Donovan, J. W. (1967), *Biochem. Biophys. Res. Commun. 29*, 734.
Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66*, 375.
Gehrke, C. W., Nakamoto, H., and Zumwalt, R. W. (1969), *J. Chromatogr. 45*, 24.
Harbon, S., Herman, G., and Clauser, H. (1968), *Eur. J. Biochem. 4*, 265.
Harbon, S., Herman, G., Rossignol, B., Jollés, P., and Clauser, H. (1964), *Biochem. Biophys. Res. Commun. 17*, 57.
Hashimoto, Y., and Pigman, W. (1962), *Ann. N. Y. Acad. Sci. 93*, 541.
Hirs, C. H. W. (1967), *Methods Enzymol. 11*, 199.
Jarboe, C. J., Noll, B. W., and Hass, L. F. (1971), *Biochem. Biophys. Res. Commun. 43*, 1029.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Marshall, R. D., and Neuberger, A. (1954), *Biochemistry 3*, 1596.
McKenzie, H. A. (1967), *Advan. Protein Chem. 22*, 55.
Mecham, D. K., and Olcott, H. S. (1949), *J. Amer. Chem. Soc. 71*, 3670.
Nisizawa, K., and Pigman, W. (1960), *Biochem. J. 75*, 293.
Österberg, R. (1964), *Acta Chem. Scand 18*, 795.
Sajdera, S. W., and Hascall, V. C. (1969), *J. Biol. Chem. 244*, 77.
Simpson, D. L., Hranisavljevic, S., and Davidson, E. A. (1971), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 30*, 1273.
Stern, E. L., Lindahl, B., and Rodén, J. (1971), *J. Biol. Chem. 246*, 5707.
Strumeyer, D. H., White, W. N., and Koshland, Jr., D. E. (1963), *Proc. Nat. Acad. Sci. U. S. 50*, 931.
Swan, J. M. (1957), *Nature (London) 180*, 643.
Tanaka, K., Bertolini, M., and Pigman, W. (1964), *Biochem. Biophys. Res. Commun. 16*, 404.
Tanaka, K., and Pigman, W. (1965), *J. Biol. Chem. 240*, PC1487.
Tettamanti, G., and Pigman, W. (1968), *Arch. Biochem. Biophys. 124*, 41.
Vercellotti, J. R., Nienaber, N., and Chang, C. J. (1970), *Carbohyd. Res. 13*, 63.
Woodward, C., Hranisavljevic, J., and Davidson, E. A. (1972), *Biochemistry 11*, 1168.

Isolation of the Peptide Core of Costal Cartilage Chondroitin 4-Sulfate Proteoglycan†

D. L. Simpson and E. A. Davidson*

ABSTRACT: The polypeptide core of the proteoglycan extracted from porcine costal cartilage has been isolated after β elimination and [^{35}S]sulfite addition. The attachment sites of chondroitin 4-sulfate chains to serine in the polypeptide backbone were specifically and quantitatively labeled by conversion to [^{35}S]cysteic acid within the structure. [^{35}S]Cysteic acid, isolated from a preparative hydrolysate, was identified by several independent chemical and chromatographic procedures. The major cysteic acid containing peptide, representing about 75% of the total protein, was resolved from a collagen-like protein on Dowex 1 or DEAE-Sephadex-A-50. The polypeptide was homogenous on Sephadex chromatography and acrylamide gel electrophoresis. There was nearly quantitative recovery of galactosamine as chondroitin 4-sulfate chains, free of protein. Collagenase digestion of the proteoglycan eliminated a protein component but the residual amino acid composition and molecular weight distribution of the product suggested that some proteolysis of the peptide core had occurred. Based on the reactions described in the preceding paper, the molecular weight of the saccharide chains recovered correlates well with that calculated from the ratio of galactosamine to cysteic acid after the elimination–addition reaction.

The structure of the major matrix proteoglycan of costal cartilage is that of a polypeptide to which are covalently attached a number of chondroitin 4-sulfate chains (Marler and Davidson, 1965). The linkage between the saccharide chains and the protein core involves the hydroxyl group of serine and an unusual trisaccharide terminating in D-xylose. The structure of the linkage region oligosaccharide has been reported (Lindahl and Rodén, 1966; Rodén and Smith, 1966) but the distribution of chains on the polypeptide core and the properties of the latter have not been completely elucidated.

