Type IX collagen from chick embryonic cartilage is a proteoglycan bearing a single chondroitin sulfate chain covalently linked to the α2(IX) polypeptide chain. We have isolated type IX collagen isolated from chick embryonic cartilage and have found that the molecule is synthesized in two forms, a collagen form (COLIX) and a proteoglycan form (PGIX). In cultured chondrocytes, the two forms of type IX collagen showed a different ability to be deposited in the matrix. We have suggested the possibility that both forms may arise from an alternative substitution of a chondroitin sulfate chain to the NC3 domain of the α2(IX) chain. Based on the reported amino acid sequence at the NC3 domain of α2(IX), we have synthesized undecapeptides containing the sequence around the glycosaminoglycan attachment site of the α2(IX) chain. Antibody against the peptide, which was raised in rabbit, only recognized COLIX and made it possible to distinguish COLIX from PGIX. Evidence shows that this could be due to a difference in antigenicity of the NC3 domain of the α2(IX) chain between COLIX and PGIX caused by the substitution of a chondroitin sulfate chain to the serine residue in this domain. Therefore, this antibody may be useful as a probe for studies on the functions of glycosaminoglycan substitution in type IX collagen.

We previously found in chick embryonic cartilage a small chondroitin sulfate proteoglycan of relatively low buoyant density and named it PG-Lt (Kimata et al., 1978). Subsequently, we demonstrated that the core protein of PG-Lt is composed of three collagenous polypeptides and the chondroitin sulfate chain is covalently linked to one of the polypeptides (Noro et al., 1983). It is now established that PG-Lt represents an intact form of type IX collagen consisting of three polypeptide chains (α1(IX), α2(IX), and α3(IX)), of which the α2(IX) polypeptide bears the chondroitin sulfate side chain (Vaughan et al., 1985; Bruckner et al., 1985; Huber et al., 1986; Konomi et al., 1986; McCormick et al., 1987; Huber et al., 1988). The glycosaminoglycan attachment site is within the nontriple helical region NC3, which separates the central collagenous domain COL2 from the amino-terminal collagenous domain COL3 (McCormick et al., 1987). This NC3 domain in α2(IX) chain is 5 amino acid residues longer than the corresponding domains in α1(IX) and α3(IX) chains, and the glycosaminoglycan is bound to a serine residue within the extra amino acid residues (McCormick et al., 1987). The data provide a simple explanation for the sharp kink at the NC3 domain of type IX collagen observed by electron microscopy (Irwin and Mayne, 1986). In cartilage, it has been shown that type IX collagen molecules are localized in a periodic manner along cartilage collagen fibrils (Müller-Glauser et al., 1986; Vaughan et al., 1988; Bruckner et al., 1988) and are cross-linked to type II collagen molecules within such fibrils (Eyre et al., 1987; van der Rest and Mayne, 1988). Due to the kink at the NC3 domain, the amino-terminal triple helical domain of type IX molecules projects out from the fibril surface and places the amino-terminal globular domain NC4 in a position to interact with other matrix components. Thus, type IX collagen molecules on the fibrils have been implicated in representing molecular bridges between individual collagen fibrils and/or between a fibril and some other matrix component (Vasios et al., 1988) as a member of a subclass of fibril-associated collagens with interrupted triple helices (FACIT collagens) (Gordon and Olsen, 1990). Relating to possible functions of the NC4 domain, it is interesting to note that in cornea, two forms of type IX collagen are generated by the synthesis of α1(IX) chains with different amino-terminal globular domains. Synthesis of the two forms, one shorter than the other, results from the alternate use of two promoters in the gene encoding the α1(IX) chain (Nishimura et al., 1989). However, in cartilage, there is another form of type IX collagen generated without a glycosaminoglycan side chain (Huber et al., 1988), and type IX collagen is therefore a "part-time" proteoglycan (Mann et al., 1990). The degree of glycosaminoglycan substitution varies with the difference in cartilage species (about 80% in chick embryo cartilage (Huber et al., 1988), <5% in bovine cartilage (Ayad et al., 1989), and 16% in Swarm rat chondrosarcoma). We previously reported the isolation and characterization of type IX collagen in chick embryonic vitreous humor and found that the type IX collagen was fully (100%) substituted with a chondroitin sulfate chain whose molecular weight was about 10 times larger than that.
in cartilage (Yada et al., 1990). These observations suggest that the glycosaminoglycan chain may be an important functional domain of type IX collagen. It is therefore possible that the post-translational modification by glycosaminoglycan chain substitution may be regulated in a tissue-specific manner. We have isolated and characterized two forms of type IX collagen in cartilage and report here for the first time the immunological and biochemical characterization of the two forms. We have also developed an approach to distinguish the two forms in situ using an anti-peptide antibody raised against the oligopeptide containing the glycosaminoglycan attachment site.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals of reagent grade were purchased from Nakarai Tesque, Tokyo, Japan; fetal eggs (White Leghorn) were obtained from a local supplier; Hanks' balanced salt solution, Ham's F-12 medium, and the amino acid and vitamin mixture for Eagle's minimal essential medium were from Nissui Seiyaku Co., Tokyo, Japan; phenylmethylsulfonyl fluoride and soybean trypsin inhibitor were from Sigma; chondroitinase ABC, chondroitinase AC, and protein A Cellulofine were from Seikagaku Corp., Tokyo, Japan; DEAE-cellulose were from Sigma; chondroitinase ABC, chondroitinase AC, and protein A Cellulofine were from Seikagaku Corp., Tokyo, Japan; L-[2,3-3H]proline and sodium [35S]sulfate were from Du Pont-New England Nuclear; complete and incomplete Freund's adjuvant and skim milk were from Difco; horseradish peroxidase-conjugated protein A was from E-Y Laboratories, San Mateo, CA.; x-ray film (Fuji RX) was from Fuji Photo Film Co., Kanagawa, Japan; Ribi adjuvant system (monophosphoryl lipid A Cellulofine were from Seikagaku Corp., Tokyo, Japan; 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droxyxycyctimine ester activated-keyhole limpet hemocyanin was then mixed with 2 mg of the peptide in 0.2 ml of 0.1 M sodium borate buffer, pH 9.0. After lowering the pH to 7-7.5, the reaction continued for 3 h with stirring at room temperature. A mixture of 1 mg of the peptide-hemocyanin complex and 250 μg of monophosphoryl lipid A, 250 μg of trehalose dimycolate (Ribi Immunochemicals, Lot 1055, lot no. 1000050), and 2 mg of the peptide in 0.2 ml of 100 M NaCl, was injected subcutaneously into a rabbit. After 2 weeks, a booster injection was carried out with a mixture of 0.5 mg of the peptide and 2 mg of the peptide-hemocyanin complex, emulsified with 200 pl of complete Freund's adjuvant. After the final booster, blood was collected. One week after the final booster, blood was collected. The antibody was purified by affinity chromatography. 10 mg of the purified synthetic peptide was coupled to a chondroitin sulfate-bound short peptide derived from a chondroitin sulfate proteoglycan by the method of Oike et al. (1980). The column was washed with 10 column volumes of phosphate-buffered saline and 5 volumes of 10 M Tris-HCl, pH 6.8, successively. The adsorbed antibodies were eluted with 9 volumes of 50 M glycine HCl, pH 2.8, and immediately neutralized with 1 volume of 1 M Tris-HCl, pH 8.0. The antibodies were then dialyzed against distilled water at 4 °C for 24 h three times and lyophilized.

