Degradation of Cartilage Proteoglycan by Human Leukocyte Granule Neutral Proteases—A Model of Joint Injury

II. DEGRADATION OF ISOLATED BOVINE NASAL CARTILAGE PROTEOGLYCAN

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ABSTRACT Extracts of human peripheral blood polymorphonuclear leukocyte granules, and two purified proteases derived from such extracts, an elastase and a chymotrypsin-like enzyme, degrade isolated bovine nasal cartilage proteoglycan at neutral pH. Viscosity studies indicate that the leukocyte granule extracts lack hyaluronidase activity and that their degradative effect on proteoglycan at physiological pH is due entirely to proteolytic action. Sepharose 4B gel chromatography and SDS-polyacrylamide gel electrophoresis of proteoglycan fractions treated with leukocyte granule enzymes at pH 7.0 indicate that they degrade one of the proteoglycan link proteins, release a fragment from the hyaluronic acid-binding portion of the proteoglycan subunit core protein, and break down the remainder of the proteoglycan subunit molecule into peptide fragments with varying numbers of chondroitin sulfate chains. Immunodiffusion studies indicate that the antigenic determinants of the proteoglycan subunit core protein and the link proteins survive treatment with granule proteases. Similar degradation of human articular cartilage proteoglycan by granule neutral proteases can be presumed to occur, in view of the similarity of structure of human articular and bovine nasal cartilage proteoglycans. The release of granule enzymes in the course of neutrophil-mediated inflammation can thus result in the degradation of cartilage matrix proteoglycan, leading to cartilage destruction and joint injury.

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INTRODUCTION

In the preceding paper (1), it was shown that enzymes in polymorphonuclear leukocyte (PMN)[1] granule extracts are capable of penetrating into cartilage and of causing the release of $^{35}SO_4$-labeled material thought to be derived from cartilage proteoglycan. In this paper we further demonstrate that crude PMN granule extract (G) and two purified enzymes isolated from it, an elastase (E) and a chymotrypsin-like enzyme (CT), degrade bovine nasal cartilage proteoglycan in vitro at neutral pH.

The main structural features of bovine nasal cartilage proteoglycan, as established by recent biochemical, physicochemical, immunological, and electron-microscopic studies (2-11), are illustrated in Fig. 1. The cartilage proteoglycan molecule is a huge aggregate composed of up to 140 proteoglycan subunits linked noncovalently to a linear hyaluronic acid chain in association with two link proteins. The proteoglycan subunit,

[1] *Abbreviations used in this paper:* AAACK, *N*-acetyl-(L-alanine)$_3$-chloromethyl ketone; CT, chymotrypsin-like enzyme; E, PMN granule elastase; G, PMN granule extract; GPL, proteoglycan glycoprotein link fraction; MES, 2-(*N*-morpholino) ethane sulfonic acid; PGC, proteoglycan complex; PGS, proteoglycan subunit fraction; PMN, polymorphonuclear leukocytes; SDS, sodium dodecyl sulfate.

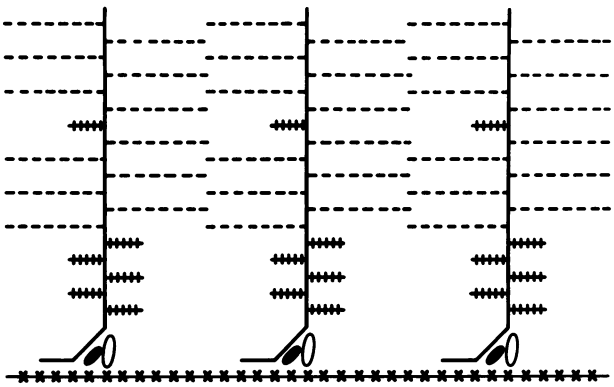

FIGURE 1 Structural model of bovine nasal cartilage proteoglycan complex. ——, core protein; - - - -, chondroitin sulfate; +++, keratan sulfate; X-X-X, hyaluronic acid; ●○, link proteins.

