade" were obtained from Research Plus Laboratories, Inc.

Analyses—The methods used were: uronate (20), hexose (21), hexosamine (22), sialic acid (23), sulfate (24), protein (25), hydroxyproline (26), glucosamine and galactosamine (27), and amino acid analyses (28). Fractions for amino acid analyses were hydrolyzed at 110° for 20 h in 6 N HCl containing 0.1% phenol. Half-cystine was determined as cysteic acid on separate samples after performic acid oxidation.

Extraction of Proteoglycan—Cartilage slices were rapidly removed from the articular surfaces of the proximal humeri of 1- to 2-year-old cattle and added immediately without dicing to ice-cold 2 M CaCl₂, 0.15 M potassium acetate, pH 6.3, containing 0.005 M iodoacetate and 2 mg of pepstatin/liter. The cartilage was extracted by slow stirring at 5° for 48 h. Extracts were filtered through nylon mesh, coarse sintered by guest on March 23, 2020http://www.jbc.org/Downloaded from
glass, and filter paper, then dialyzed against 10 volumes of 0.15 M potassium acetate, pH 6.3, at 5°C for 16 h.

Equilibrium Density Gradient Centrifugation under Associative Conditions—To 250 ml of reassociated extract, 177 g of CsCl was added slowly with gentle stirring while the pH was maintained at 6.3 at 5°C. If required, the final volume of the solution was brought to 300 ml with 0.15 M potassium acetate, pH 6.3. The final solution was 3.5 M CsCl, and its density at 10°C was 1.46 to 1.47 g/ml. Equilibrium density gradient centrifugation was then carried out at 40,000 rpm (125,000 x g) for 60 h at 10°C, in polyallomer tubes (1 x 3½ inch) with high-force cap assemblies. The gradient was divided into six equal fractions by gentle suctioning into common pools from the top of the gradient. The fractions were exhaustively dialyzed against 0.15 M potassium acetate, pH 6.3, at 5°C, precipitated with 3 volumes of ethanol, washed with ethanol and then ether, and dried in a vacuum. In the nomenclature of Hascall and Heinegard (9), the fractions from the bottom to the top of the associative gradient are called Al through A6. A mixture of proteoglycan subunit and aggregate is concentrated in fraction Al at the bottom of this gradient.

Equilibrium Density Gradient Centrifugation under Dissociative Conditions—Six hundred milligrams of A1 were dissolved in 220 ml of 5.5 M GdmCl, 0.15 M potassium acetate, pH 6.3, and stirred for 16 h at 5°C to ensure complete dissociation of the proteoglycan aggregate. CsCl (152 g) was added slowly with gentle stirring while the pH was maintained at 6.3. The volume was adjusted to 300 ml with 0.15 M potassium acetate, pH 6.3. The final solution contained 4 M GdmCl, 3 M CsCl, 2 mg of proteoglycan/ml and its density at 5°C was 1.51 g/ml. Centrifugation was carried out for 60 h at 40,000 rpm and 5°C as described above. The gradient was divided into six equal fractions. The fractions were exhaustively dialyzed against 0.15 M potassium acetate, pH 6.3, at 5°C, precipitated with 3 volumes of ethanol, washed with ethanol and ether, and dried in a vacuum. In the nomenclature of Hascall and Heinegard (9), the fractions from the bottom to the top of this dissociative gradient are called A1-D1 through A1-D6.

Analytical Ultracentrifugation—Sedimentation velocity studies were carried out as previously described (4). Runs were made at 40,000 rpm and 20°C in 0.1 M KCl, 0.02 M KH₂PO₄, 0.03 M K₂HPO₄, pH 6.95 (ρ₀ = 1.0092 g/ml; nₑ𝑙ₑ = 1.0189). All additions of solvent to dry samples and dilutions were made by weight. Schlieren patterns were photographed on Kodak metallographic plates and read with a Nikon microcomparator. Extrapolations to ω₀ were made from a least squares curve fit of plots of s against c by computerized regression analysis.

