Pure & Appl. Chem., Vol. 63, No. 4, pp. 545–554, 1991.
Printed in Great Britain.

ADONIS 2069222091000684

Glycosaminoglycan chains of proteoglycans: approaches to the study of their structure and function

Sakaru Suzuki, Akihiro Mizutani, Yasushi Koike, Masato Kato, Keiichi Yoshida, and Koji Kimata

Institute for Molecular Science of Medicine, Aichi Medical University, Nagakute, Aichi 480-11, Japan

Abstract - Monoclonal antibodies capable of recognizing glycosaminoglycan (GAG) fine structures are likely to be of immense value in isolating and characterizing GAG chains with specific functional domains. We previously described the characterization of a monoclonal antibody directed against the chondroitin sulfate moiety of chick embryo fibroblast proteoglycan. This antibody is highly specific for GlcA(2-SO_4)β1-3GalNAc(6-SO_4), the minor disaccharide unit occasionally found in some chondroitin sulfate chains. More recently, we have prepared a monoclonal antibody (HK-249) to the heparan sulfate moiety of EHS-tumor proteoglycan. This antibody recognizes a fine structure which is abundant in the tumor heparan sulfate but rare in heparan sulfate preparations purified from healthy mammalian tissues. Chemical parameters of various heparan sulfate preparations and their respective activities suggest that the exceedingly high concentration of non-O-sulfated $GlcNSO_3$ residues in the tumor heparan sulfate plays an important role.

Many applications of anti-GAG monoclonal antibodies can be foreseen in both detecting and separating specific GAG subpopulations by virtue of their ability to recognize characteristic GAG fine structures or sequences.

INTRODUCTION

Proteoglycans are macromolecules composed of sulfated glycosaminoglycan (GAG) chains that are covalently attached to protein. Species differ with regard to the nature of the core protein and the amount and type of GAG chains (Fig.1) (for reviews see refs.1-6). In multicellular organisms the near-ubiquitous distribution of these components in the extracellular matrix, on the cell surface, or inside intracellular granules, suggests their involvement in a wide range of biological processes, including cell proliferation, cell migration, extracellular matrix deposition, cell-matrix interaction, and tissue morphogenesis and repair. Several rare proteoglycans probably involved in specific nonstructural functions have also been described, e.g. the precursor for Alzheimer's disease-related amyloid protein (refs. 15, 16), the invariant chain that associates with mouse class II major histocompatibility gene products (ref. 17), and the type III TGF-β receptor (ref. 18). These findings raise interesting questions about the possible occurrence in either the core protein or GAG moieties of specific determinant groups which may well be related to the potentially important functions associated with proteoglycans. Recent studies have shown that some of the interactions of proteoglycan with other substances are mediated by the

protein moiety. For example, the large aggregating cartilage proteoglycan (Fig. 1, a) contains a hyaluronic acid-binding domain and a domain that functions as a lectin capable of binding simple sugars (ref. 7). A similar hyaluronic acid-binding domain is present in the large fibroblast proteoglycan (Fig. 1, b) (ref. 8) and in a lymphocyte membrane proteoglycan which mediates lymphocyte binding to the high endothelium of venules in lymphoid organs (refs. 19, 20). The binding of a small fibroblast-proteoglycan to fibronectin and collagen (ref. 21) and the binding of TGF-β to type III receptor proteoglycan (ref. 18) also appear to be of the core protein-dependent kind. Rapidly increasing amounts of research activity aiming at the elucidation of core protein structure will make it possible to identify novel functional domains involved in proteoglycan core protein moieties.