which has a mol wt of approximately 2.5×10^6, consists of a linear core protein with approximately 110 chondroitin sulfate and 50 keratan sulfate side chains covalently attached. It includes two functionally, structurally, and immunologically distinct portions: a hyaluronic acid-binding segment at one end that contains only protein and keratan sulfate, and a much larger chondroitin sulfate-containing segment. In terms of its overall composition by weight, bovine nasal cartilage proteoglycan is 86% chondroitin sulfate, 6% keratan sulfate, 8% protein, and less than 1% hyaluronic acid (2).

The studies presented here indicate that PMN granule enzymes act only on the protein components of bovine nasal cartilage proteoglycan. The peptide fragments produced have been characterized by chromatographic, gel-electrophoretic, and immunological techniques previously used to study trypsin and trypsin-chymotrypsin digests of bovine proteoglycan (7, 12, 13). Because its structure is similar to that of bovine nasal cartilage proteoglycan (14), it is highly likely that human articular cartilage proteoglycan is similarly affected by these enzymes and that this process plays a role in the pathogenesis of joint destruction in inflammatory arthritis.

METHODS

Proteoglycan fractions: bovine nasal cartilage proteoglycan complex (PGC), proteoglycan subunit fraction (PGS), and proteoglycan glycoprotein link fraction (GPL). Bovine nasal septa were extracted with 4 M guanidine-HCl and fractionated by successive CsCl density gradients in 0.4 M and 4 M guanidine-HCl, by the method of Hascall and Sajdera (2, 3), as described in detail previously (5, 14). PGC (aggregate fraction) represents the bottom two-fifths of the initial (associative) gradient, PGS (subunit fraction) the bottom two-fifths, and GPL (link fraction) the top fifth of the second (dissociative) gradient. The PGS preparation used for viscosometry studies showed a single peak on analytical ultracentrifugation with an S_o value of 21.8S, and the PGC contained two peaks, the aggregate component having an S_o value of 86.8S. Analytical studies by column chromatography, to be described in detail elsewhere, indicated that the PGC contained 72% aggregate and 28% monomer.

Keratan sulfate and chondroitin sulfate-fragment fractions. The preparation of these fractions by trypsin-chymotrypsin digestion of PGS followed by DEAE-cellulose chromatography, and their chemical and immunological properties, have been described in detail previously (13).

Chondroitin sulfate "singlets". PGS was dissolved in 0.4 M NaOH and kept at room temperature for 24 h. After neutralization with dilute HCl and dialysis against distilled water, the free chondroitin sulfate chains were isolated by DEAE-cellulose chromatography, as described previously (13).

Hyaluronic acid and chondroitin sulfate. The human umbilical cord hyaluronic acid and whale cartilage chondroitin sulfate used in viscosity experiments were purchased from Schwarz/Mann Div., Becton, Dickinson & Co., (Orangeburg, N. Y.) and Miles Laboratories, Inc., Miles Research Div. (Elkhart, Ind.), respectively. They contained no significant amounts of protein, as indicated by the lack of absorbance at 280 nm.

PMN granule enzymes and inhibitors. The preparation of G and the isolation of E and CT and their properties have been described in detail previously (15–17). The activity of the preparation of E used in these studies on tertiary-butyloxyl-carbamyl L-alanine paranitrophenyl ester was 6.9 μmol/min/mg protein, and the activity of the CT preparation on benzoyl tyrosine ethyl ester was 11.0 μmol/min/mg protein.

Human alpha 1-antitrypsin was purchased from Worthington Biochemical Co. (Freehold, N. J.). *N*-Acetyl-(L-alanine)$_3$-chloromethyl ketone (AAACK) was provided by Drs. J. C. Powers and P. M. Tuhy under contract from the Division of Lung Diseases, National Heart and Lung Institute, National Institutes of Health.