The lability of the seryl glycosides to alkali elimination has been utilized as the basis for an addition reaction permitting the localization of the substituted serine residues (Simpson *et al.*, 1972). In addition, the introduction of new charged residues (cysteic acid) into the polypeptide permits ready separation from other proteins as well as from the eliminated saccharide chains. This paper describes the isolation, purification, and properties of the polypeptide core of the predominant porcine costal cartilage proteoglycan. A preliminary report has been presented (Hranisavljevic *et al.*, 1971).

Materials and Methods

Chondroitin 4-sulfate proteoglycan was prepared by a modification of the procedure of Sajdera and Hascall (1969) using either 2 M $CaCl_2$ or 4 M guanidinium chloride as extractant.

† From the Department of Biological Chemistry, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, Pennsylvania 17033. *Received December 28, 1971.* This work was supported by Public Health Service Grant AM12074.

* To whom all correspondence should be addressed.

[^{35}S]Sodium sulfite was obtained from New England Nuclear Corp. Bacterial collagenase was obtained from Worthington Biochemical Corp. All other chemicals and chromatography media were the best commercially available.

Analyses for amino acids and amino sugars was performed on a Beckman Model 120C amino acid analyzer following hydrolysis *in vacuo* in 6 N HCl for 24 hr. Corrections for destruction of serine and threonine were employed following evaluation over a 72-hr hydrolysis period and extrapolation to zero time. Residue compositions were calculated using a computer program as previously described (Simpson *et al.*, 1972).

Uronic acid was analyzed by the orcinol (Brown, 1946) or carbazole methods (Dische, 1947).

Elimination–sulfite addition reactions were performed at pH 11.5 in the presence of *freshly* prepared 0.2 M sodium sulfite. The proteoglycan concentration was 1.0 mg/ml. Optimal conditions for the reaction were established by examining the rate of cysteic acid formation as a function of pH and sulfite concentration. When appropriate, [^{35}S]sulfite was employed to permit labeling of the polypeptide chain.

A series of experiments were carried out to eliminate serine *O*-sulfate as a possible contaminant since this compound and cysteic acid have identical mobilities on the amino acid analyzer. Hydrolysis of serine in 6 N HCl for 24 hr in the presence of excess sodium sulfate results in the formation of serine *O*-sulfate in about 10% yield. This would result from esterification under the highly acidic, dehydrating conditions employed to concentrate samples prior to amino acid analysis. Hydrolysis of serine *O*-sulfate in 6 N HCl at 104° was shown to be complete in 4 hr, in contrast to the ^{35}S-labeled product isolated from radioactive peptide, which demonstrated complete acid stability for 48 hr with no detectable conversion to serine.

Acrylamide disc gel electrophoresis was performed for 75 min at a current of 2.5–3.0 mA/tube in a Shandon electrophoretic cell SAE-2731. Gels were prepared with 7.5% acrylamide and 3.6% *N,N'*-methylenebisacrylamide (based on acrylamide content), and polymerization was initiated with ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine. Gels were equilibrated in Tris–borate buffer (pH 9.5) and the dye marker method was employed for staining proteins (Weber and Osborn, 1969). Details are given in the appropriate figure legend.

Optical rotatory dispersion data were obtained on a Cary Model 60 spectropolarimeter.

Radioactivity was determined following development by sectioning the gel into 20 disks of equal diameter. Following overnight dehydration, the gels were counted in a liquid scintillation spectrometer in toluene containing 2,5-diphenyloxazole (4 g/l.) and 1,4-bis[2-(5-phenyloxazole)]benzene (0.1 g/l.) as scintillators.

Amino acid analyzer chromatography of hydrolysates of ^{35}S-labeled peptide utilizing the stream-splitting device permitted collection of labeled eluent from the column at specific elution times. An aliquot of [^{14}C]glycine containing 250,000 dpm was added to the hydrolysate as a chromatographic marker.