RESULTS

Table I gives the chemical composition of Fractions Al-D1 through Al-D6 separated from two preparations of Al by equilibrium density gradient centrifugation under dissociative conditions. The two sets of data represent the extremes in the values for the yields and analytical data on Fractions Al-D1 through Al-D6 separated from different preparations of Al. Providing Fractions Al-D1 through Al-D6 are isolated from bovine proximal humeral articular cartilage as described under "Experimental Procedures," the composition of the fractions should fall between the limits given in Table I. Fraction Al-D6 contained mainly two proteins (link proteins) approximately 45,000 and 50,000 in molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The properties of these proteins will be described in a separate report. Fractions A1-D1 through A1-D5 represent proteoglycan fractions separated on the basis of differences in composition and buoyant density. A still finer separation was achieved by recycling Fractions A1-D1 through A1-D5 at low concentration (<1 mg/ml) in a second dissociative gradient. The yields and chemical composition of the eight fractions obtained on recycling are given in Columns 2 through 9 of Table II. As indicated at the top of Table II, A1 D1 and A1 D2 were individually recycled, while A1 D3, A1 D4, and A1 D5 were pooled and recycled. In Column 1 of Table II is given the amino acid composition of the hyaluronic acid-binding region of proteoglycan subunit isolated from bovine nasal cartilage by Heinegard and Hascall (11).

Sedimentation velocity studies revealed that each fraction contained one component of relatively low molecular weight whose sedimentation coefficient (sₑ) varied from 5.7 S (Column 2) to 14.3 S (Column 9). The concentration dependence of the sedimentation coefficients of these species are shown in Fig. 1, and representative schlieren patterns are shown in Fig. 2. We suggest that the 5.7 S to 14.3 S species represent relatively monodisperse fractions of proteoglycan subunit separated on a basis of differences in chemical composition and buoyant density from the polydisperse population of proteoglycan subunits present in Al.

In two fractions recovered at the buoyant density of hyaluronic acid (Columns 3 and 4), a second species of larger size (18.8 S, 32.1 S) were also present under associative conditions. The 18.8 S and 32.1 S species were reversibly dissociable in 4 M

Table I

Yields and chemical composition of proteoglycan fractions Al-D1 through Al-D6 separated from two preparations of Al by equilibrium density gradient centrifugation under dissociative conditions.

| Preparation | Fraction |
|-------------|----------|
|            | D1       | D2       | D3       | D4       | D5       | D6       |
| Yield, g/g  | 0.760    | 0.126    | 0.050    | 0.025    | 0.022    | 0.018    |
| Density, g/ml | 1.645    | 1.562    | 1.520    | 1.467    | 1.414    | 1.371    |
| Uronate, % | 18.5     | 14.5     | 10.9     | 9.0      | 6.8      | 2.4      |
| Galactosamine | 18.60    | 14.34    | 9.12     | 7.79     | 5.14     | 3.39     |
| Hexose | 10.9     | 11.9     | 12.6     | 11.1     | 9.7      | 6.5      |
| Glucosamine | 6.36     | 7.89     | 9.44     | 10.50    | 8.48     | 4.87     |
| Sialate | 1.8      | 2.5      | 3.1      | 3.1      | 2.9      | 2.1      |
| Protein | 8.6      | 13.3     | 19.9     | 23.8     | 42.7     | 57.6     |
| Uronate, % | 0.683    | 0.215    | 0.034    | 0.021    | 0.022    | 0.025    |
| Galactosamine | 21.0     | 18.1     | 12.7     | 9.7      | 7.1      | 2.5      |
| Hexose | 9.7      | 11.3     | 12.2     | 15.8     | 14.9     | 8.0      |
| Glucosamine | 3.98     | 5.48     | 7.62     | 7.96     | 7.49     | 4.16     |
| Sialate | 1.4      | 2.0      | 2.9      | 3.0      | 2.7      | 2.1      |
| Sulfate | 14.2     | 12.3     | 12.4     | 11.3     | 11.3     | 4.8      |
| Protein | 9.0      | 12.4     | 19.4     | 24.4     | 40.6     | 70.0     |
| Hypro | 0.017    | 0.018    | 0.036    | 0.046    | 0.075    | 0.215    |
Polydispersity of Proteoglycan Subunit