Specificity and Characterization of Anti-NC3 Peptide Antibodies—The titer and specificity were examined by enzyme-linked immunosorbent assay (ELISA) (Rennard et al., 1981) and by immunoblotting (Towbin et al., 1979), respectively. To examine the effect of chondroitinase or hyaluronidase treatment of PGIX, the peptide was coupled to colloidal gold with the aid of 1 mg of protein A-Sepharose (Takagaki et al., 1990). PGIX was digested with collagenase, and the GAG-containing peptides were isolated as described previously (Huber et al., 1988). Brieﬂy, purified chick embryonic vitreous type IX collagen was digested with 10 units of chondroitinase ABC in the enriched buffer containing protease inhibitors (Oike et al., 1980) or 0.1 mg of testicular hyaluronidase in 0.01 M sodium acetate, acetic acid, pH 5.0, 0.15 M NaCl was added to the PGIX-coated ELISA plates and then incubated for 1 h at 37 °C. The plates were rinsed and subjected to ELISA. For competitive inhibition tests, 5 μg of the peptide as a competitor in 100 μl of phosphate-buffered saline, pH 7.4, were added to the well. The antibodies were then added and allowed to react with the antigens. β-Elimination of COLIX and PGIX was performed with 0.2 M NaOH at 4 °C for 12 h. After neutralization with acetic acid, eluted core proteins were collected by trichloroacetic acid precipitation. The precipitates were dissolved in carbonate buffer and used for the substrate for ELISA.