Antisera. Rabbit antisera to PGS and GPL were the same used in previous studies (5, 9, 13, 14). They were concentrated three times by vacuum ultrafiltration and absorbed with lyophilized bovine serum before use.

Viscosity measurements. The viscosity studies were performed in calibrated Cannon-Ubbelohde semi-micro viscometers (Cannon Instrument Co., State College, Pa.) at 36°C in pH 7.2 phosphate buffer, ionic strength 0.3, or in 0.05 M 2-(*N*-morpholino)ethane-sulfonic acid (MES) buffer, pH 5.3, with 0.1 M NaCl. The viscometer was charged with 1.1–2.9 ml of a solution of the substrate and, after thermal equilibration, the appropriate amount of enzyme solution was added to attain the final concentrations listed in Table I. For inhibition studies, the enzymes were preincubated for 15 min at 22° with the inhibitor before being added to the substrate solution in the viscometer. Transit times were measured to ±0.01 s at 6–10-min intervals over a 2-h incubation and were converted to specific viscosity values with correction for solution density. Curves such as those shown in Fig. 2 were plotted for each experiment, and the data in Table I represent the reduction in specific viscosity after a 100-min incubation as determined from these plots.

Digestion of PGS and GPL with G, E, and CT. PGS, 12 mg/ml in 0.01 M phosphate buffer, pH 7.0, and 0.5 M NaCl, was incubated at 37°C in plastic vials with 8–40 μg protein of G, 1–21 μg protein of E, or 0.6–15 μg protein of CT/ml final solution. Samples for gel chromatography, sodium dodecyl sulfate (SDS)-gel electrophoresis, and immunodiffusion studies were removed after 0.5, 1, 2, and 4 h incubation, and immediately frozen at −80°C. Several drops

TABLE I

The Effect of PMN Granule Enzymes and their Inhibitors on the Specific Viscosity of Polysaccharide and Proteoglycan Solutions after a 100-min Incubation at pH 7.2 and 36°C

Enzyme	Concentration	Substrate	Inhibitor	Reduction η_{sp}
	μg protein/ml	*mg hexuronate/ml*		%
G	19	Hyaluronic acid (0.35–0.57)	—	<0.2
	19	Chondroitin sulfate (8.4)	—	<0.4
	19	PGS (0.8)	—	39.7
	19	PGC (0.59)	—	27.2
	26	PGS (2.82)	—	13.7
	26	PGS (2.82)	Alpha 1-antitrypsin, 133 μg/ml	<0.3
E	19	PGS (1.04)	—	54.2
	19	PGS (1.04)	AAACK, 1.7 mM	20.2
	19	PGC (0.59)	—	62.7
	0.58	PGS (0.44)	—	47.2
	0.58	PGS (0.44)	Alpha 1-antitrypsin, 3.8 μg/ml	30.3
	0.58	PGS (0.44)	Alpha 1-antitrypsin, 28.8 μg/ml	6.8
CT	17.8	PGS (1.04)	—	35.8
	17.8	PGC (0.59)	—	30.8

of toluene were added and incubation continued for up to 20 h, at which time the remaining digest was frozen.

GPL, 2.5 mg/ml in 0.01 M phosphate buffer, pH 7.0, 0.5 M NaCl, or GPL at the same concentration previously incubated with 750 USP U/ml of bovine testicular hyaluronidase (HSEP grade, Worthington Biochemical Corp., Freehold, N. J.) at 37°C for 6 h, were incubated with granule enzymes in plastic test tubes at concentrations in the range noted above for 4 h at 37°C and then frozen at −20°C.

