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Characteristics of the Core Protein of the Aggregating Proteoglycan From the Swarm Rat Chondrosarcoma

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A ternary complex of hyaluronic acid-binding region and link protein bound to hyaluronic acid was isolated from limit clostripain digests of proteoglycan aggregates isolated from the Swarm rat chondrosarcoma. Under these conditions, the hyaluronic acid-binding region has a molecular weight of $\cong$65,000 (HA-BR_{65}). N-terminal amino acids in the complex were selectively ^{14}C-carbamylated. The resulting derivatized HA-BR_{65} was isolated, and tryptic peptide maps were prepared and developed on two-dimensional TLC sheets. A single, labeled peptide was obtained which gave a M_r by $\cong$8,000 by SDS-PAGE. Chymotrypsin digestion of the ternary complex reduced the molecular weight of HA-BR_{65} to a polypeptide of $\cong$55,000 (HA-BR_{55}) which still retains the same N-terminal tryptic peptide. Partial digestion of proteoglycan aggregates with clostripain generated a series of larger intermediates with the hyaluronic acid-binding region. Direct SDS-PAGE analysis revealed one major intermediate with M_r $\cong$109,000 (HA-BR_{109}) as well as HA-BR_{65}. After chondroitinase digestion, two additional prominent intermediates were observed on a SDS-PAGE gel at M_r $\cong$120,000 (HA-BR_{120}) and $\cong$140,000 (HA-BR_{140}). All the intermediates were recognized by a monoclonal antibody specific for the hyaluronic acid-binding region, and all of them contained the same N-terminal tryptic peptide. The results indicate that the N terminus of the core protein is at the hyaluronic acid-binding end of the proteoglycan and that the chondroitin sulfate chains are first present on the core protein in a region between 109,000 and 120,000 molecular weight away from the N terminus.

Key words: proteoglycan, core protein N terminus, carbamylation

Abbreviations used: aA1 is that used in describing the associatively extracted proteoglycans purified by associative CsCl density gradient centrifugation [6]; HA, hyaluronic acid; LP_{45}, native link protein. HA-BR indicates the distinct domain on the proteoglycan monomer that contains the binding site for hyaluronic acid. The subscripts attached to abbreviated domains of proteoglycan—ie, HA-BR_{65}, HA-BR_{109}—indicate molecular weights of those generated through proteolytic cleavage of the proteoglycan monomer. For example, HA-BR_{65} indicates a hyaluronic acid-binding region with a 65,000 molecular weight species generated with clostripain.

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Proteoglycans from the Swarm rat chondrosarcoma have been extensively studied, in terms of both their structure [1–8] and their biosynthesis [9–18]. This system has been particularly valuable since the tumor has provided a convenient source for large amounts of purified proteoglycans for chemical studies as well as for large numbers of chondrocytes for primary cultures to investigate details of biosynthesis. The structures of the complex carbohydrates assembled on the core protein, namely chondroitin-4-sulfate [1], O-linked oligosaccharides [4,5], and N-linked oligosaccharides [5] have been determined. The proteoglycans from the tumor lack the keratan sulfate chains characteristic of proteoglycans from mature hyaline cartilages. Recent evidence [8] has also indicated that a large portion of the xylose residues in the xylosyl-serine linkage regions for the chondroitin sulfate chains carry a phosphate group on the C-2 position. Further, the intracellular sites of assembly of these substituents on the protein core have been inferred from the kinetics of incorporation of various radiolabeled precursors into the macromolecules [summarized in 19], and from cell fractionation studies [18].

However, very little is known about the structure of the core protein. The initial biosynthetic precursor identified in the rough endoplasmic reticulum has an apparent molecular weight of about 370,000 and is present in only small amounts, approximately 1 $\mu g/10^6$ cells [12]. Once the chondroitin sulfate chains and oligosaccharides are added, most of the core protein becomes highly substituted with carbohydrate derivatives which make the direct analysis of the protein difficult. Previous work has shown that a portion of the core protein, the hyaluronic acid-binding region (HA-BR), is relatively unsubstituted with carbohydrate structures and that this portion can be isolated after selective proteolysis of intact proteoglycan aggregating species with trypsin [20,21] or clostripain [7]. Proteolytic digestion of the aggregating proteoglycan yields an intact ternary complex consisting of HA-BR (derived from the proteoglycan monomer unit), a slightly modified link protein (LP), and hyaluronic acid (HA). For the rat chondrosarcoma, clostripain digestion yields a uniform polypeptide fragment of about 65,000 molecular weight, HA-BR_{65}, which retains its ability to interact specifically with HA and with the LP originally involved in aggregate formation.

We are investigating the protein chemistry of HA-BR_{65} and of other defined fragments from the core protein which still contain the hyaluronic-acid binding site. ^{14}C-carbamylation of the N terminus has been used to identify the N-terminal peptide in tryptic digests and to show that the N terminus of the intact core protein is located at the end that contains the hyaluronic acid-binding site. Furthermore, evidence is provided that the N terminus of the newly synthesized and secreted proteoglycans is blocked, while with time in the tumor, limited degradation presumably occurring in the matrix exposes the N terminus observed on HA-BR_{65}.