To examine the effect of treatment of PGIX with endo-β-xylosidase on antibody reactivity, we isolated the peptide-containing GAG fraction. The antibody was purified by affinity chromatography. 100 mg of the peptide-hemocyanin complex and 250 pg of monophosphoryl lipid A, 250 pg of trehalose dimycolate, and 200 μl of incomplete Freund's adjuvant. Two additional booster injections were given at 2-week intervals. One week after the final booster, blood was collected.

Preparation of COLIX-rich Fraction—7-day-old chick embryo sternal cartilages were homogenized in Solution E and left at 4 °C for 16 h with stirring. Insoluble residues were removed by centrifugation at 2,000 × g for 30 min. Collagens in the supernatant were precipitated with 45% saturation of ammonium sulfate. Precipitates were dissolved in 0.05 M Tris-HCl, pH 7.5, 0.5 M NaCl and then subjected to sequential salt fractionation with 2.0 and 4.0 M NaCl. Precipitates with 4.0 M NaCl were dissolved in 0.5 M acetic acid and then subjected to further sequential salt fractionation with 0.7, 1.2, 2.0, and 3.0 M NaCl. The precipitate with 1.2 M NaCl was redissolved in 0.5 M acetic acid, dialyzed against 0.05 M acetic acid, and freeze-dried. This fraction was termed as a collagen form of type IX collagen (COLIX)-rich fraction.

RESULTS

Two Forms of Type IX Collagen in Cartilage—17-Day-old chick embryo sternal cartilage was labeled with L-[2,3-3H]proline under lathyrinnc conditions, as described under "Experimental Procedures." Extraction of the cartilage with 1 M NaCl solubilized the majority of radiolabeled type IX collagen with only an additional 5% left in the residue, which was solubilized by subsequent extraction with 4 M guanidine. The tissue extract was then subjected to affinity chromatography using an anti-type IX collagen antibody-coupled Sepharose 4B gel column. Bound molecules were eluted with 3 M KSCN and analyzed by SDS-PAGE. A double band consisting of a diffuse band with a low mobility and a sharp one with slightly higher mobility was observed (Fig. 1, lane b). In comparison with mobilities of known types of collagen, the former had an estimated molecular weight of 300,000 and appeared to correspond to a typical form of type IX collagen, and the latter had a molecular weight of 270,000. Neither band was observed in the unbound fraction (Fig. 1, lane d). In addition, rechromatography of the unbound fraction using the affinity column never gave such bands (data not shown). Both bands were sensitive to digestion with highly purified bacterial collagenase (protease-free preparation) (Fig. 1, lane c). The bound molecules were further fractionated by DEAE-Sephacel column chromatography. 3H radioactivity was largely separated into two fractions, the passed through and the bound ones. Major radioactivity of the latter was eluted at high ionic strength (0.45 M NaCl) (Fig. 2). Fractions were pooled as indicated in Fig. 2 (I-IV), digested with chondroitinase ABC, and then subjected to SDS-PAGE (Fig. 3). Fraction I, the passed through fraction, gave a single band of 270 kDa, regardless of chondroitinase ABC treatment (Fig. 3, lanes a and b). However, Fractions II-IV, the bound fractions with the higher concentrations of NaCl for elution, gave the more diffuse bands with the lower mobilities but equally gave a single 270 kDa band after treatment with chondroitinase ABC.
Two Forms of Type IX Collagen in Cultured Chondrocytes—
Chick chondrocytes were cultured and metabolically labeled with L-[2,3-³H]proline as described under "Experimental Procedures." 1 M NaCl extract was prepared from both cell layers and media and subjected to immunoprecipitation with the anti-type IX collagen antibodies as described above. Fluorographs of the immunoprecipitates (Fig. 4) showed that the type IX collagen from the cell layers consisted of three components of 300, 270, and 265 kDa (Fig. 4, lane c), whereas the one from media contained only the 300-kDa component (Fig. 4, lane a). When the immunoprecipitation was further repeated with the supernatants obtained after the first immunoprecipitation, radioactive precipitates were never formed (data not shown). When pulse-chase labeling studies were performed on the L-[2,3-³H]proline-labeled chondrocytes to investigate any possible biosynthetic precursor/product relationships between the 265-kDa component and the other two components of type IX collagen, the 265-kDa component disappeared during the first 10-h chase. But significant increases in the radioactivity of the other two components could be detected after the chase (data not shown). The results suggested that the 265-kDa component was a biosynthetic precursor for either COLIX or PGIX, or both. The fluorographs also showed that digestion with chondroitinase ABC completely diminished the radioactivity from the 300 kDa band, and the measurement of the radioactivity revealed a parallel increase in the intensity of the 270-kDa component in the digested samples (Fig. 4, lanes b and d). Further, when chick chondrocytes were labeled with [³⁵S]sulfate and the labeled immunoprecipitates were prepared as above, the labeled band was only observed at 300 kDa in the fluorograph. The bands completely disappeared after chondroitinase ABC digestion (data not shown). These results again confirmed that the 300- and 270-kDa components corresponded to PGIX and COLIX, respectively, and suggest that both PGIX and COLIX may be deposited in the matrix surrounding chondrocytes, but only PGIX could be released into the medium.