Alkaline sulfite-treated proteoglycan was resolved into peptide core and saccharide chains by chromatography on DEAE-Sephadex A-50 or Dowex 1-X2. A column (2 × 45 cm) of DEAE-Sephadex A-50 was equilibrated with 0.4 M NaCl–4 M urea (pH 7.0). The elimination–addition reaction mixture was applied to the column in the same salt–urea solution. Labeled peptide was washed from the column with a 600-ml volume of 0.4 M NaCl–4 M urea and routinely located by addition of labeled marker. The saccharide chains were eluted with a linear salt gradient (1200 ml) consisting of 0.4–1.5 M NaCl–4 M urea. Saccharide containing fractions were located by spotting on paper and staining with Alcian Blue dye; positive fractions were quantitated by the orcinol procedure.

TABLE I: Amino Acid Composition of Proteoglycan before and after Reaction with Alkaline Sulfite.

Amino Acid[a]	Zero Time	8-hr Elimination–Addition
Lysine	15.9	14.8
Histidine	11.2	9.1
Arginine	17.9	16.9
Aspartic	39.8	38.7
Threonine	25.2	20.0
Serine	52.9	32.2
Glutamic	60.1	59.8
Proline	41.0	45.0
Glycine	67.0	63.0
Alanine	32.4	34.2
Valine	24.6	25.0
Isoleucine	16.1	15.2
Leucine	38.8	40.0
Tyrosine	9.3	8.1
Phenylalanine	14.1	11.0
Cysteic	0.0	20.1
Glucosamine	34.3	33.0
Galactosamine	487.2	507.5

[a] Values in residues per 1000 residues.

Dowex 1-X2 200–400 mesh anion-exchange resin was equilibrated with 0.2 M NaCl–4 M urea at pH 7.0. Reaction mixtures were applied to the column in a solution of identical salt–urea concentration and peptide eluted with three volumes of 0.2 M NaCl–4 M urea. The saccharide chains were then eluted with a linear gradient of 0.2–1.5 M NaCl–4 M urea in a total volume of 1500 ml. As a rule peptide was located by monitoring ^{35}S activity and test-spotting fractions with Bromophenol Blue. Saccharide chains were located with Alcian Blue and uronic acid content quantitated as above.

The molecular weights of the saccharide chains and peptide core were determined by sedimentation equilibrium in a Beckman Model E analytical ultracentrifuge using Rayleigh interference optics. Data were analyzed using a computer program generously donated by Dr. Dennis Roark of this department (Woodward *et al.*, 1972).

Results

The amino acid composition of a typical preparation of cartilage proteoglycan before and after treatment with alkaline sulfite is given in Table I. The only significant changes are those in serine and cysteic acid which are in excellent agreement; a small loss of threonine was observed.

A kinetic study of the effects of alkaline sulfite on the content of cysteic acid, serine, and threonine in the proteoglycan over a 72-hr period is summarized in Table II. Threonine is very slightly affected during this time whereas there is very close correspondence between serine loss and cysteic acid formation. The reaction appears to be complete after 8 hr.

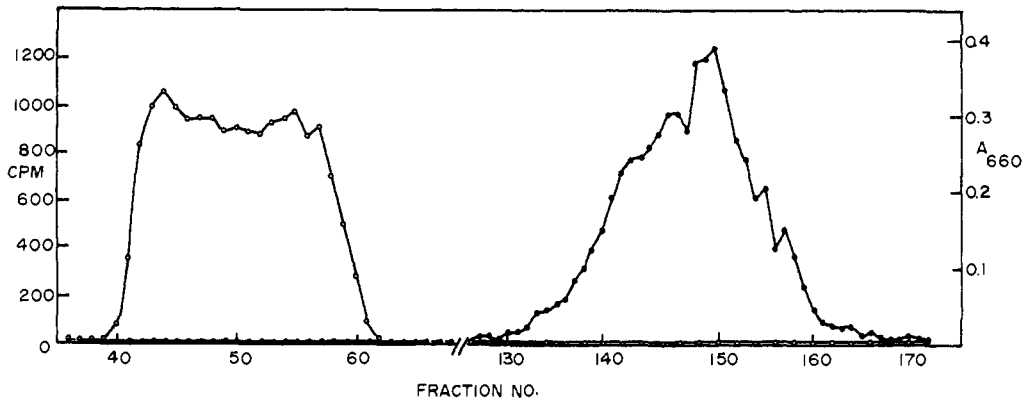

FIGURE 1: Chromatographic profile of a typical 8-hr elimination–addition reaction of cartilage proteoglycan fractionated on Dowex 1-X2. Uronic acid content was assessed by the orcinol reaction (A_{660}) and peptide was monitored by ^{35}S radioactivity. Closed circles are a plot of A_{660} and open circles represent radioactivity in counts per minute.