MATERIALS AND METHODS

Proteoglycans were isolated from the Swarm rat chondrosarcoma under associative extraction conditions followed by CsCl isopycnic centrifugation as described by Faltz et al [6]. Isolation of the ternary complex (HA-BR_{65}, LP_{43}, HA) and subsequent purification of the HA-BR_{65} from the LP_{43} were accomplished following methods

described by Caputo et al [7]. ^{14}C-carbamylation procedures were taken from Stark [22] with some modifications. Generally, carbamylation was done in 0.5 M guanidine HCl or 4 M guanidine HCl in 0.2 M sodium phosphate, pH 8.0, at 37°C for 6–24 hr. Concentrations of 6 mg/ml (aAl) and 0.8 mg/ml (ternary complex) were used for the carbamylation conditions. A final concentration of 0.22 mCi/ml of ^{14}C-potassium cyanate (50.5 mCi/mmole) was used for carbamylation. Following incubation, the ^{14}C-carbamylated species were freed of unincorporated ^{14}C-cyanate by desalting on a small Sephadex G-50 column.

Two-dimensional mapping of tryptic digested ^{14}C-carbamylated species was done as described by Oike et al [23] with some modifications. ^{14}C-carbamylated species were resolved on SDS-PAGE gels. The individual bands were cut out, fixed, equilibrated with NH_4HCO_3 buffer, pH 7.7, and digested with trypsin (1:100 w/w). The digests were evaporated, dissolved in the thin-layer electrophoresis buffer, and applied to silica 60 TLC plates. The first dimension was electorphoresed at 600 V for 60–90 min, 6°C, pH 3.5. The second dimension was developed in an ascending chromatographic buffer of n-butanol:pyridine:acetic acid:water (13:10:2:8) for 3.5 hr. Fluorograms were developed with New England Nuclear En^{3}Hance using Kodak X-Omat AR films exposed at −70°C.

A discontinuous buffered SDS-PAGE system was used with slab gels as described by Laemmli [24]. However, 3% N, N′diallyltartardiamide was used as a cross-linking agent, permitting solubilization of the gel with 1% periodic acid for determination of radioactivity [12]. When species in the SDS-PAGE were to be processed further for two-dimensional peptide maps, an adjoining lane was sliced and analyzed for radioactivity. Labeled species were then cut out of adjacent lanes and processed as described above.

Electroblotting of polypeptides (Western Blotting) from SDS-PAGE gel to nitrocellulose sheets was done as described by Towbin et al [25] as modified by Caterson and Hampton [26]. A monoclonal antibody, designated 12/21/1-C-6, was made against the reduced and alkylated proteoglycan monomer unit from the rat chondrosarcoma. The antibody was shown to be specific for the HA-BR domain of the molecules. It was used in ELISA assays as described by Rennard et al [27] and Thonar et al [28]. The second antibody used was peroxidase-conjugated goat antimouse immunoglobulins (IgA+IgG+IgM), and o-phenylenediamine was used as the substrate.

Aggregating species of proteoglycan were isolated from the tumor aA1 preparation and from the cell culture medium aAl preparation as described by Kimata et al [2] by Cs_2SO_4 rate zonal centrifugation. The gradient consisted of a 2 M Cs_2SO_4 cushion below a linear gradient from 0.15 to 0.50 M. The aggregating proteoglycan bands in the 2 M Cs_2SO_4 cushion while the nonaggregated species are found at the top of the gradient. The cell culture system yields approximately 30–40 μg proteoglycan/10^6 cells over 24 hr [12] in an A1 preparation.

RESULTS AND DISCUSSION

Identification of the N-Terminal Peptide in Tryptic Digests of the Hyaluronic Acid–Binding Region

More than half of the proteoglycans in the Swarm rat chondrosarcoma can be extracted with associative solvents (typically 0.5 M guanidine HCl in the presence of protease inhibitors), and purified in an associative CsCl density gradient to yield a