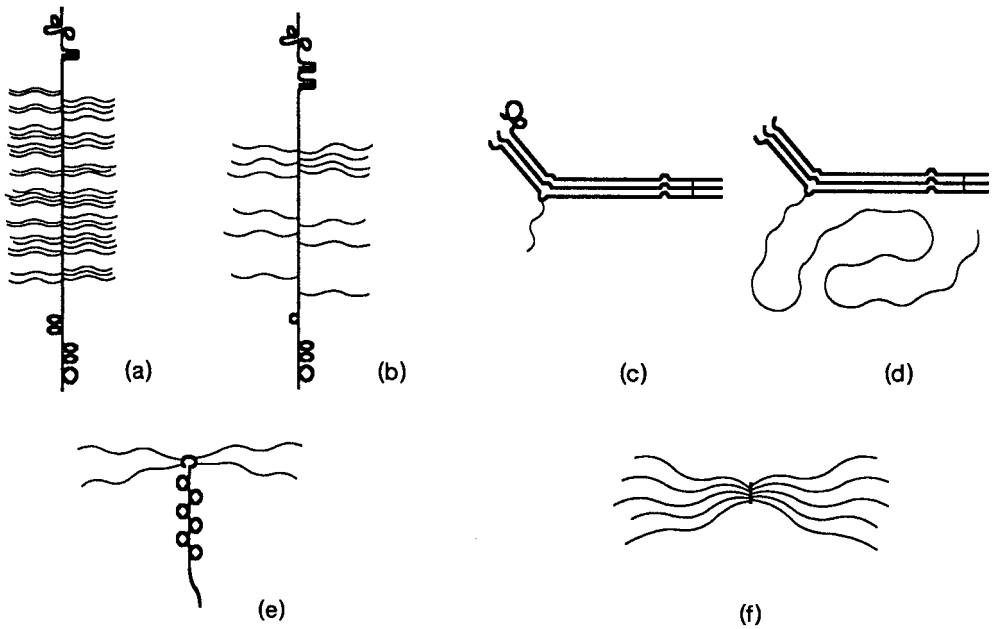

Fig. 1. Designs in proteoglycan structure. (a) rat chondrosarcoma chondroitin sulfate proteoglycan (ref. 7); (b) human fibroblast chondroitin sulfate proteoglycan (ref. 8); (c) embryonic chick cartilage type IX collagen proteoglycan (refs. 9-11); (d) embryonic chick vitreous type IX collagen proteoglycan (ref. 12); (e) EHS-tumor (basement membrane-type) heparan sulfate proteoglycan (ref. 13); (f) rat skin heparin proteoglycan (ref. 14). The core proteins are drawn thicker than the attached GAG chains.

The GAG moieties of proteoglycan have also been shown to mediate many of the interaction of proteoglycan with other substances. For example, different types of GAG have abilities to bind to various proteins including extracellular matrix macromolecules (e.g. fibronectin, thrombospondin, collagens, laminin), cell adhesion molecules (e.g. neural cell adhesion molecule), growth factors (e.g. acidic and basic FGF, TGF-β), and serum protease modulators (e.g. antithrombin III, heparin cofactor II, tissue plasminogen activator). Some of these reactivities are likely to depend on specific sugar sequences or sulfation pattern in the GAG structure (for reviews see refs. 3,4 and 6).

The early work of Meyer and many others (for a review see ref. 22) have established the structure of the basic repeating units of every known group of sulfated GAGs. It is obvious however that the knowledge of their basic

features (or average structures) provides little basis for understanding the potential abilities of sulfated GAGs which may be of biological importance. Much more detailed information on the fine structure of GAG molecules is needed. In the present communication, we summarize the results of our recent studies utilizing monoclonal antibodies that specifically recognize GAG fine structure, offering a clue to the problem concerning the structure-function relationship of GAG chains.

STRUCTURAL DIVERSITY OF GLYCOSAMINOGLYCANS

In the late 1950s, the sulfated GAGs composed of alternating hexuronic and hexosaminidic groups were classified as chondroitin sulfate A, B, and C (subsequently designated chondroitin 4-sulfate, dermatan sulfate, and chondroitin 6-sulfate, respectively), heparitin sulfate (subsequently designated heparan sulfate), and heparin. At that time the chondroitin sulfate A, B, and C were regarded as isomeric polymers differing only in C_5-epimerization (D-glucuronic for A and C, and L-iduronic for B) or in the position of the sulfate residues (N-acetylgalactosamine 4-sulfate for A and B, and 6-sulfate for C). However, it soon became apparent that the structure of these polysaccharides is far more heterogeneous in regard to position and distribution of D-glucuronic and L-iduronic groups and the content and location of sulfate groups. In an attempt to employ definitive degradative enzyme systems to unravel the complexity of chondroitin sulfate structure, Suzuki and coworkers purified two bacterial endoeliminases (designated chondroitinase ABC and chondroitinase AC) and related sulfatases (designated chondro-4-sulfatase and chondro-6-sulfatase) (ref. 23). The enzymes with defined substrate specificity have been useful tools for structural analysis of chondroitin sulfate/dermatan sulfate-type chains. Thus, degradation of chondroitin sulfate and dermatan sulfate preparations from various sources have been shown to release a number of novel disaccharides and higher oligosaccharides, some of which are listed in Fig. 2.