Following a preparative scale elimination–addition reaction utilizing $[^{35}S]SO_3^{2-}$, the radioactive peptide fraction was resolved from eliminated chondroitin 4-sulfate chains by chromatography on either DEAE-Sephadex A-50 or Dowex 1-X2 acetate form ion-exchange resin. The marked difference in charge density permits ready separation of peptide from the amino acid free saccharide chains. A typical chromatogram of such a reaction is illustrated in Figure 1.

The predominant cysteic acid containing peptide, representing about 75% of the total peptide fraction, was desalted on Sephadex G-15. In experiments employing [^{35}S]sulfite, the radioactive peptide was well separated from salt by this procedure and contained all of both the peptide and radioactivity applied to the column.

The amino acid composition of a reaction mixture and the purified peptide and saccharide chains derived from it is shown in Table III. Note that separation from a glycine-, proline-, and alanine-rich fraction has been achieved and that the saccharide chains, in which the recovery of galactosamine was greater than 90%, are essentially free of amino acid and glucosamine. Hydroxyproline was present in the starting material but absent in the isolated peptide and chondroitin 4-sulfate chains. The glycine rich polypeptide, presumably soluble collagen, was eluted from the column with the sodium chloride–urea wash. The recovery of cysteic acid in the main peptide peak was greater than 70% while the galactosamine content of the peptide was below detectable limits. The molecular weights of the isolated chains and core peptide are given in Table IV. The optical rotatory dispersion behavior of the isolated peptide is illustrated in Figure 2.

TABLE II: A Kinetic Study of the Alkaline Sulfite Elimination–Addition Reaction.[a]

Amino Acid[b]	0 hr	0.5 hr	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr	72 hr
Serine	178	152	135	108	79	68	69	68	68
Threonine	50	52	52	51	50	46	45	46	46
Cysteic	0	12	18	45	77	98	98	96	98

[a] Amino acid values from a kinetic study of cartilage proteoglycan treated over a 72-hr period with alkaline sulfite. [b] Values in residues per 1000 residues of amino acids.

The per cent recovery of peptide and amino sugar based on composition and weight recovery of the individual fractions resolved on DEAE-Sephadex A-50 chromatography is summarized in Table V. A second peptide peak was resolved and represented about 18% of the total peptide content. The amino acid composition was similar to that of the major fraction and it was not studied further. Approximately one-half of the glucosamine content was not recovered and probably represents keratan sulfate chains which were not removed from

TABLE III: Chromatography of Elimination–Addition Reaction.[a]

Amino Acid[b]	8-hr Elimination–Addition	Peptide[c]	Chondroitin 4-Sulfate Chains[d]
Lysine	45.5	32.2	
Histidine	20.6	18.3	
Arginine	52.8	49.8	
Aspartic	92.7	129.7	Trace
Threonine	42.1	49.9	
Serine	16.8	59.8	Trace
Glutamic	107.1	135.8	
Proline	94.5	70.6	
Glycine	196.0	87.8	22.4
Alanine	83.8	69.3	
Valine	36.9	45.3	
Isoleucine	25.5	40.5	
Leucine	74.9	99.4	Trace
Tyrosine	19.8	26.2	
Phenylalanine	22.5	28.6	
Cysteic	23.5	32.6	9.8
Glucosamine		9.7	17
Galactosamine		Trace	930

[a] Amino acid analysis of an 8-hr elimination–addition reaction mixture of cartilage proteoglycan and the purified peptide and saccharide chains derived from subsequent chromatography. [b] Values in residues per 1000 residues; corrected for amino sugars. [c] Values in residues per 1000 residues. Cysteic acid recovery was greater than 70%. [d] Trace represents less than 10 residues/1000 residues. Galactosamine recovery was greater than 90%.