Sepharose gel chromatography. 0.9-ml samples of thawed granule enzyme digests of PGS were applied to a 2.5 × 60-cm glass column packed with Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The column was eluted with 0.2 M NaCl at a flow rate of 20 ml/h at room temperature. The 2-ml fractions collected were tested for the presence of chondroitin sulfate by the carbazole reaction. V_o was determined by the elution maximum of PGC and V_T by the elution maximum of glucuronolactone. Pooled eluates from the fractions indicated in Fig. 3 were dialyzed against distilled water and lyophilized and their molecular weight was determined by equilibrium sedimentation, as described by Heinegård and Hascall (12).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis with SDS was performed as previously described (5, 14). Gels were stained for protein with 0.25% Coomassie blue in water and for polysaccharide with 0.03% toluidine blue in 3.5% acetic acid, and were destained with 7% acetic acid.

Immunodiffusion. Immunodiffusion was performed in 0.6% agarose at pH 8.6 as previously described (5, 14). PGS and chondroitin sulfate-fragment fractions were used at concentrations of 10 mg/ml and GPL and keratan sulfate-fragment fractions at 2.5 mg/ml. PGS and GPL were pretreated with hyaluronidase as described above.

Analyses. The glucuronic acid of chondroitin sulfate was determined by the Bitter and Muir modification of the carbazole reaction (18), with glucuronolactone as standard.

RESULTS

Viscosity studies. Incubation at 36°C with crude PMN G had no effect on the viscosity of solutions of hyaluronic acid or chondroitin sulfate at either pH 5.3 or pH 7.2. However, the viscosity of solutions of PGC or PGS were markedly reduced by incubation at pH 7.2 with similar amounts of G. The action of G on PGC and PGS was abolished by alpha 1-antitrypsin (Table I).

The purified PMN granule neutral proteases E and CT reduced the viscosities of PGC and PGS solutions

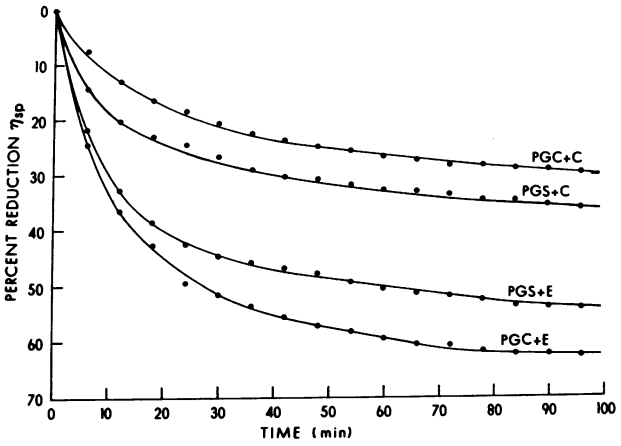

FIGURE 2 The effect of purified human leukocyte granule proteases on the specific viscosity of solutions of cartilage proteoglycan fractions at pH 7.2 and 36°C. PGS was used at a concentration of 4.45 mg/ml, PGC at 0.59 mg hexuronate/ml, and E and C at 0.58 μg protein/ml.