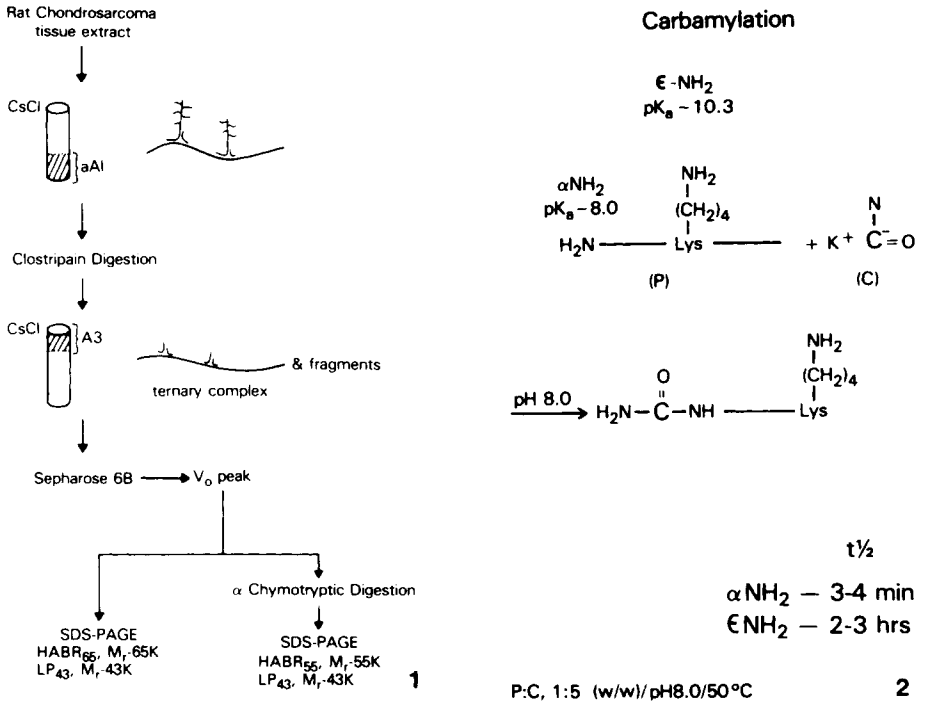

Fig. 1. Schematic flow diagram for the isolation of the HA-BR domain from the Swarm rat chondrosarcoma proteoglycan. Tissue is extracted under associative conditions (a), and the bottom third of an isopycnic CsCl density gradient (A1) is removed and dialyzed against associative buffer appropriate for clostripain digestion. The clostripain digest is then run on a CsCl gradient and the top third of the gradient (A3) is removed. The ternary complex is further purified from protein fragments and enzyme by molecular sieve chromatography (Sepharose 6B).

Fig. 2. Schematic model for carbamylation of amino groups of peptides. Carbamylation favors the nonprotonated amino group as indicated by the kinetics at pH 8.0 under optimum concentration ratio between peptide (P) and potassium cyanate (C).

fraction referred to as aA1 [6]. When proteoglycan aggregates in aA1 are digested with clostripain, the interactions between the HA-BR of the core protein, the LP, and HA are sufficiently protective that a clostripain-resistant species is reached that contains HA-BR_{65} and a modified LP, LP_{43}, still bound to HA (Fig. 1). LP_{43} is slightly smaller than the native LP, LP_{45}, indicating that the protease has removed a small peptide(s). Associative CsCl density gradients are used to separate the dense chondroitin sulfate peptides from the less dense ternary complex. The very large ternary complex is then purified from degradation products and enzyme by molecular sieve chromatography [7].

Portions of purified ternary complex were ^{14}C-carbamylated in the presence of ^{14}C-potassium cyanate under conditions which favor derivitization of amino terminal residues rather than ϵ-amino groups of lysines (Fig. 2) [22]. The ^{14}C-labeled complex was then reisolated from the reaction mixture, and portions were run on SDS-PAGE