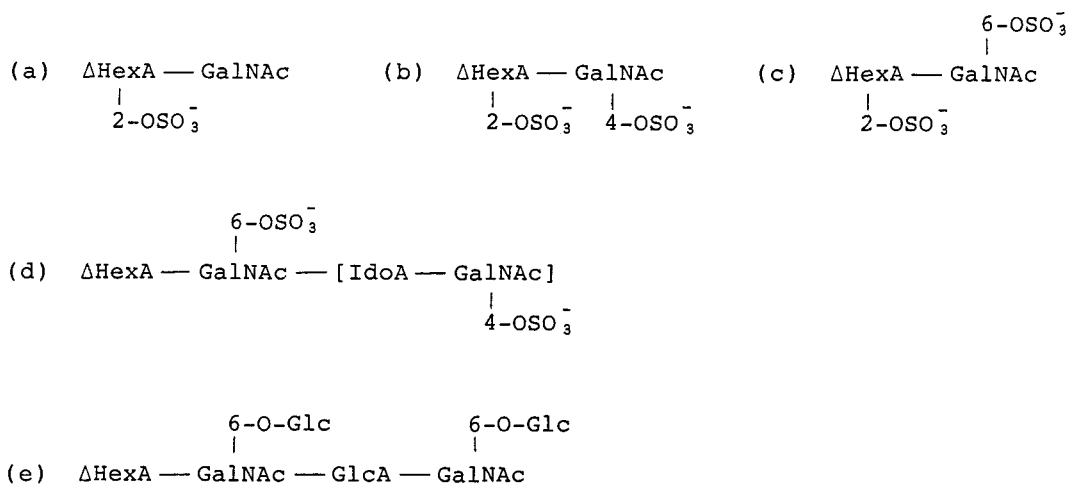

Fig. 2. Structure of some oligosaccharides derived from chondroitin sulfate/dermatan sulfate-type chains by chondroitinase digestion. ΔHexA, Δ^4-unsaturated hexuronosyl residue generated by chondroitinase digestion. See refs. 24-26 for (a)-(c); refs. 27, 28 for (d); and ref. 29 for (e).

The results unequivocally demonstrate the presence of oversulfated regions and copolymeric D-glucuronic/L-iduronic sequences in this class of GAGs. Rare chondroitin sulfate chains with glucose branches have also been

demonstrated in some invertebrate cartilages (ref. 29). Another example of the variation of chondroitin sulfate-type chains is the occurrence of 4,6-bissulfated N-acetylgalactosamine predominantly at the nonreducing terminal (ref. 30). Concurrent with this finding, a novel enzyme (terminal 6-sulfotransferase) capable of introducing a sulfate group almost exclusively into the nonreducing GalNAc 4-SO_4 end group has been demonstrated in several tissues and sera (ref. 31). The oversulfation at the terminal position might play a key role in terminating the elongation of chondroitin sulfate chain or in modifying the nonreducing terminal sugar to yield a potentially bioactive domain.

The structural heterogeneity of heparan sulfate/heparin-type chains has also been widely documented (for a review see ref. 32). The heterogeneity arises from a stepwise series of polymer-level modifications of the precursor molecule composed of alternating N-acetylglucosamine and D-glucuronic acid residues (ref. 33). The precursor chains can be uniquely N-sulfated in the glucosaminyl moiety which is the signal for epimerization of glucuronic acid. The polymer is then further modified by O-sulfation at C_3 and C_6 on the glucosamine and at C_2 on the D-glucuronic or L-iduronic residues. As the extent and location of both epimerization and sulfation are variable, the final products display a high degree of microheterogeneity.