TABLE IV: Molecular Weight of Proteoglycan Components Derived from β Elimination and Sulfite Addition Reactions.[a]

Component	Mol Wt (M_w)
Chondroitin 4-sulfate chains	13,000
Polypeptide core	9,000–11,000

[a] Weight-average molecular weights determined by sedimentation equilibrium in 0.5 M NaCl. The chains were essentially homogeneous (M_z/M_w = 1.04) while the peptide core exhibited $\bar{M}_z$ to $\bar{M}_w$ of 1.25. Partial specific volumes and details of the computational procedures employed have been described (Woodward *et al.*, 1972).

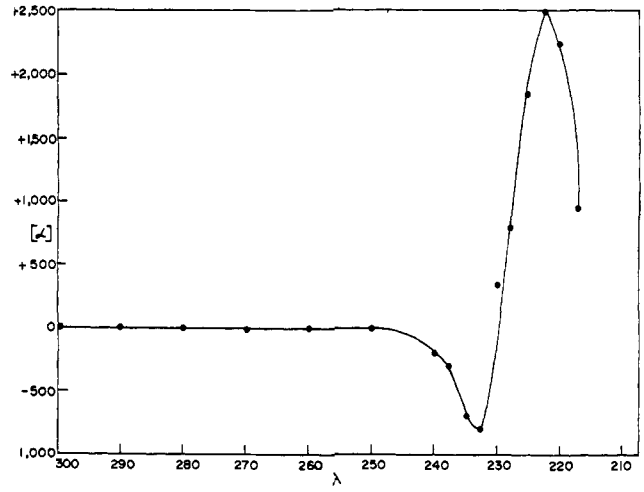

FIGURE 2: Optical rotatory dispersion of the purified cysteic acid containing peptide core from cartilage proteoglycan. Protein concentration is 5 mg/ml in 0.5 M NaCl.

the column under the elution conditions employed. On a weight basis, this fraction would represent less than 3% of the saccharide content.

The identity of the presumed cysteic acid peak observed on amino acid analysis was confirmed by several independent methods. ^{35}S-Labeled peptide was hydrolyzed for 24 hr in 6 N HCl and aliquots of the hydrolysate chromatographed on the amino acid analyzer at a series of temperatures (12, 36, 40, and 50°) following the addition of standard cysteic acid. In all cases, symmetrical peaks were detected for cysteic acid which contained all of the applied radioactivity.

Proteoglycan was treated at pH 11.5 in the absence of sulfite or at pH 7.0 in the presence of 0.2 M bisulfite and, following acid hydrolysis, subjected to amino acid analysis. There was no peak observed corresponding to cysteic acid. In a separate experiment, ^{35}S-labeled peptide product was isolated, hydrolyzed, and an aliquot of the hydrolysate chromatographed on a Dowex 50-X8 cation-exchange column (0.5 × 8 cm) and equilibrated with 0.2 M citrate buffer (pH 3.4). A single symmetrical radioactive peak was obtained which was examined on the amino acid analyzer following addition of standard cysteic acid. The radioactivity was eluted coincidentally with cysteic acid in a peak of constant specific activity. A second aliquot from the preparative hydrolysate was chromatographed on a (0.5 × 8 cm) column of Dowex 1-X8 acetate form anion-exchange resin with standard aspartic and cysteic acids. Results are illustrated in Figure 3. Final confirmation was obtained by gas–liquid chromatography of the Me_3Si deriv-

TABLE V: Per Cent Recovery of Peptide and Amino Sugar.[a]

Amino Acid	Peptide I	Peptide II	Chains
Serine	75	25	Trace
Threonine	78	20	
Glycine	80	20	Trace
Cysteic	72	25	Trace
Glucosamine	10	8–10	25
Galactosamine	None	2.5	92

[a] Calculations based on residue composition and weight recovery of individual fractions. The elimination–addition reaction mixture was resolved by chromatography on DEAE-Sephadex A-50 employing a NaCl gradient in 4 M urea. Peptide I was eluted first and represented 80% by weight of the total peptide. The low recovery of glucosamine suggests the presence of keratan sulfate chains not eluted from the column. Analyses for peptide I and chains are given in Table III. The sample employed in this experiment had been fractionated on DEAE-Sephadex A-50 following isolation from cartilage (Hranisavljevic *et al.*, 1971, unpublished data).