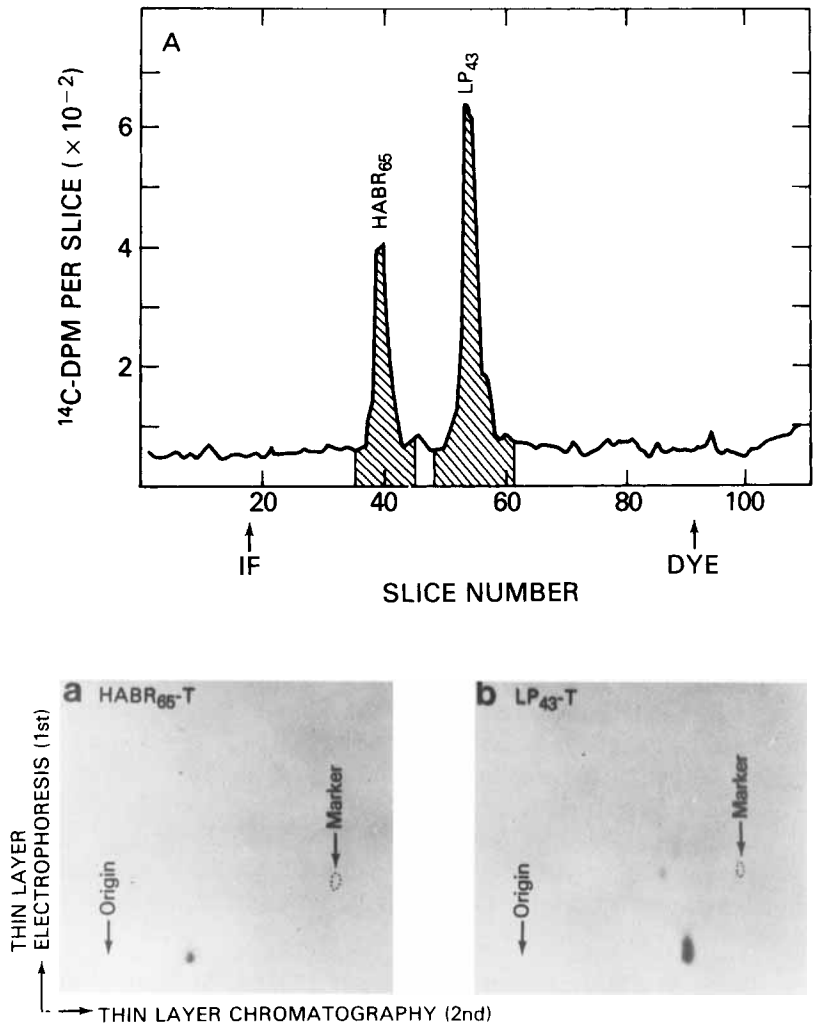

Fig. 3. Upper panel A) 10% SDS-PAGE gel of the ternary complex following ^{14}C-carbamylation for 23 hr in the presence of 4 M guanidine HCl. Panels a and b) Tryptic peptide maps of the ^{14}C-carbamylated HA-BR$_{65}$ and LP$_{43}$ developed on TLC sheets following isolation from the SDS-PAGE gel in an adjoining lane to that in which the radioactivity was determined in the upper panel.

gels. Both the HA-BR$_{65}$ and LP$_{43}$ protein bands contained radioactivity (Fig. 3), and the extent of substitution, calculated from the specific activity of the ^{14}C-cyanate and the protein concentration, was estimated to be about 0.9 moles per mole of HA-BR$_{65}$ molecule and 1.4 moles per mole of LP$_{43}$ molecule. Proteins of each radiolabeled band from a corresponding lane were digested with trypsin [23] and the digests displayed on two-dimensional maps. A single, predominant labeled spot was observed for HA-BR$_{65}$ (Fig. 3). Separate analyses of this labeled peptide on a calibrated SDS-

TABLE I. Competitive ELISA of the HA-BR Domain on Proteoglycans for the Monoclonal Antibody 12/21/1-C-6

	50% Inhibition points (μg) Coating antigen	
Competing antigen	HA-BR_{65}-R+A	PG-T-R+A
HA-BR_{65}	233[d]	—
HA-BR_{65}R+A	3.6[d]	3.5[d]
PG[a]	>9,000[e]	>9,000[e]
PG-R+A[b]	59[e]	44[e]
PG-R+A-T[c]	—	49[e]
PG-T-R+A	—	67[e]

[a]PG—aA1 preparation of proteoglycans.
[b]R+A—reduced with mercaptoethanol followed by alkylation using iodoacetamide.
[c]T—trypsin-digested.
[d]Based on protein content.
[e]Based on proteoglycan weight.

Competitive ELISA was performed as described by references 27 and 28; however, polyvinyl plates were coated with PG-T-R + A (15 μg/well) or HA-BR_{65}-R + A (0.3 μg/well). Values in the table represent the 50% inhibition points extrapolated from plots of varying concentration of the competitive antigen.

PAGE gel indicated an approximate molecular weight of 8,000 (data not shown). The map of trypsin-digested LP_{43} also showed a single, predominant, labeled spot which migrated to a different location from that for the radiolabeled peptide from HA-BR_{65}. SDS-PAGE analysis of the radiolabeled peptide from LP_{43} revealed an approximate molecular weight of 11,000 (data not shown). Limited sequence analysis of HA-BR_{65} by conventional Edman degradation gave the sequence Gln-Gln-Val-Pro-Asn-His through the first six residues.* Limited sequence analysis through the first four residues of the ^{14}C-carbamylated HA-BR_{65} indicated that the N-terminal amino acid was radiolabeled and that the sequence of the following three residues was the same as for HA-BR_{65}.

Characteristics of a Monoclonal Antibody (12/21/1-C-6) Against an Epitope in HA-BR_{65}

A monoclonal antibody isolated from a mouse immunized against monomer proteoglycan (aA1-D1) [6] that had been reduced with dithiothreitol and alkylated with iodoacetamide was characterized (Table I). In an inhibition ELISA assay, the antibody showed a high, nearly identical reactivity toward reduced and alkylated monomer before and after trypsin digestion. The activity was much less for intact, unreduced monomer or for unreduced HA-BR_{65}, suggesting that the epitope is at least partially sequestered when the HA-BR site is folded into its native conformation involving disulfide bonds. The antibody is not directed against the acetamido-substituted cysteine residues because HA-BR_{65} and monomer reduced and derivitized with N-ethylmaleimide were equally reactive. Furthermore, electrotransfer blots of HA-BR_{65} from SDS-PAGE gels, where the HA-BR is presumably extensively denatured,

*N-terminal sequence work on the HA-BR_{65} was done by Dr. Jeff Stevens in the laboratory of Dr. Peter Feitzek at the New Jersey School of Medicine and Dentistry. Present address: Carl Freidenberg/Collan, Postfach 1369, D-6940 Weinheim, West Germany.

reacted readily with the antibody. Thus, the epitope seems to be a portion of the polypeptide sequence, possibly in or near a region containing disulfide bonds.