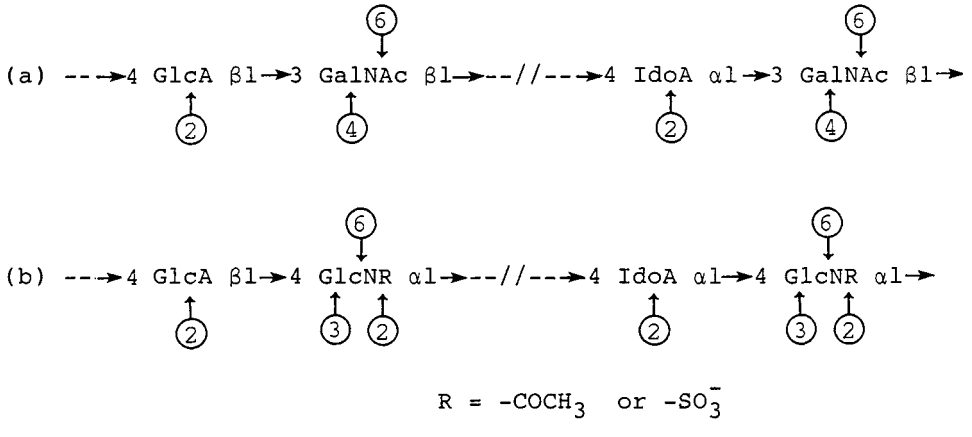

Fig. 3. Structural variation of two types of GAG chain. Portions of chondroitin sulfate/dermatan sulfate-type chain (a) and heparan sulfate/heparin-type chain (b) are depicted. The position numbers with arrow indicate the possible sites of sulfate substitution.

Fig. 3 depicts the general structure and variation of chondroitin sulfate/dermatan sulfate-type chains and heparan sulfate/heparin-type chains, the two major classes of GAG chain differing in the nature of carbohydrate backbone. Both types of chain vary in the proportion of D-glucuronic to L-iduronic acid and the content and arrangement of the sulfate groups, thus forming diverse polymer chains with structural variability.

MONOCLONAL ANTIBODIES THAT SPECIFICALLY RECOGNIZE CHONDROITIN SULFATE FINE STRUCTURES

The microheterogeneity of GAG appears to distinguish this class of biopolymers sharply from the proteins, in which the number of permissible variations seems very limited. Previous attempts to isolate and characterize determinant groups or functional domains of GAGs have been hampered by this structural variability. Taking advantage of the fact that animals

preferentially make antibodies to the regions of biopolymers which may vary from species to species, we have examined the possibility that a series of monoclonal antibodies may be produced against the atypical structures contained in chondroitin sulfate/dermatan sulfate-type chains (see Figs. 2, 3). Such antibodies may find application in selectively isolating a subpopulation of GAG chains or oligosaccharide fragments with a specific determinant group, thus allowing us to look at their structure and function in more detail.

When we started on this study (∿1983), it was generally accepted that chondroitin sulfate in its native state is effectively non-antigenic, so to elicit an immune response it must in some way be modified. Thus, in 1980, Christner et al. (ref. 34) had been successful in producing antigenic determinants, nonreducing Δ^4-uronosyl end groups, by digestion of bovine nasal chondroitin sulfate proteoglycan with chondroitinase ABC or AC. Subsequently, however, Avnur and Geiger (ref. 35) prepared in mice a monoclonal antibody (designated CS-56) directed to native chondroitin sulfates by immunization with ventral membranes of cultured chick fibroblasts. This would indicate that GlcA-GalNAc(4-SO_4) and/or GlcA-GalNAc(6-SO_4), the common repeat units of most avian and mammalian chondroitin sulfates, may be the focus of an immune response in mice. Using commercially available GAG preparations, the above authors have indeed shown that CS-56 antibody bound preferentially to chondroitin 4- and 6-sulfate (ref. 35). Since, however, the GAG preparations used in those studies should have a wide range of microheterogeneity, there is an alternative possibility that the epitope recognized by this antibody resides in an atypical structure rather than the common disaccharide repeat structure.