TABLE II

Chemical and physical properties of proteoglycan fractions isolated from AI-D1 through AI-D5 by equilibrium density gradient centrifugation under dissociative conditions

| Column | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
|--------|----|----|----|----|----|----|----|----|----|
| FRACTIONS | HA-PGS* | A1-D3,4,5 | A1-D3,4,5 | A1-D3,4,5 | A1-D3,4,5 | A1-D3,4,5 | A1-D3,4,5 | A1-D3,4,5 | A1-D3,4,5 |
| Yield, g/g | 0.19 | 0.39 | 0.93 | 0.05 | 0.74 | 0.53 | 0.20 | 0.19 | 0.45 |
| Uronate, Z | 9.7 | 10.3 | 11.5 | 15.3 | 16.1 | 17.1 | 19.0 | 20.1 |
| Galactosamine | 6.6 | 8.1 | 12.7 | 14.3 | 15.4 | 14.8 | 17.5 | 18.7 |
| Hexose | 12.5 | 13.5 | 12.9 | 14.3 | 13.3 | 11.7 | 11.8 | 12.2 |
| Glucosamine | 10.4 | 11.1 | 10.0 | 9.1 | 8.6 | 5.6 | 6.5 | 6.0 |
| Sialate | 3.0 | 3.1 | 2.9 | 2.4 | 2.8 | 1.8 | 1.8 | 1.4 |
| Protein | 30.7 | 23.9 | 17.3 | 13.0 | 14.9 | 10.3 | 11.1 | 9.9 |
| Density, g/ml | 50* subunit | A1-D3 | A1-D3 | A1-D3 | A1-D3 | A1-D3 | A1-D3 | A1-D3 | A1-D3 |
| Aspartic acid | 57 | 45 | 34 | 17 | 11 | 7 | 5 | 4 | 3 |
| Threonine | 60 | 61 | 65 | 68 | 63 | 65 | 62 | 62 | 61 |
| Serine | 72 | 69 | 77 | 90 | 105 | 103 | 115 | 123 | 125 |
| Glutamic acid | 122 | 139 | 138 | 149 | 147 | 141 | 150 | 146 | 150 |
| Proline | 75 | 84 | 96 | 101 | 111 | 110 | 104 | 105 | 101 |
| Glycine | 80 | 81 | 87 | 93 | 102 | 102 | 117 | 114 | 118 |
| Alanine | 85 | 75 | 76 | 77 | 74 | 76 | 71 | 73 | 70 |
| Half-cystine | 21 | 20 | 21 | 17 | 14 | 17 | 12 | 13 | 12 |
| Valine | 60 | 60 | 56 | 56 | 59 | 56 | 59 | 59 | 57 |
| Methionine | 14 | 14 | 14 | 10 | 6 | 6 | 7 | 5 | 5 |
| Isoleucine | 48 | 35 | 34 | 33 | 32 | 31 | 32 | 33 | 40 |
| Leucine | 70 | 81 | 78 | 74 | 73 | 74 | 74 | 78 | 78 |
| Tyrosine | 48 | 42 | 27 | 33 | 29 | 20 | 27 | 25 | 24 |
| Phenylalanine | 33 | 40 | 45 | 41 | 43 | 41 | 39 | 38 | 38 |
| Lysine | 24 | 32 | 28 | 24 | 19 | 19 | 15 | 15 | 13 |
| Histidine | 14 | 14 | 14 | 12 | 11 | 11 | 12 | 13 | 13 |
| Arginine | 58 | 58 | 55 | 51 | 44 | 47 | 42 | 41 | 37 |
| Amino Acid Composition residues/1000 |

*HA-PGS: Hyaluronic acid binding region of PGS core protein, isolated by Heinegard and Hancall (11).

GdmCl or 2 M CaCl$_2$. Based on interpretations given in the discussion, we suggest that the 18.8 S and 32.1 S species represent small proteoglycan aggregates formed when the 7.8 S and 8.8 S proteoglycan subunits and isopycnic hyaluronate, selectively separated into the same fraction of a dissociative gradient, are brought to associative conditions.

As indicated in Table II, the uronate and galactosamine contents of the proteoglycan fractions increase in proportion to the sedimentation coefficient of the proteoglycan subunit species present in each fraction. The chondroitin sulfate content of each fraction, calculated as the potassium salt by multiplying the uronate values by a factor of 2.8, increases from 27.2 to 56.3%, as the sedimentation coefficient of the proteoglycan subunit decreases from 14.3 S to 5.7 S.

Of particular interest are the changes in the amino acid composition of the proteoglycan subunit fractions with increasing molecular weight. Column 1 of Table II shows the amino acid composition of the hyaluronic acid-binding region of proteoglycan subunit (11). The amino acid composition of the proteoglycan subunit of lowest molecular weight (Column 2) closely resembles that of the hyaluronic acid-binding region, in regard to the relatively high aspartic acid, cystine, and methionine contents, and the relatively low serine and glycine contents. As the size of the proteoglycan subunit increases from 5.7 S to 14.3 S, there is a progressive increase in the serine and glycine contents of the fractions. The potassium salt by multiplying the glucosamine values by a factor of 2.7, increases from 16.2 to 28.1% as the sedimentation coefficient of the proteoglycan subunit decreases from 14.3 S to 5.7 S.