Characterization of Core Protein Fragments Containing the Hyaluronic Acid–Binding Site

As indicated in the schematic drawing (Fig. 1), α-chymotrypsin digestion of the purified ternary complex decreases the size of HA-BR_{65} to a polypeptide with M_r = 55,000, HA-BR_{55}. A portion of ^{14}C-carbamylated ternary complex was treated with α-chymotrypsin and the digest run on SDS-PAGE. The radioactivity originally present in HA-BR_{65} was quantitatively recovered in HA-BR_{55} (data not shown). Further, a two-dimensional map of a tryptic digest of the resulting HA-BR_{55} was identical to that shown for HA-BR_{65}, in Figure 3. (data not shown). Thus the ~10,000-MW peptide(s) removed from HA-BR_{65} by α-chymotrypsin are located in the carboxyl terminal portion of HA-BR_{65}. In a separate experiment an electroblot showed that HA-BR_{55} was reactive with the 12/21/1-C-6 monoclonal antibody (data not shown), indicating that the epitope is still contained in this fragment. Thus, the epitope is located somewhere between the N terminus and the peptide bond in HA-BR_{65} susceptible to α-chymotrypsin (see schematic Fig. 7 below).

A portion of aA1 from the tumor was ^{14}C-carbamylated directly, or after partially digesting with clostripain for sufficient time to generate a series of intermediates in the core protein larger than the limit HA-BR_{65} fragment. Portions of each sample were treated with chondroitinase ABC and analyzed on SDS-PAGE gels. Coomassie Blue staining revealed two major core protein molecules in the starting material, core I and core II (Fig. 4a), as well as a band near where HA-BR_{65} migrates. These results are similar to those observed previously in which two predominant chondroitinase-generated core protein species were observed [3] and in which polyclonal antibodies against HA-BR_{65} detected some immunoreactive species of nearly identical size to HA-BR_{65} in aA1-D4 samples from the tumor [7,29]. After partial clostripain digestion, a series of intermediate fragments were observed (Fig. 4b). Electroblot revealed that several of these were reactive with the monoclonal antibody (Fig. 4c). Both core I and core II react with the antibody. However, the apparent weakness of the reaction in this experiment occurred because of poor transfer and binding of the larger fragments to the nitrocellulose, particularly for those with residual chondroitin sulfate linkage oligosaccharides remaining after the chondroitinase treatment. Other fragments at 140,000 and 120,000 reacted positively with the antibody in the partial clostripain digest, and a strongly reacting fragment at 109,000 was particularly prominent (Fig. 4c).

The analyses of radioactivity in the SDS-PAGE gels from the ^{14}C-carbamylated samples (Fig. 4e) indicated that the HA-BR_{109} fragment is present even when the digest is not treated with chondroitinase, whereas the HA-BR_{120} fragment is only apparent after chondroitinase treatment (Fig. 4d). Since both of these fragments contain the hyaluronic acid–binding region, this result indicates that the chondroitin sulfate chains are first present on the core protein somewhere on the polypeptide in the HA-BR_{120} which is extended beyond the HA-BR_{109} fragment. Each of the radiolabeled fragments HA-$BR_{65,\ 109,\ 120,\ 140}$ was recovered from the gel, trypsin-digested, and displayed on two-dimensional maps. In each case, a predominant, radiolabeled spot was located at the position characteristic of that shown for the N-terminal tryptic peptide from the HA-BR_{65} in Figure 3 above (data not shown).