TABLE 1. Relative abilities of various chondroitin sulfate samples to bind to MO-225 monoclonal antibody

Source of chondroitin sulfate	IC_{50}[a] (μg/ml)	Content of GlcA(2-SO_4)-GalNAc(6-SO_4)unit (% of total HexA)
Shark skin		
Fraction III	0.004	21.4
Fraction II	0.02	17.5
Fraction I	0.08	13.9
Shark scapular cartilage	0.43	8.4
Whale cartilage	4.3	3.0
Embryonic chick cartilage	170	2.0
Human umbilical cord	430	0.5
Swarm rat chondrosarcoma	>3300	0

[a]The concentration (μg/ml) of chondroitin sulfate required for a 50 % inhibition of MO-225 binding to the immunogen (embryonic chick chondroitin sulfate proteoglycan) (see Ref. 36 for details).

Our studies, on the other hand, have shown that a BALB/c mouse immunized with chondroitin sulfate proteoglycan isolated from embryonic chick limb bud produces a monoclonal antibody (MO-225) directed against the chondroitin sulfate moiety (ref. 36). Specificity studies using oligosaccharides of defined structure have shown that MO-225 antibody specifically recognizes a GlcA(2-SO_4)-GalNAc(6-SO_4)-containing determinant (unit D) in the embryonic chick chondroitin sulfate chains. Apparently, this oversulfated disaccharide

unit is foreign to the BALB/c mouse (recipient), as it is absent from the chondroitin sulfate prepared from the whole body of a BALB/c mouse. Table 1 shows that unit D is present in variable amounts in many tissues of different species and that the content of unit D correlates with reactivity to MO-225. The content of other disaccharide units (not shown) shows no correlation with the reactivity.

More recently, Sorrell *et al.* (ref. 37) reported five monoclonal antibodies, 7D4, 4C3, 6C3, 4D3, and 3C5, that recognize native chondroitin sulfate epitopes. All were raised in mice against high buoyant density proteoglycans isolated from embryonic chick bone marrow. Immunocytochemical studies have indicated that the epitopes identified by these monoclonal antibodies are differentially distributed in adult human skin. Immunostaining patterns in the skin using these fine monoclonal antibodies were distinctly different from those obtained using monoclonal antibodies against the stubs of Δ^4-oligosaccharides produced by chondroitinase digestion, suggesting that the epitopes of the five monoclonal antibodies do not represent the typical unsulfated, 4-, or 6-sulfated disaccharide repeats that constitute the bulk of chondroitin sulfate chains. Although the data presented do not specifically identify the antigenic structures recognized by these antibodies, it appears that atypical structures, including the oversulfated units present in variable amounts in chondroitin sulfates from different sources (see Figs. 2, 3), may serve as determinant groups. If this is so, the distribution of these epitopes in functionally distinct regions of a tissue implies that these atypical structures have functional significance.

PREPARATION OF A MONOCLONAL ANTIBODY (HK-249) AGAINST A HEPARAN SULFATE-ASSOCIATED EPITOPE THAT IS HIGHLY EXPRESSED IN A MOUSE TUMOR BUT SCARCELY IN NORMAL TISSUES

Approaches to the structural study of heparan sulfate-type GAGs have included chromatographic separation of different types of GAG followed by enzymatic and chemical degradation of the resulting heparan sulfate fraction to small oligosaccharide fragments for identification. Although these approaches have proved useful for demonstrating both similarities and differences among different heparan sulfate preparations in their average structure, further progress in the elucidation of heparan sulfate fine structure or functional domain has been limited, owing to the lack of techniques that enable high resolution of heparan sulfate chains based on differences in fine structure or functional domain. One notable exception is the elucidation of the saccharide sequence in heparin that binds to antithrombin III, *i.e.* in 1976, three groups independently showed that only about one-third of the molecules in commercially available heparin preparations bound with high affinity to immobilized antithrombin III (refs. 38-40). Subsequent structural analysis has shown that the high affinity binding to antithrombin III is mediated by a unique pentasaccharide sequence, in which a novel 3-*O*-sulfated *N*-sulfoglucosamine residue is an essential component (ref. 33). This discovery provided a useful idea as to how to approach more closely the problem of GAG structure-function relationships. Thus, recent studies using an antithrombin III affinity column have shown that antithrombin-binding regions are not restricted to heparin but also occur in heparan sulfate-type chains (refs. 41, 42). Interestingly, the high-affinity chains in endothelial cell heparan sulfate amount to only a few per cent of the total polysaccharide, but they account for practically all of the anticoagulant activity and the 3-*O*-sulfated glucosamine residues of the starting material (ref. 41).