The chondroitin sulfate to protein ratio of the fractions increases from 0.9 to 5.7 and appears to be largely responsible for the difference in the buoyant density of the fractions. Conversely, the glucosamine and sialate contents of the fractions vary inversely with the size of the proteoglycan subunit species. The keratan sulfate content of the fractions, calculated as the potassium salt by multiplying the glucosamine values by a factor of 2.7, increases from 14.8 to 28.1% as the sedimentation coefficient of the proteoglycan subunit decreases from 14.3 S to 5.7 S.
Polydispersity of Proteoglycan Subunit

Figure 1. Concentration dependence of sedimentation coefficients of the proteoglycan species present in the fractions isolated by equilibrium density gradient centrifugation under dissociative conditions. The sedimentation velocity data given in this diagram and the chemical composition of the fractions given in Table II are related by the value of $s_{0w}$ given in the upper right-hand corner of each graph.

Figure 2. Left, sedimentation velocity pattern of the 12.7 S proteoglycan fraction (Column 8, Table II) at 3.008 mg/ml and 40 min. The 5.7 S, 9.7 S, 10.3 S, 10.8 S, and 14.3 S proteoglycan fractions (Columns 2, 5, 6, 7, and 9, Table II) showed similar unimodal sedimentation velocity patterns. Right, sedimentation velocity pattern of the 7.8 S and 18.8 S proteoglycan species present in the proteoglycan fraction whose composition is given in column 3 of Table II, at 1.607 mg/ml and 24 min. The proteoglycan fraction whose composition is given in column 4 of Table II showed a similar bimodal sedimentation velocity pattern.

Core protein. With increasing molecular weight, a region of core protein rich in serine and glycine, providing attachment sites for chondroitin sulfate chains, comprises an increasing proportion of the core protein. With decreasing molecular weight, the hyaluronic acid-binding region, rich in aspartic acid, cystine, and methionine, comprises an increasing proportion of the core protein.

Discussion

Equilibrium density gradient centrifugation under dissociative conditions has become a standard method for separating proteoglycan aggregate into its component species (2, 4, 14, 15–19, 29). Link proteins are separated into the top of such gradients (2, 8, 11). Hyaluronic acid distributes in the middle of 4 M GdmCl, 3 M CsCl dissociative gradients (29, 30). As indicated by the data given in Table II, proteoglycan subunits from bovine articular cartilage distribute throughout the dissociative gradient, individual members of the polydisperse population of proteoglycan subunits banding at buoyant densities determined mainly by their chondroitin sulfate to protein ratios. Most of the proteoglycan subunit from cartilage is of high molecular weight and high chondroitin sulfate to protein ratio and is recovered at buoyant densities considerably higher than those at which hyaluronic acid bands in 4 M GdmCl, 3 M CsCl.
Since hyaluronic acid is not present in these fractions, no aggregate is reformed under associative conditions. Therefore, relatively monodisperse fractions of the highest molecular weight proteoglycan subunits are readily isolated in pure form, free of proteoglycan aggregate.

While high molecular weight proteoglycan subunit, free of aggregate, is recovered from A1-D1 at the base of dissociative gradients, two proteoglycan species are invariably found when fractions of intermediate buoyant density from the middle of dissociative gradients are brought to associative conditions.

The following interpretation is offered to explain this phenomenon. Proteoglycan subunits of relatively low molecular weight and hyaluronic acid band at the same intermediate buoyant densities in dissociative gradients. When dissociative gradient fractions containing isopycnic proteoglycan subunits and hyaluronic acid are brought to associative conditions, proteoglycan aggregates will reform. The aggregates will be smaller than the aggregates originally present in A1 since they are formed from relatively monodisperse fractions of a proteoglycan subunit of low molecular weight rather than from the entire polydisperse population of proteoglycan subunits. Further, the actual size of the aggregate will be directly related to the size of the proteoglycan subunit separating into a particular fraction. Thus, in the results reported here, 7.8 S and 8.8 S proteoglycan subunits distributed at buoyant densities at which hyaluronate bands (29, 30); 18.8 S and 32.1 S aggregates were reformed from these fractions when they were brought to associative conditions.