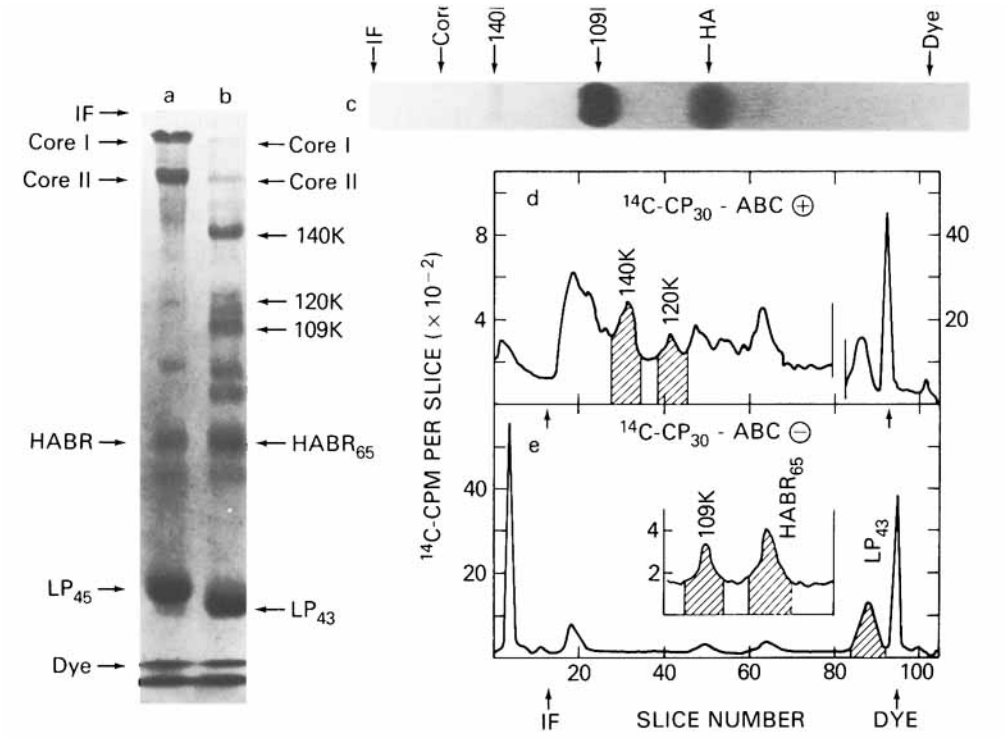

Fig. 4. Partial clostripain digestion of proteoglycans. An aA1 preparation was digested with clostripain to generate intermediate species ranging from the size of the HA-BR_{65} to core I. A portion of the partial digest was ^{14}C-carbamylated and run on an 8% SDS-PAGE gel directly or following chondroitinase ABC digestion. Lanes a and b are Coomassie Blue stains of a) an aA1 preparation and b) a partial clostripain digest of aA1; both applied following chondroitinase ABC digestion. Panel c) Electroblot of an adjoining lane to b, using 12/21/1-C-6 monoclonal antibody specific for the HA-BR. Panels d and e) radiolabeled profile from SDS-PAGE of ^{14}C-carbamylated partial clostripain digest applied directly (e) or following chondroitinase ABC digestion (d). Barred regions indicated locations of species processed for two-dimensional tryptic peptide mapping.

Thus, all of these intermediates, which contain the HA-BR as indicated by reactivity with the 12/21/1-C-6 antibody, also contain the same ^{14}C-carbamylated N-terminal peptide. This indicates that the HA-BR is located in the N-terminal portion of the core protein.

Evidence for Blocked Amino Terminus in Newly Synthesized and Secreted Aggregating Proteoglycans

Aggregating proteoglycans were purified by rate zonal sedimentation on shallow Cs_2SO_4 gradients [2] as outlined in Figure 5. Usually, 60–80% of the proteoglycans in aA1 from tumor are found as aggregate species. After ^{14}C-carbamylation, portions of each aggregate species were digested with chondroitinase ABC and analyzed on SDS-PAGE gels. For the aggregating proteoglycan species sample from the tumor,

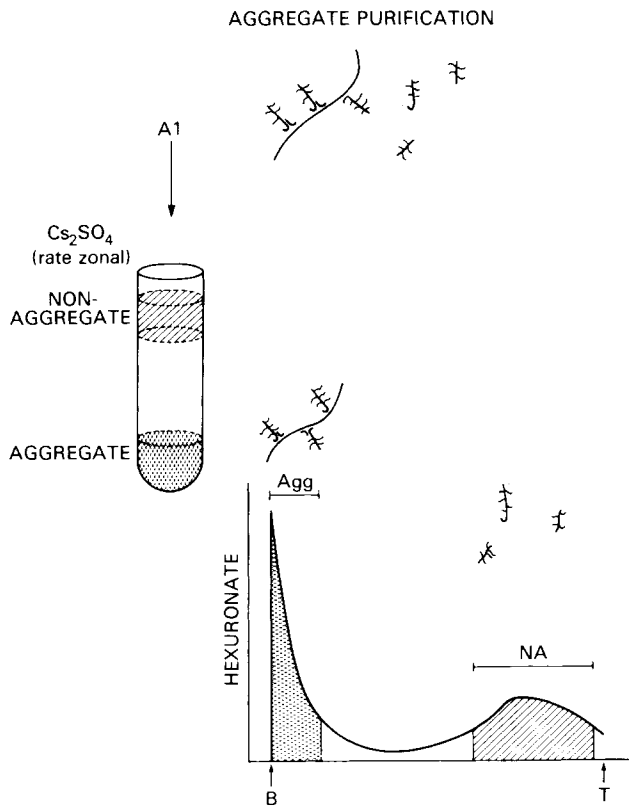

Fig. 5. Isolation of aggregating proteoglycans. An A1 preparation was layered on a linear gradient (0.15 M to 0.5 M Cs_2SO_4) formed on a cushion of 2 M Cs_2SO_4, centrifuged in a Beckman SW 27 rotor at 27,000 rpm, at 10°C for 6 hr.

radioactivity revealed primarily core I and core II bands and a radiolabeled LP_{45} band (Fig. 6). Chondroitinase-digested aggregating species isolated from the cell cultures contains only core I, and a small amount of radiolabel in the region of LP_{45} (data not shown). Previous results have shown that the chondroitinase digests of endogeneously radiolabeled proteoglycans from the cell cultures contain essentially only a single core protein species of apparent MW somewhat greater than 400,000 [12]. The core I bands for both samples and that for LP_{45} from the tumor were trypsin-digested and displayed on two-dimensional maps (Fig. 6). The map for core I from the tumor showed two spots of nearly equal intensity, one at the origin presumably representing background derivitization of lysines and a second at the location for the trypsin generated N-terminal peptide from $HA\text{-}BR_{65}$. For core I from the cell culture, only the spot at the origin was seen; no spot was found in the location of the N-terminal peptide. Quantitation indicated that 1.25 mole of ^{14}C was incorporated per mole of core I from the tumor compared with 0.57 mole incorporation for the core I from the cell culture. The results suggest that the N terminus on the newly synthesized and secreted aggregating proteoglycan monomer is blocked to carbamylation. On the