Previous study of MO-225 monoclonal antibody (see above) suggested that, if heparan sulfate-type GAGs contain atypical fine structures, a series of monoclonal antibodies might be produced, directed against those atypical structures. In 1986, we (ref. 43) and Kure and Yoshie (ref. 44) independently prepared monoclonal antibodies against heparan sulfates (designated HK-249 and HepSS-1, respectively). The heparan sulfate proteoglycan prepared from mouse EHS-tumor was used for the immunization of rats for HK-249 production and the fibrosarcoma cells from methylcholanthrane-induced mouse for the immunization of mice for HepSS-1 production. Subsequent studies of the antigen specificity of HepSS-1 showed that the antibody reacts with bovine kidney heparan sulfate and to a lesser degree, with heparin (ref. 45). This would indicate that the HepSS-1 epitope resides in a typical saccharide structure, i.e. the common structure predominantly found in ordinary heparan sulfate preparations. Since, however, the concentration of the heparan sulfate sample required for a 50 % inhibition of HepSS-1 binding to the immunogen (fibrosarcoma cell) is very high (∿2.5 mg/ml) and since the nature of antigen (heparan sulfate proteoglycan ?) on the fibrosarcoma cell surface has not been described, the definitive structure of the HepSS-1 epitope, whether typical or atypical, remains to be determined.

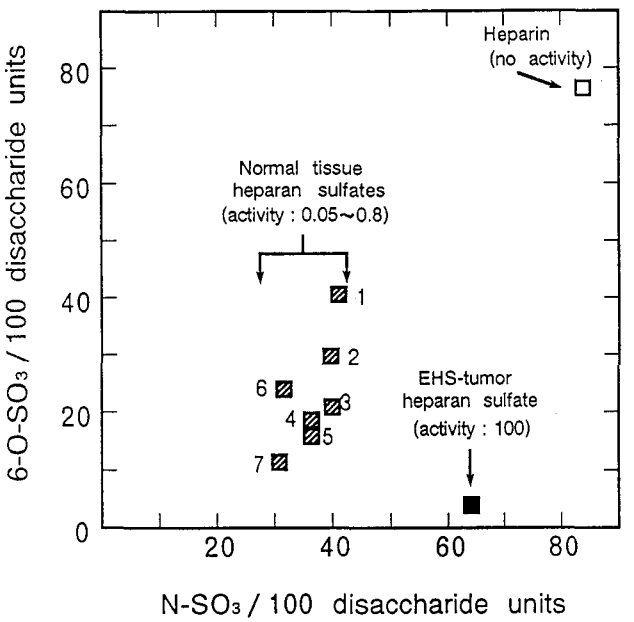

Fig. 4. Correlation between the chemical parameters of heparan sulfates from different sources and their antigenic activity for HK-249. The plots of N-sulfation against 6-O-sulfation indicate the distinction in sulfation pattern between EHS-tumor heparan sulfate (with high antigenic activity) and normal tissue heparan sulfates or heparin (with low antigenic activity). The sources of normal tissue heparan sulfates are: 1, bovine kidney; 2, bovine liver; 3, bovine small intestine; 4, porcine liver; 5, porcine lung; 6, bovine lung; and 7, bovine aorta.

Our studies of HK-249, on the other hand, have shown that it specifically recognizes a heparan sulfate-associated epitope that is highly expressed in the EHS-tumor but to a far lower degree in normal tissues (Fig. 4). The heparan sulfate from EHS-tumor is unique in that, whereas 6-O-sulfate groups are scarce, the N-sulfate content is significantly above the range for the heparan sulfates prepared from normal tissues. One could predict then that