If one accepts the interpretation given above, then the data presented in Table II provides a description of essentially the entire polydisperse population of proteoglycan subunits from bovine proximal humeral articular cartilage in terms of size and chemical composition of relatively monodisperse proteoglycan subunit fractions, the only reservation being the effect of the presence of hyaluronic acid on the chemical composition of two of the eight fractions.

The question is now raised, what is the structural basis for the pattern of polydispersity observed? One possible structural basis would be that all proteoglycan subunit molecules contained the same core protein, i.e. polypeptide chains identical in molecular weight and composition, to which chondroitin sulfate of different average chain lengths was attached. However, certain observations immediately rule out the possibility that individual members of the polydisperse population of proteoglycan subunits contain a core protein identical in size and composition. First, electron microscopic studies show that proteoglycan subunit core protein is of variable length (12, 13). Second, the amino acid composition of proteoglycan subunit molecules of different molecular weight is not constant. As shown in Table II, the amino acid composition of proteoglycan subunit varies in a characteristic fashion with molecular weight. The molecules of lowest molecular weight are highest in cystine, methionine, and aspartic acid, and lowest in serine and glycine. As the molecular weight of proteoglycan subunit increases, there is a progressive increase in serine and glycine and a decrease in cystine, methionine, and aspartic acid. Three recent studies (31-33) lead to an interpretation of the significance of these parallel increases in serine, glycine, chondroitin sulfate content, and molecular weight.

In 1971, based on studies of trypsin/chymotrypsin-degraded cartilage proteoglycans, Mathews suggested that proteoglycan subunit core protein might be composed of two types of amino acid sequences, which alternated regularly in the polypeptide chain (31). One type (sequence X) consisted of 9 to 10 amino acid residues to which two chondroitin sulfate chains were attached. Sequence X contained 2 serine and 2 glycine residues, at least 1 of each as Ser-Gly. The other type (sequence Y) consisted of about 35 amino acid residues and included serine residues to which no chondroitin sulfate chains were attached. Mathews (31) suggested that glycosylation of serine residues might be limited to specific regions of the polypeptide chain by requirements for enzyme specificity.

The concept of a proteoglycan subunit core protein composed of variable numbers of possibly homologous peptides, each containing Ser-Gly within a region with the primary structure and conformation required for the initiation of chondroitin sulfate synthesis, is particularly supported by two other studies (32, 33). Xylosyltransferase catalyzes the first in the series of reactions involved in the synthesis of the chondroitin sulfate linkage region and chondroitin sulfate chains (32, 33), i.e., the transfer of xylose from UDP-xylose to serine in proteoglycan subunit core protein. Native proteoglycan, in which roughly one-half of the serine residues are not substituted, is not a xylose acceptor. The failure of free serine in native proteoglycan to function as a xylose acceptor is a result of steric effects due to the presence of chondroitin sulfate chains. Removal of chondroitin sulfate chains with hyaluronidase does not give native proteoglycan xylose acceptor activity. The free serines are contained in polypeptide regions, analogous to Mathews’ sequence Y (31), lacking the conformation and substrate specificity required by xylosyltransferase.

Smith degradation of proteoglycan subunit yields free serine residues contained within polypeptide regions possessing the primary structure, conformation, and substrate specificity required by xylosyltransferase, analogous to Mathews’ sequence X (31). While “macromolecular” Smith-degraded proteoglycan has the greatest activity as a xylose acceptor, Smith-degraded, trypsin-degraded proteoglycan has substantial activity, and even Ser-Gly has some activity as a xylose acceptor. These studies (31-33), viewed in context with the variation in the length of core protein observed electron microscopically (12, 13) and the parallel increases in serine and glycine, chondroitin sulfate, and molecular weight reported here, suggest the following interpretation. Proteoglycan subunit is a polydisperse population of molecules in which the core protein varies in length in proportion to the numbers of peptide sequences possessing the primary structure and conformation required for the attachment of chondroitin sulfate chains; as the numbers of such sequences increases and the length of core protein increases, so does the chondroitin sulfate content and the molecular weight of the proteoglycan subunit.