Production of Anti-NC3 Peptide Antibodies (Immune Probe for COLIX)—The amino acid sequence deduced from the cDNA encoding α2(IX) chain (McCormick et al., 1987), as well as the primary sequence of GAG-containing peptide from collagenase-digested type IX collagen (Huber et al., 1988), showed that the NC3 domain of the α2(IX) chain was 5 amino

![Fig. 2. DEAE-Sephacel column chromatography of affinity-purified [³H]proline-labeled type IX collagen. The bound fraction of affinity chromatography was applied to a DEAE-Sephacel column as described under "Experimental Procedures." After unbound materials were washed out with the starting buffer, bound materials were eluted with a linear gradient of 0.02-0.6 M NaCl. Elution profiles of [³H]proline radioactivity are shown (■). Fractions were pooled for further analysis, as indicated by the Roman numerals.](image)

![Fig. 3. Fluorographs of SDS-PAGE (5% gel) of pooled fractions obtained by DEAE-Sephacel column chromatography before and after chondroitinase ABC digestion. Lanes a, b, and i, Fraction I; lanes c and d, Fraction II; lanes e and f, Fraction III; lanes g, h, and j, Fraction IV. Samples in lanes g, h, and j were digested with chondroitinase ABC; samples in lanes i and j were treated with 5% (w/v) 2-mercaptoethanol prior to the electrophoresis.](image)

![Fig. 4. A fluorograph of SDS-PAGE (5% gel) of immunoprecipitates with anti-type IX collagen antibodies of the [³H]proline-labeled extracts prepared from cultured chick embryonic sternum cartilage. Immunoprecipitates with anti-type IX collagen antibodies of the NET buffer extracts prepared from the spent culture media (lanes a and b) and the chondrocyte cell layer (lanes c and d) were analyzed by SDS-PAGE after the treatment with buffer alone (lanes a and c) or chondroitinase ABC (lanes b and d) for 1 h at 37 °C.](image)
acid residues longer than that of the α1(IX) chain and that the serine residue in this extra sequence of α2(IX) chain (Val-Glu-Gly-Ser-Ala) could serve as the GAG attachment site. This extra sequence is thought to form an external loop-like structure such that it may be antigenic. When the GAG substitution occurs at the serine residue in this extra sequence, the substitution may yield the antigenically different structure. It is likely, therefore, that PGIX may be immunologically different from COLIX in this region. The undecapeptide having the partial sequence of the NC3 domain, including the extra sequence (Gln-Gly-Val-Glu-Gly-Ser-Ala) could serve as the GAG attachment site.