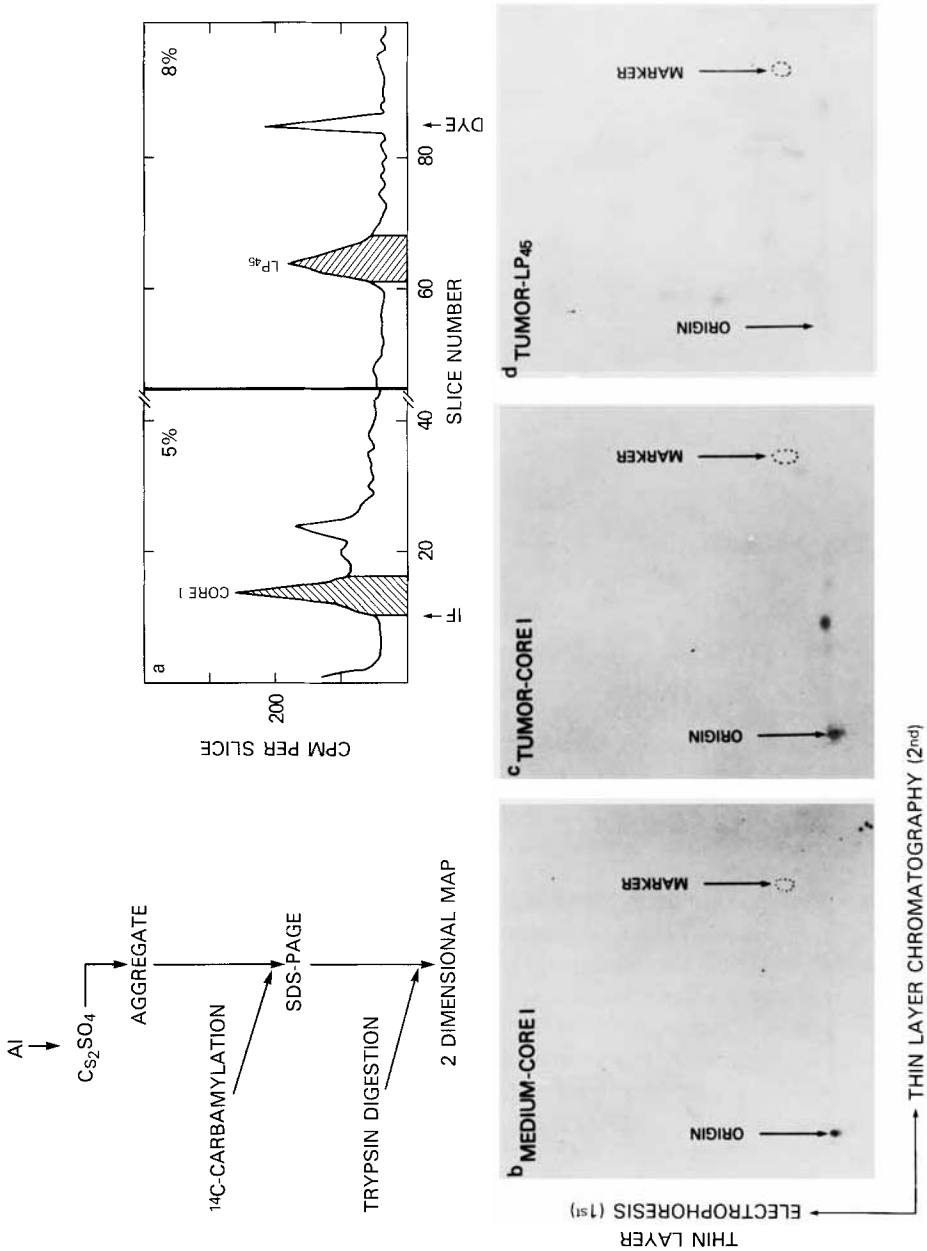

Fig. 6. Tryptic two-dimensional peptide maps of $_{14}$C-carbamylated aggregates from the tumor or cell culture following resolution of species on SDS-PAGE gels. Core I was isolated from a 5% SDS-PAGE gel whereas the LP_{45} was resolved, separately, on an 8% SDS-PAGE gel.