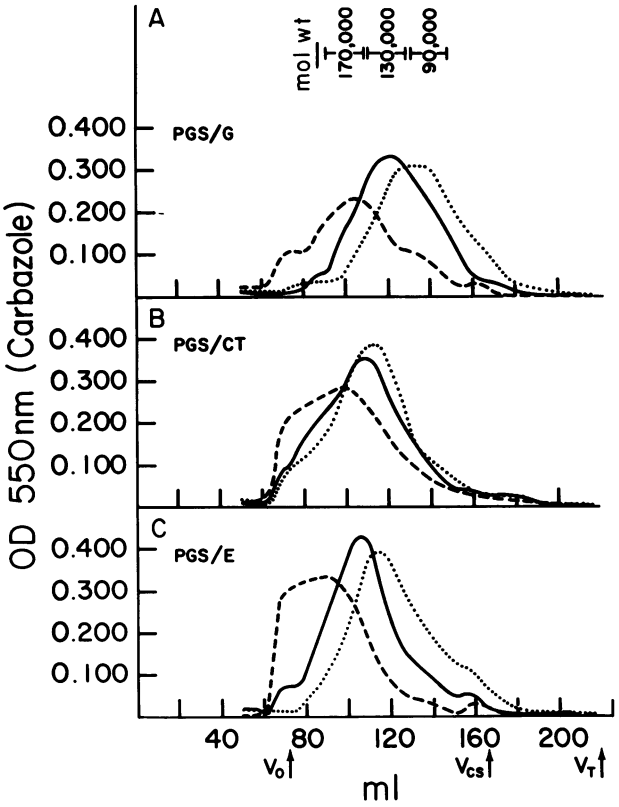

FIGURE 3 Sepharose 4B column chromatography of PGS incubated at 37°C and pH 7.0 for the indicated times with crude PMN granule extract (G) and purified granule proteases CT and E. The average molecular weight of the fragments eluting in the indicated pooled fractions was determined by equilibrium sedimentation. V_{cs} denotes the elution peak of single chondroitin sulfate chains. ----, 0.5-h incubation; ——, 4-h incubation; ····, 20-h incubation.

upon incubation at 36°C and pH 7.2, as shown in Fig. 2. When approximately the same amounts of the two enzymes were used, E caused a greater reduction in viscosity of PGC or PGS solutions than did CT (Fig. 2). The action of E on PGS solutions could be inhibited by alpha 1-antitrypsin and by the specific elastase inhibitor AAACK (Table I).

Sepharose gel chromatography studies. Digestion of the proteoglycan subunit core protein with proteases yields peptide fragments whose apparent size, as indicated by elution position upon Sepharose gel chromatography, is determined by the number of chondroitin sulfate chains per peptide. Thus, exhaustive digestion of PGS with trypsin and chymotrypsin yields an assortment of peptide fragments containing 1–10 chondroitin sulfate chains, broadly distributed over the elution volume of a Sepharose 6B column (12, 13). Because of the highly extended configuration of its glycosaminoglycan chains, the intact proteoglycan subunit is almost entirely excluded from Sepharose 2B, the Sepharose gel of largest pore size (7).

After incubation of PGS with G at a concentration of 8 μg protein/ml at 37°C for 0.5 h, most of the resulting peptide fragments were small enough to elute within the included volume upon Sepharose 4B chromatography (Fig. 3A). With increasing time of incubation, the peptide fragments became progressively smaller, as indicated by the finding of chondroitin sulfate-containing material in progressively later-eluting fractions (Fig. 3A).

Digestion of PGS with CT, 0.6 μg protein/ml at 37°C for 0.5 h, produced some fragments small enough to elute within the included volume of Sepharose 4B (Fig. 3B). With longer incubations, or with higher concentrations of enzyme, the proportion of fragments so large as to elute in the Sepharose 4B excluded volume decreased, but the size of the smallest peptide fragments produced was not appreciably changed (Fig. 3B). The average molecular weight of the PGS fragments in pooled fractions from several portions of the Sepharose 4B column eluate was estimated by equilibrium sedimentation (12). The elution position of the fragments produced by digestion with CT corresponded to that of peptides with approximately 5–12 chondroitin sulfate chains (Fig. 3).

Incubation of PGS with E, 1 μg protein/ml, for 0.5 h at 37°C yielded fragments that eluted partly in the included volume and partly in the excluded volume upon Sepharose 4B chromatography (Fig. 3C). With longer incubations, or with more enzyme, progressively smaller fragments were produced (Fig. 3C). After incubation at 37°C for 20 h with E at a concentration of 21 μg protein/ml, the Sepharose 4B elution pattern of the PGS digest indicated that it consisted entirely of peptide fragments with one to five chondroitin sulfate chains.

SDS-polyacrylamide gel electrophoresis studies. SDS-polyacrylamide gel electrophoresis of PGS yields a proteinpolysaccharide band at the gel origin, since the proteoglycan subunit molecule is too large in effective size to enter the 5% polyacrylamide gel (5). Digestion of PGS with CT or E produced a major protein band within the gel, the fragment produced by E being somewhat smaller than that produced by CT (Fig. 4). Digestion of PGS with G yielded a protein fragment similar in size to that produced by E. Varying the concentration of protease or the duration of the incubation did not affect the migration position of the major protein band or result in the production of additional protein bands.