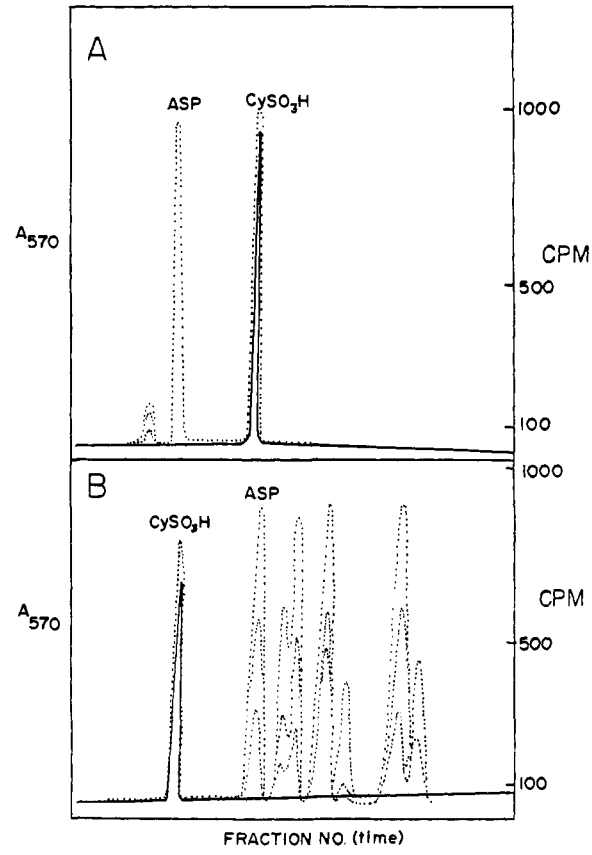

FIGURE 3: Chromatographic profiles of [^{35}S]cysteic acid derived from labeled peptide core obtained by β elimination and sulfite addition of cartilage proteoglycan. The dotted line is a plot of A_{570} while the solid line represents radioactivity in counts per minutes. (A) Chromatography of [^{35}S]cysteic acid on Dowex 1-X8 with standard cysteic and aspartic acids. (B) Chromatography of [^{35}S]cysteic acid on Dowex 50-X8.

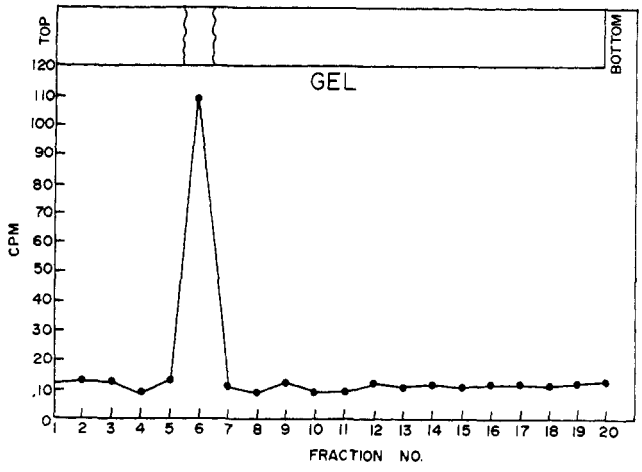

FIGURE 4: Polyacrylamide disc gel electrophoresis of the purified ^{35}S-peptide core in the presence of sodium dodecyl sulfate. A sample mixture consisted of 100 μl of ^{35}S-labeled peptide 2 mg/ml, 40 μl of H_2O, 10 μl of 0.2 N NaOH, 15 μl of 1% Coomassie Blue Dye with either 20 μl of 3% sodium dodecyl sulfate in 0.1% NaN_3 or 5% sodium deoxycholate in 0.1% NaN_3. A sample (100 μl) of the above mixture was applied to the gel.

ative of the sulfonic acid compared to standard Me_3Si–cysteic acid (Simpson *et al.*, 1962). These data establish cysteic acid as the product of the alkali elimination–sulfite addition reaction described.

Intact ^{35}S-labeled peptide was examined by acrylamide disc gel electrophoresis in the presence of sodium dodecyl sulfate. A single protein-staining band was observed which migrated well into the gel and contained 95% of the original radioactivity as shown in Figure 4. A small amount of residual protein-staining material, estimated at less than 5% of the major fraction, was excluded from the gel but contained no radioactivity.