The change in the amino acid composition and chondroitin sulfate to protein ratio of proteoglycan subunit with increasing molecular weight is not explained by the interpretation given so far. If proteoglycan subunit core protein consisted of no other regions, and solely of homologous sequences containing acceptor serines (Mathews’ sequence X, Ref. 31) alternating regularly with equal numbers of homologous connecting peptides (Mathews’ sequence Y, Ref. 31), the chondroitin sulfate to protein ratio and amino acid composition of proteoglycan subunit would remain constant as the length of core protein increases. Yet, with decreasing molecular weight, proteoglycan subunit shows a progressive decrease in chondroitin sulfate to protein ratio, a decrease in serine and glycine, and a relative increase in cystine, methionine, and aspartic acid. A relative increase in glucosamine and sialic acid is also observed. With
decreasing molecular weight, a progressively larger proportion of the proteoglycan molecule appears to be composed of a region of core protein rich in cystine, methionine, and aspartic acid to which some keratan sulfate but little or no chondroitin sulfate is attached. Recent work by Heinegard and Hascall (11) has shed light on the significance of these observations.

Heinegard and Hascall (11) isolated the hyaluronic acid-binding region of proteoglycan subunit of bovine nasal cartilage. Proteoglycan aggregate (A1) was degraded with chondroitinase and trypsin. The region of proteoglycan subunit core protein containing the attachment sites for most of the chondroitin sulfate and some of the keratan sulfate (polysaccharide attachment region) was readily degraded with trypsin, resulting in its conversion to small fragments easily separated from the essentially unaltered remainder of the proteoglycan aggregate. The remainder of the aggregate consisted of the hyaluronic acid-binding region of proteoglycan subunit core protein, noncovalently associated with link protein and hyaluronic acid. The hyaluronic acid binding region of core protein was isolated from this complex. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, in the presence or absence of mercaptoethanol, it consisted of a single polypeptide fragment 90,000 in molecular weight comprising approximately one-half the molecular weight of core protein. Compared to core protein, the hyaluronic acid-binding region was relatively high in cystine, methionine, and aspartic acid and contained about 15% keratan sulfate.

Heinegard and Hascall (11) speculated on the significance of the variation in chondroitin sulfate to protein ratio and molecular weight of proteoglycan subunit fractions. Since chemical binding studies indicated that all proteoglycan subunit fractions contain subunit molecules with functional hyaluronic acid-binding regions, they suggested that the observed pattern of polydispersity might result from variations in the length of the polysaccharide attachment region attached to a hyaluronic acid-binding region of constant size and composition in different molecules. This suggestion is in perfect accord with the results of electron microscopic studies of proteoglycan aggregates (12) which show proteoglycan subunit molecules varying in length from 1000 to 4000 Å bound to hyaluronic acid at one terminus. In proteoglycan aggregates, all proteoglycan subunit molecules contain functional hyaluronic acid-binding regions, but appear to contain polysaccharide attachment regions of variable length.

Column 1 of Table II gives the amino acid composition of the hyaluronic acid binding region isolated by Heinegard and Hascall (11). Column 2 of Table II gives the composition of proteoglycan subunits of lowest molecular weight from bovine articular cartilage. The amino acid composition of native proteoglycan subunits of the lowest molecular weight is almost identical to that of the hyaluronic acid-binding region. The hyaluronic acid-binding region and the lowest molecular weight native subunits are also relatively rich in keratan sulfate, compared to subunits of higher molecular weight. Native proteoglycan subunits of the lowest molecular weight appear to consist of the hyaluronic acid-binding region and an adjoining keratan sulfate-rich peptide. As the molecular weight of proteoglycan subunit increases, there is a progressive increase in serine, glycine, and chondroitin sulfate, and a relative decrease in cystine, methionine, aspartic acid, and keratan sulfate.

The pattern of polydispersity observed supports the concept that proteoglycan subunit core protein contains the following regions: (a) a hyaluronic acid-binding region of constant size and composition, rich in cystine, methionine, and aspartic acid, located at one terminus of the molecule, protected from tryptic degradation in proteoglycan aggregates by the shielding effect of link protein and hyaluronic acid; (b) a polysaccharide attachment region of variable length, extending towards the other terminus of the molecule, composed in part of variable numbers of Ser-Gly-containing peptides providing attachment sites for chondroitin sulfate chains; (c) a peptide region rich in keratan sulfate but containing little or no chondroitin sulfate, located between the hyaluronic acid-binding region and the polysaccharide attachment region. The polydispersity of proteoglycan subunit appears to be determined largely by the variable length of the polysaccharide attachment region of proteoglycan subunit core protein.

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