The major protein fragments produced by digestion of PGS with PMN granule enzymes migrate to approximately the same portion of the SDS-polyacrylamide gels as do single chondroitin sulfate chains. How-

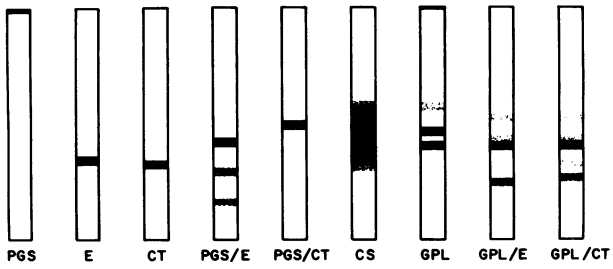

FIGURE 4 SDS-polyacrylamide gel electrophoresis of proteoglycan fractions, purified PMN granule neutral proteases, and proteoglycan fractions incubated with the indicated protease at 37°C for four hours. The gels were stained for protein with Coomassie blue, except for the gel with single chondroitin sulfate chains (CS), which was stained with toluidine blue.

ever, the toludine blue-stained band of isolated chondroitin sulfate chains is much broader than these protein bands (Fig. 4). Furthermore, as discussed in the previous section, peptide fragments with single chondroitin sulfate chains are found only after prolonged incubation with relatively large amounts of E or G and are not found after digestion with CT. Thus, these SDS-gel protein bands must represent fragments derived from a portion of the proteoglycan subunit core protein lacking chondroitin sulfate side chains. Similar fragments, containing protein and keratan sulfate but not chondroitin sulfate, are produced by trypsin or papain digestion of cartilage proteoglycan, and it has been shown that these fragments are derived from the hyaluronic acid-binding segment of the proteoglycan subunit (7, 8).

SDS-polyacrylamide gel electrophoresis of GPL has been shown to yield two major protein bands within the gel (5, 7); the more rapidly migrating band has been termed link *a* and the slower migrating band link *b*. After digestion with GPL with E or CT the link *a* band was unchanged, the link *b* band was absent, and a new protein band of more rapid mobility appeared (Fig. 4).

Immunodiffusion studies. The proteoglycan subunit core protein has been shown on immunodiffusion studies to contain at least two antigenic determinants (13). As illustrated in the upper part of Fig. 5A, both are present in peptide fragment fractions of trypsin-chymotrypsin digests of PGS containing only keratan sulfate, whereas only one is present in chondroitin sulfate-fragment fractions. Although only a single precipitin line was formed by the reaction of antisera to PGS with CT- or G-digested PGS, both core protein antigenic determinants survived, as indicated by the smooth merger of the precipitin lines in the immunodiffusion plate shown in Fig. 5A. Similar results were obtained with PGS incubated with E for up to 6 h. The formation of only a single precipitin line after digestion with PMN granule neutral proteases suggests that the antigenic fragments produced by these enzymes are similar in their diffusion rate or that the two determinants remain on the same fragment.