The amino acid composition of the major peptide fraction isolated after sulfite addition suggested that a protein high in glycine, alanine, and proline had been separated during the fractionation procedure. Since these amino acids are present in high concentrations in collagen, the proteoglycan after extraction from cartilage was digested in two separate experiments with purified collagenase preparations from different sources. The products were fractionated on DEAE-Sephadex A-50. The results, summarized in Table VI and Figure 5, suggest removal of protein but the amino acid composition of the reisolated proteoglycan and its molecular weight distribution indicated that cleavage of some bonds in the polypeptide core had occurred. Whether these are normally collagenase susceptible or reflect the presence of other proteolytic activity in the enzyme preparations is not known at present.

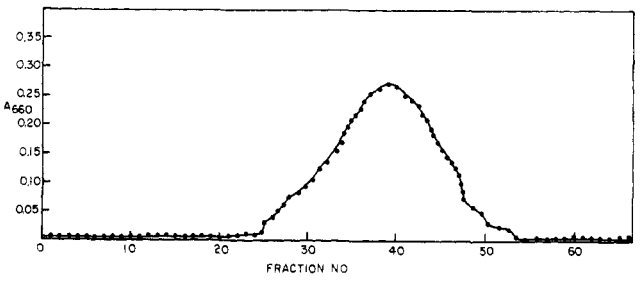

FIGURE 5: DEAE-Sephadex A-50 chromatography of collagenase-digested cartilage proteoglycan.

TABLE VI: Collagenase Treatment of Cartilage Proteoglycan.[a]

Amino Acid[b]	Proteoglycan before Collagenase Treatment	Expt I	Expt II
Lysine	31.0	20.0	28.6
Histidine	21.9	20.8	23.3
Arginine	34.9	28.9	20.5
Aspartic acid	77.6	64.2	66.3
Threonine	49.1	40.4	37.0
Serine	103.1	167.5	172.5
Glutamic	117.2	129.3	135.9
Proline	79.9	81.0	74.7
Glycine	131.6	157.0	163.5
Alanine	63.2	61.3	62.4
Valine	48.0	44.2	42.6
Isoleucine	31.4	33.4	38.3
Leucine	75.7	65.6	63.9
Tyrosine	18.2	11.0	9.4
Phenylalanine	23.4	20.1	17.6
Glucosamine	66.8	47.3	42.4

[a] Amino acid analysis of cartilage proteoglycan digested in separate experiment with collagenase preparations from two different sources and chromatographed, subsequent to digestion, on DEAE-Sephadex A-50. The hydroxyproline content of the proteoglycan was 0.2% by weight. [b] Residues per 1000 residues corrected for galactosamine content. The amino acid content represents 7% by weight of the total. Galactosamine recovery was essentially quantitative.

Discussion

Employing the β elimination and sulfite addition reaction described in the preceding paper, the main peptide fraction obtained from porcine costal cartilage represents about 75% of the protein as evidenced by the weight recovery of several amino acids. This peptide fraction was completely free of any detectable galactosamine, but consistently contained a rather constant amount of glucosamine. The recovery of cysteic acid within the main peptide fraction was in accord with the recovery of total protein upon elimination–addition and fractionation. This major protein fraction, in which cysteic acid was identified by several chromatographic and chemical procedures, is presumed to be the polypeptide core of the complex to which chondroitin 4-sulfate chains are covalently linked. The weight recovery of the chondroitin 4-sulfate chains, as represented by galactosamine, was greater than 90% and when appropriate corrections were made for destruction of amino sugar, was essentially quantitative. These data suggest that the proteoglycan preparation was free of other chondroitin 4-sulfate containing protein–polysaccharides but did contain some extraneous protein as indicated by the weight recoveries of amino acids.

The molecular weight of the recovered saccharide chains, approximately 13,000, is in excellent agreement with that calculated from the composition of the elimination–addition re-

action mixture summarized in Table I. Based on the assumption that every cysteic acid residue formed represented the site of attachment of a saccharide chain, it can be estimated from the galactosamine content that the chain should contain 25 disaccharide units on the average, representing a molecular weight of approximately 13,000. It should be noted that this procedure represents a convenient means of estimating the average molecular weight of saccharide chains in such complexes. The optical rotatory dispersion behavior of the peptide was characteristic with a trough at 230 nm and distinctly different from that of chondroitin 4-sulfate chains. There was a shift toward a positive rotation at higher wavelengths probably due to the loss of asymmetry at the substituted seryl residues.