When the GAG peptide and COLIX on an ELISA plate (Fig. 5C, g and j). These reactions were competitively inhibited by the addition of free synthetic peptide (Fig. 5C, i and k). The antibodies also recognized alkali-treated COLIX (Fig. 5C, h). PGIX was not reactive to anti-NC3 peptide antibodies. However, neither of the glycosaminoglycan-free derivatives of PGIX prepared by treatment with glycosaminoglycanase or alkali was reactive (Fig. 5C, a-d). Since the former treatment left the linkage structure attached to the serine residue and the latter gave the dehydroalanine residue in place of the serine residue, no reactivity of the derivatives was probably due to such modifications of the serine residues by the treatments. Therefore, we took another way to remove the GAG chain without any modifications of the serine residue. It has been shown that endo-β-xilosidase from the Patinopecten midgut gland hydrolyzes the xylosyl serine linkages of various glycosaminoglycan-peptides (Takagaki et al., 1990), which may remove the GAG chain from PGIX to give the antigen peptide sequence. However, it has also been shown that the enzyme only hydrolyzes the linkage of GAG chain on a short peptide, but not on an intact proteoglycan (Takagaki et al., 1990). Therefore, the collagenase-resistant short peptides containing the GAG chain, GAG-NC3 peptides, were prepared from PGIX as described under “Experimental Procedures” (Huber et al., 1988). The peptides thus obtained were treated with endo-β-xilosidase. As expected, the treatment resulted in the positive reactivity with anti-NC3 peptide antibodies (Fig. 5C, f). The treatment with the heat-denatured enzyme gave a weak reactivity (Fig. 5C, e), which might be due to a cross-reactivity, with the short peptide relating to the antigen peptide. On the other hand, anti-type IX collagen antibodies that were raised in rabbit by the injection of HMW collagen (pepsin-resistant collagenous fragment of type IX collagen) (Yada et al., 1990) distinctly recognized both COLIX and PGIX (Fig. 5A, a-d and g-i) but neither the GAG-NC3 peptide (Fig. 5A, e and f) nor synthetic NC3 peptide (Fig. 5A, j and k). LY111, a monoclonal antibody (mouse IgM) that recognized intact chondroitin sulfate chains of PGIX only showed positive reaction to the intact PGIX and GAG-NC3 peptide (Fig. 5B, a and e), but none of the GAG-free derivatives (glycosaminoglycanase-treated or alkali-treated PGIX, GAG-NC3 peptide, intact or alkali-treated COLIX, or synthetic NC3 peptide) were reactive (Fig. 5B, b-d and f-h). Taken together, the results suggest that anti-NC3 peptide antibodies recognize COLIX, which is the type IX collagen, without any modification of the serine residue in the NC3 domain.

Reactivity of COLIX and chondroitinase ABC-treated PGIX (PGIX(-CS)) to these antibodies was further examined by immunoblotting. When COLIX and PGIX(-CS) were run under nonreduced conditions on SDS gel and then subjected to immunoblotting with anti-type IX collagen antibodies, the 270 kDa bands from both COLIX and PGIX( CS) were stained with this antibody (Fig. 6, lanes a and b). When, however, anti-NC3 peptide antibodies was used for the blotting, the 270 kDa band only of COLIX was detected (Fig. 6, lanes a and c). When COLIX and PGIX(-CS) were run under reduced conditions on SDS gel and then subjected to immunoblotting with anti-type IX collagen antibodies, respectively, two stained bands of 84 (α1(IX)) and 68 kDa (α2(IX) plus α3(IX)) were obtained from both COLIX and PGIX(-CS) (Fig. 6, lanes e and f). However, when stained with anti-NC3 peptide antibodies, only the 68 kDa band from COLIX was detected (Fig. 6, lanes g and h), which corresponded to α2(IX) chain.

**DISCUSSION**

Proteoglycans are a group of molecules consisting of a central core protein and covalently bound glycosaminoglycan.
side chains. However, recent studies have indicated that certain proteins exist both as unsubstituted proteins and as proteoglycans, for example type IX collagen (Huber et al., 1988), the transforming growth factor-β type III receptor (Cheifetz et al., 1988), thrombomodulin (Bourin et al., 1986), a lymphocyte-homing receptor (Jalkanen et al., 1988), and a melanoma-associated proteoglycan antigen (Bumol et al., 1984).

Derivatives of xylose, such as β-D-xyloside, inhibit proteoglycan synthesis in chondrocytes (Kato et al., 1978; Lohmander et al., 1979), and human melanoma cells (Harper et al., 1986). Experiments that analyzed the capacity of GAG-synthesizing enzymes using p-nitrophenyl β-D-xyloside suggested that the availability for core protein as an initiator for GAG chain synthesis is a limiting factor in proteoglycan synthesis in chondrocytes (Kato et al., 1978) and human melanoma cells (Harper et al., 1986). The results suggest that glycosaminoglycan-free core proteins in the extracellular matrix never appear simply due to the excess over the capacity of the GAG-synthesizing machinery.

The first step for the initiation of glycosaminoglycan substitution on a core protein is xylosylation of the hydroxyl group of a serine/threonine residue. Transfer of xylose residue from UDP-xylose to the hydroxyl group is catalyzed by xylosyltransferase (EC 2.4.2.26). Indicative of the specificity of this catalysis for serine residues within a specific protein context is the fact that neither all proteins containing serine residues are substituted with glycosaminoglycans, nor are all serine residues within a proteoglycan core protein substituted, and rather, only specific serine residues within relatively few proteins are found substituted with glycosaminoglycans.