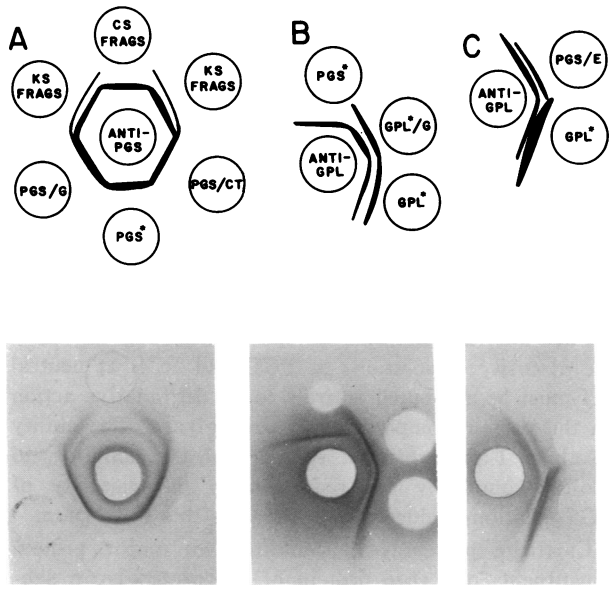

FIGURE 5 The effect of treatment with PMN granule neutral proteases on the immunodiffusion reactivity of proteoglycan fractions. Incubation was carried out at 37°C and pH 7.0 for 6 h with G and CT and for 20 h with E. These digests are denoted PGS/G, PGS/CT etc. The well labeled "CS frags" contained chondroitin sulfate-peptides and the wells labeled "KS frags" contained keratan sulfate-peptides isolated from trypsin-chymotrypsin digests of PGS. PGS and "CS frags" were used at 10 mg/ml, GPL* and "KS frags" at 2.5 mg/ml. * denotes hyaluronidase-treated proteoglycan fractions.

Immunodiffusion of hyaluronidase-digested GPL and antisera to GPL yields two precipitin lines, the line nearer to the antigen well being related to the proteoglycan link proteins (5). The immunodiffusion reaction with anti-GPL sera was not affected by incubation of hyaluronidase-digested GPL at 37°C with G (Fig. 5B), E, or CT.

After incubation of PGS with E for 20 h, two precipitin lines formed upon immunodiffusion with antisera to PGS and GPL, rather than the single line found after shorter incubations. The immunodiffusion reaction with anti-GPL sera, shown in Fig. 5C, suggests that this new antigenic component may be partially related to the link protein component of GPL.

DISCUSSION

The viscosity of a solution of a macromolecule is a function of its effective hydrodynamic size and configuration, so that the reduction in molecular size resulting from the degradative action of an enzyme is reflected by a loss of viscosity. The failure of G to reduce the vis-

cosity of solutions of hyaluronic acid and chondroitin sulfate at pH 5.3 and pH 7.2 therefore indicates that PMN granules lack hyaluronidase or chondroitinase activity. Hyaluronidase has been reported in numerous tissues (19) and in rat liver lysosomes (20), but we are unaware of a previous report of its presence or absence in PMN granules. The failure of G to rapidly produce single chondroitin sulfate chains upon incubation with PGS (Fig. 3A) indicates that enzymes attacking the neutral sugar bridge between the polysaccharide chains and the proteoglycan core protein at pH 7.0 are likewise absent. Thus, the ability of G to reduce the viscosity of solutions of PGC and PGS at neutral pH must be attributed entirely to its degradative action on the protein components of proteoglycan. The ability of alpha 1-antitrypsin, a serum protease inhibitor, to abolish completely the reduction in the viscosity of PGS solutions induced by G supports this conclusion.

Cartilage proteoglycan contains two major protein constituents, the core protein of the proteoglycan subunit and the link proteins of proteoglycan aggregates. G, CT, and E each degrade the larger of the two link proteins without affecting the smaller and without altering the immunological reactivity of the GPL fraction in which they are isolated. Although they serve to stabilize the proteoglycan aggregate under certain conditions in vitro (7, 8, 21), the link proteins are not necessary for aggregate formation (6). The link proteins appear to share antigenic determinants with PGS and it has been suggested that they may, in fact, be derived from PGS (21*a*); this suggestion is supported by the finding described here that prolonged digestion of PGS with E produces a link protein-like precipitin line on immunodiffusion. The physiological role of the link proteins in cartilage, if any, is unknown and the consequences of the degradation of a link protein on cartilage integrity are unclear.