The low recovery of glucosamine may be due to the presence of keratan sulfate chains not eluted from the column. Although chromatography of the alkali sulfite-treated complex on DEAE-Sephadex A-50 or Dowex 1-X2 or prior digestion of the extracted complex with collagenase resolved it from a collagenase-susceptible material, this latter fraction contained no cysteic acid.

The cysteic acid not recovered in the major peptide fraction, approximately 25% of that present in the alkali sulfite-treated complex and not present in the collagenase-susceptible fraction, is most likely retained in the polysaccharide fraction as suggested in Table III. Any peptide, or peptide fragments, which may have been incompletely eliminated during sulfite treatment would contain at least a single chondroitin 4-sulfate chain or perhaps noneliminated keratan sulfate thus affecting their chromatographic behavior. This might occur if the chain were at an amino- or carboxyl-terminal end of the peptide core or if a small amount of peptide-bond cleavage took place.

Dissociative extraction of cartilage with either 2.2 M calcium chloride or with 4 M guanidinium chloride yielded a proteoglycan of essentially identical composition which appears to have associated with it a minor protein, collagen like in composition and collagenase susceptible. Hydroxyproline analysis confirmed the presence of a small amount, approximately 2% by weight, of collagen in the starting extract. Separate experiments with collagenase preparations from *Clostridium histolyticum* and a marine organism gave products with essentially identical analyses upon subsequent chromatography. Molecular weight measurements on the collagenase-treated samples showed about 25% of the sample present as chains (mol wt 13,000), with a relatively small fraction exceeding 40,000. These data suggest that some proteolysis of the peptide core had occurred. However, since the absolute purity of the collagenase preparations is not known, the nature of the susceptible linkages remains in doubt.

Both chromatographic procedures utilized for resolving components of the proteoglycan after elimination–addition provide essentially identical recoveries of peptide and saccharide. The identity of the amino acid and amino sugar compositions and the cysteic acid stoichiometry support the contention that the related peptide represents the predominant if not exclusive core to which the chondroitin 4-sulfate chains are attached.

The glucosamine retained in the peptide core throughout alkaline elimination–addition is presumably linked N-glycosidically to asparagine. Additional substituted neutral monosaccharides, not detected on amino acid analysis, may be present as well.

β elimination and sulfite addition as described appears far superior to other methods available for identification of substituted serines. The conditions employed are such as to minimize peptide-bond cleavage (Simpson *et al.*, 1972) and permit isolation of the peptide core from costal cartilage proteoglycan. The sites of chondroitin 4-sulfate chain attachment can be labeled in a specific and quantitative fashion which enables them to be located on subsequent amino acid sequence analysis.

Acknowledgment

The authors thank Dr. Dennis Roark for providing the computer program utilized in the molecular weight calculations. We also thank Dr. N. Tudball for providing a pure sample of serine *O*-sulfate and Dr. J. R. Merkel for the gift of purified marine collagenase.

References

Brown, A. (1946), *Arch. Biochem. Biophys. 11*, 269.

Dische, Z. (1947), *J. Biol. Chem. 167*, 189.

Hranisavljevic, J., Stewart, J., Simpson, D. L., and Davidson, E. A. (1971), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29*, 3654.

Lindahl, U., and Rodén, L. (1966), *J. Biol. Chem. 241*, 2113.

Marler, E., and Davidson, E. A. (1965), *Proc. Nat. Acad. Sci. U. S. 54*, 648.

Rodén, L., and Smith, R. (1966), *J. Biol. Chem. 241*, 5949.

Sajdera, S. W., and Hascall, V. C. (1969), *J. Biol. Chem. 244*, 77.

Simpson, D. L., Hranisavljevic, J., and Davidson, E. A. (1972), *Biochemistry 11* (in press).

Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.

Woodward, C., Hranisavljevic, J., and Davidson, E. A. (1972), *Biochemistry 11*, 1168.