It is likely, therefore, that a proteoglycan core protein contains a signal for its recognition that allows the subsequent xylosylation of specific serine residues within it. Sequence comparisons support this hypothesis by revealing a sequence motif surrounding the glycosaminoglycan attachment site among several small chondroitin/dermatan sulfate proteoglycan core proteins (Bourdon et al., 1987). Synthetic peptides have also been used to define a putative signal for the attachment of xylose. Earlier results from the in vitro xylosyltransferase assays showed that tripeptides containing Ser-Gly sequence are the best acceptors (Roden et al., 1988). However, the glycosylated sequence Glu-Gly-Ser-Ala-Asp in the non-collagenous NC3 domain of α2(IX) does not have the Ser-Gly-containing sequence that normally functions as a GAG attachment site. The assays showed that inversion of this sequence, i.e. Gly-Ser-Ala for Ala-Ser-Gly, resulted in an approximately 10-fold reduction in the acceptor activity, as measured by $V_{max}/K_m$ in the in vitro assays (Roden et al., 1986; Huber et al., 1988). The amino acid residues neighboring the serine residue may generate a conformation that is bound poorly by xylosyltransferase and may result in the substitution site with a low efficiency (Mann et al., 1990).

The present experiments using various antibodies for the first time suggested that most if not all serine residues of COLIX were not substituted with xylose (Fig. 5C, g and h). If the core molecules of PGIX and COLIX have identical structures in the amino acid sequence, the substitution may be regulated by the efficiency of the xylosyltransferase reaction or assortments of the core molecule to the compartments where a set of GAG-synthesizing enzymes exist.

Type IX collagen in chick embryonic vitreous humor has been recently characterized as a high buoyant density proteoglycan (Yada et al., 1990). All molecules of vitreous type IX collagen appeared to bear an extraordinarily large chondroitin sulfate chain in the α2(IX) chain. Thus, structural characteristics of vitreous type IX collagen are obviously different from those in cartilage. Such characteristics are also seen in type IX collagen of adult chick vitreous humor as well (Yada et al., 1990) and may be a reflection of the functional difference of the vitreous molecule from that of the cartilage. In fact, the extraordinarily large chondroitin sulfate chains are thought to be responsible for the gel-like matrix of this tissue. Therefore, the substitution with the GAG chains to determine either form may be regulated in a tissue-specific manner.

Type IX collagen, once thought to be a cartilage-specific molecule like type II collagen, has now been shown to also be a component of the extracellular matrices in other tissues at various developmental stages: primary corneal stroma, neural retina (Fitch et al., 1988), vitreous (Fitch et al., 1988; Yada et al., 1990), notochordal sheath (Fitch et al., 1989), sclera, and feather pulp. However, it remains to be determined whether type IX collagen in these tissues, except for vitreous, is a proteoglycan or not.

The observed difference in the deposition of the two forms of type IX collagen (COLIX and PGIX) in cultured chondrocyte matrix is also suggestive of possible roles of the GAG substitution of this molecule. A similar difference was observed in the case of human melanoma-associated antigen proteoglycan (Bumol et al., 1984). The core protein (250 kDa) and the proteoglycan (>450 kDa) forms are both synthesized and expressed on the cell surface, but only the proteoglycan form is released into the culture media. Taken together, it is likely that a modification by glycosaminoglycan substitution may regulate interactions of type IX collagen with the cell surfaces or pericellular matrix in cultured chondrocytes. Since the localization of type IX collagen has been found on the surface of collagen fibrils in cartilage (Vaughan et al., 1988), the regulation might be involved in determining the deposition of the fibrils into the extracellular matrix of chondrocytes. Alternatively, since it has been shown that the chondroitin sulfate chain may be one of the potential sites through which proteoglycans could interact with other matrix components or cells (Yamagata et al., 1989), the modification might affect some other functions of type IX collagen to yield the difference.

The chondroitin sulfate chains of type IX collagen may thus be an important functional domain. Anti-NC3 peptide antibodies to which COLIX is only reactive may serve as a probe to investigate this possibility indirectly. Furthermore, anti-chondroitin sulfate antibody such as LY111 could also offer a useful and direct probe for functional studies.

Acknowledgments—We are very grateful to K. Tanabe (Aichi Cancer Institute) for his kind help in the synthesis and purification of peptides, N. Takahashi (Nagoya City University) for the analysis of amino acid compositions, K. Takagaki and M. Endo (Hirosaki University) for the generous gift of endo-β-xylosidase, and R. Mayne (University of Alabama) for helpful criticisms and improvement of the manuscript.

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