Analysis of the fragments of PGS produced by PMN granule enzymes at neutral pH indicates that the enzymes act on both functional portions of the proteoglycan subunit, the hyaluronic acid-binding segment, and the chondroitin sulfate-containing segment, while preserving the immunodiffusion reactivity of the two known antigenic determinants of the proteoglycan core protein. The purified granule proteases CT and E, at concentrations as low as 0.6–1 μg protein/ml, rapidly produce major fragments that lack chondroitin sulfate and therefore must be derived from the hyaluronic acid-binding portion of the proteoglycan subunit molecule. The production of such fragments results in the breakdown of proteoglycan aggregates, inasmuch as the chondroitin sulfate-containing segments of the proteoglycan subunits are no longer linked to hyaluronic acid and are free to diffuse away.

The chondroitin sulfate-containing segment of the proteoglycan subunit is also rapidly degraded by leukocyte granule enzymes at low concentration. CT and E differ in the extent of degradation they produce. Even with extended incubations and large amounts of enzyme, the fragments produced by digestion of PGS with CT never contained fewer than about five chondroitin sulfate chains. Digestion with E under similar conditions resulted in the production of fragments as small as chondroitin sulfate singlets. This difference probably reflects a difference in the range of specificity of these enzymes, similar to that demonstrated in their pancreatic counterparts (22, 23). Thus, CT may cleave the proteoglycan core protein at a few specific amino acid residues and then be incapable of proceeding further, whereas E may rapidly degrade it into relatively large fragments at preferred cleavage sites and then more slowly into smaller fragments by cleavage at less preferred sites. This manner of degradation, along with the broad size distribution of the resulting fragments, suggests that the chondroitin sulfate-containing segment of the proteoglycan subunit core protein does not have a completely uniform amino acid sequence between polysaccharide chains, even though the amino acids immediately adjacent to the point of attachment of chondroitin sulfate chains may be constant (24).

Although it differs in the relative proportions of chondroitin 4-sulfate, chondroitin 6-sulfate, and keratan sulfate (25), human articular cartilage proteoglycan is similar, if not identical, in overall structure to the proteoglycan of bovine nasal cartilage (14). The antigenic determinants of bovine cartilage proteoglycan, which reside solely in its protein constituents (13), are shared by human cartilage proteoglycans (14). There is, therefore, every reason to expect that the actions of PMN granule enzymes on the proteoglycan of human articular cartilage are the same as found in these studies with bovine nasal cartilage proteoglycan.

According to a useful, though perhaps oversimplified, conceptual model, cartilage matrix consists of an intertwining meshwork of collagen fibers surrounding and entrapping proteoglycan aggregate molecules and the water associated with them (26). The gross structure and tensile strength of cartilage are due to its collagen, and its ability to absorb stress is related to its proteoglycan and water content (27, 28). On the basis of their in vitro actions described here and in the preceding paper (1), PMN granule neutral proteases, released during the neutrophil-mediated inflammatory response and locally in excess over inhibitory factors, can be presumed to diffuse into cartilage and cause the breakdown of the huge proteoglycan aggregates into chondroitin sulfate-containing fragments too small to be entrapped by the collagen meshwork. These fragments can diffuse out

of the cartilage matrix, resulting in the depletion of its proteoglycan content. The loss of proteoglycan may render cartilage more susceptible to the disruptive effect of forces arising from joint motion and weight bearing (28). Collagenase present in leukocyte granule extract (29) may also participate in this process (30), by loosening the collagen meshwork so as to allow large proteoglycan fragments to diffuse out, or by disrupting the structural integrity of cartilage matrix resulting in its gross physical destruction. Finally, since the proteoglycan fragments produced by these enzymes have been found to retain their antigenic determinants, it is possible that immunological responses to degradation products of cartilage may play a role in the causation and perpetuation of joint inflammation, as suggested by Herman et al. (31, 32). Because of their ability to initiate all these degradative processes at the pH actually found in inflamed joints (33), PMN granule neutral proteases may play a central role in the pathogenesis of joint injury in inflammatory arthritis.

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