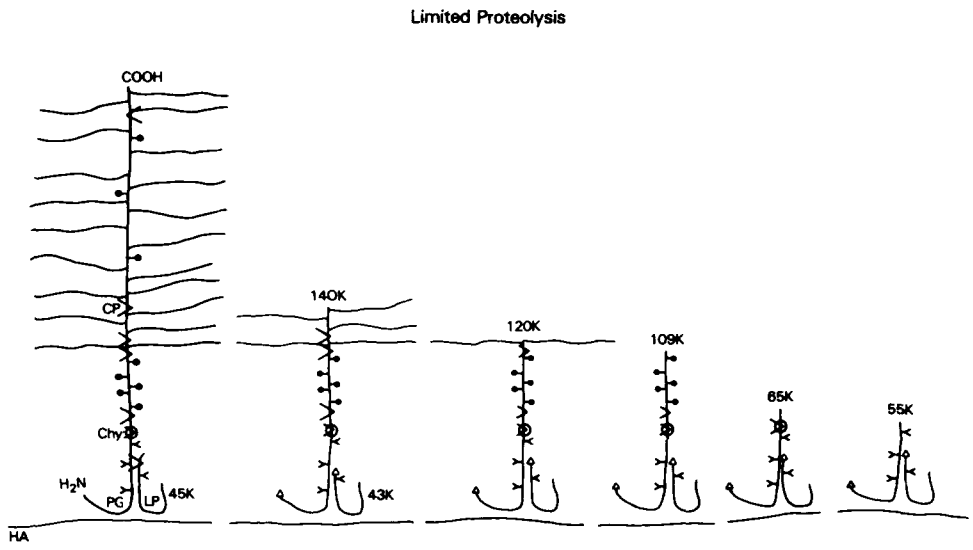

Fig. 7. Schematic model of susceptible sites on the aggregating proteoglycan species to cleavage: < —site of cleavage seen in tumor; > — selected sites of clostripain cleavage; and ⋑ —site of α-chymotrypsin cleavage. Abbreviations: HA, hyaluronic acid; PG, proteoglycan monomer unit consisting of a core protein designated with an NH_2 terminus on one end and a COOH terminal on the other; LP, link protein. Horizontal lines attached to core protein represent chondroitin sulfate chains. Solid circles, O-linked oligosaccharides and Y-shaped, N-linked oligosaccharides; △ carbamylated N-terminus on the protein. Numbers 140K, 43K, etc, represent approximate molecular weights.

other hand, aggregating proteoglycans that accumulate in the tumor with time appear to be degraded sufficiently to generate smaller fragments, core II for example, and to expose sufficient N termini to yield ^{14}C-carbamylated peptides in two-dimensional maps of trypsin digests which are indistinguishable from that present in clostripain derived HA-BR_{65}. It remains to be seen whether clostripain digestion of aggregating proteoglycans from the cell culture unblocks the N terminus observed on HA-BR_{65}.

The trypsin map of LP_{45} did not reveal a labeled peptide that migrated to the location of that observed for LP_{43} (compare Fig. 6 with Fig. 3), even after prolonged exposure in which many background spots from nonspecific substitution were revealed. This indicates that the LP_{45} N terminus is not the same as that generated by clostripain digestion, and that it might be blocked as indicated by the absence of any intense single spot in the tryptic map.

CONCLUSION

The schematic model in Figure 7 summarizes the results presented above. The initially synthesized aggregating proteoglycan appears to have a blocked N terminus. With time in the tumor and probably after treatment with clostripain, an N-terminal glutamine is exposed. This N terminus can be tagged by ^{14}C-carbamylation, and a characteristic radiolabeled tryptic peptide of ~8,000 molecular weight isolated. This peptide appears to be present on core I isolated from the tumor (Fig. 6) and on several

intermediate fragments, HA-$BR_{109, 120, 140}$ in partial clostripain digests. α-Chymotrypsin treatment of intact ternary complex removes about 10,000 MW of polypeptide from the carboxyl region of HA-BR_{65} without altering the N terminus. All of the species indicated, from HA-BR_{55} to core I, are reactive to the 12/21/1-C-6 monoclonal antibody, indicating that the epitope for this antibody is located somewhere in the HA-BR. This monoclonal antibody recognizes denatured as well a trypsin-digested HA-BR_{65}.* Other work in progress has shown that this monoclonal antibody recognizes aggregating cartilage proteoglycans from several species (data not shown). Thus, its high specificity for this class of proteoglycan should be most useful for quantitation and identification of those macromolecules with this epitope in different tissues and culture systems. The generation of the HA-BR_{109} fragment as an intermediate is also intriguing, since this fragment, unlike the next larger one, HA-BR_{120}, does not appear to contain any chondroitin sulfate chains. This defines the portion of the polypeptide where the first chondroitin sulfate chains are present with more precision than previously.

It is possible that the portion of polypeptide in the region between HA-BR_{65} and HA-BR_{109} represents a part of the keratan sulfate–enriched region identified for proteoglycans isolated from mature hyaline cartilage [21]. It remains to be seen what proportion of the O-linked and N-linked oligosaccharides reside in this region.

We hope that investigations of this type will begin to identify and characterize in much greater detail peptides located in different defined regions of the core protein and those located in functional regions such as those interacting with hyaluronic acid and link protein. Further, we hope that the development of a series of monoclonal antibodies reacting with well-defined epitopes in different regions, such as for 12/21/1-C-6, will provide useful tools for probing the structure of the core protein in many cartilagenous tissues. Sequence analysis of key peptides will also provide the requisite toehold for the molecular biologist to unravel the nucleotide sequence and eventually the gene structures for this class of extremely large macromolecules.

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*Recent work has indicated that the reduced and alkylated tryptic peptide recognized by the 12/21/1-C-6 antibody is on a different peptide from the N-terminal peptide. Thus, the epitope does not appear to be located in the 8,000 M_r N-terminal region (Oike, unpublished observation).

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