this unique structural feature is associated with the immunological properties of EHS-tumor heparan sulfate. The importance of N-sulfate groups has indeed been shown by chemical modification of EHS-tumor heparan sulfate, i.e. removal of N-sulfate groups resulted in a complete loss of the reactivity to HK-249 which was restored by resulfation but not by acetylation of the exposed free amino groups. That the scarcity of 6-O-sulfate groups is essential for an optimal reactivity has been indicated by the observations that chemical O-sulfation of EHS-tumor heparan sulfate predominantly at C_6 of the glucosamine residues resulted in an appreciable weakening of the reactivity, whereas partial de-O-sulfation caused heparin or bovine kidney heparan sulfate (which otherwise had little or no reactivity) to become somewhat more reactive. Thus, the chemical parameters of heparan sulfates and their respective reactivities suggest that the exceedingly high concentration of N-sulfated, non-O-sulfated glucosamine residues in the tumor heparan sulfate species plays an important role in the HK-249 recognition. However, pending the actual isolation of a determinant group or functional domain from EHS-tumor heparan sulfate, any prediction as to the structure of the recognition determinant must be regarded as tentative.

SEPARATION OF HIGH- AND LOW-AFFINITY HEPARAN SULFATE SPECIES BY AFFINITY CHROMATOGRAPHY ON IMMOBILIZED HK-249

As Fig. 5 shows, our affinity column complexed with HK-249 antibody separates EHS-tumor heparan sulfate into two species, one with low affinity for HK-249 and one with high affinity. The high-affinity species accounts for about 23 % of the applied material.

After dialysis, when this high-affinity fraction was reapplied to the column, it was quantitatively bound. To ensure that the flow-through fraction was devoid of high-affinity species, the unbound material was passed through the column three times more. By the third application, little or no high-affinity material was seen. The final unbound fraction accounts for about 62 % the starting EHS-tumor heparan sulfate preparation.

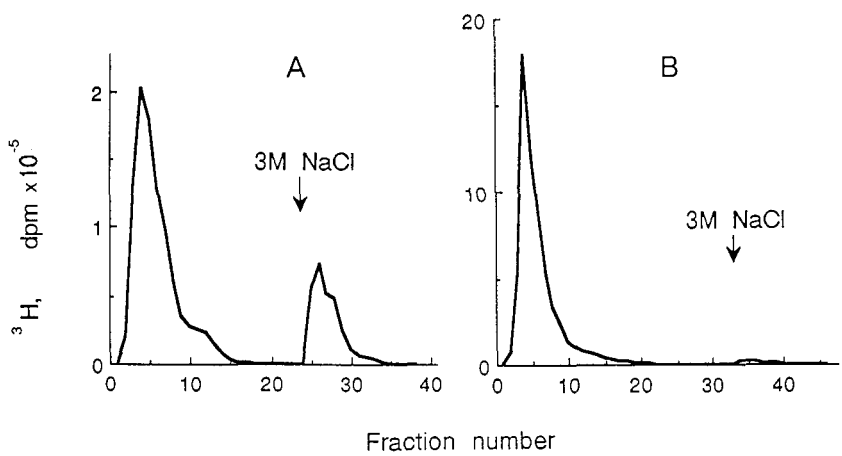

Fig. 5. Separation of high-affinity and low-affinity heparan sulfate species by immunoaffinity chromatography on immobilized HK-249 antibody. EHS-tumor heparan sulfate (A) and bovine lung heparan sulfate (B) were postlabeled with ^{3}H and separately chromatographed on HK-249-conjugated protein A-Cellulofine column eluted stepwise with phosphate-buffered saline and then with 3 M NaCl.

In contrast to the EHS-tumor heparan sulfate, bovine lung heparan sulfate, when subjected to the HK-249 affinity column, yielded a high-affinity material that accounts for only 1.6 % of the applied material. It is notable, however, that this high-affinity material accounted for practically all of the antigenic activity of the starting material. At the present time we have no knowledge of the specific features which distinguish the high-affinity species from the low-affinity one, but further analyses of these distinct species will hopefully reveal the structure of the HK-249 recognition determinant in the heparan sulfate molecules.

Application of the methods illustrated in this study to other GAGs and their partial degradation products might enable production of specific immune reagents useful for the detection and isolation of oligosaccharide species with defined sequences.

Acknowledgement - This work was supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We thank Dr. J. E. Scott, University of Manchester for reading the manuscript and for helpful advice.

REFERENCES

1. J.R. Hassell, J.H. Kimura and V.C. Hascall, Ann. Rev. Biodhem. 55, 539-567 (1986).
2. A.R. Poole, Biochem. J. 236, 1-14 (1986).
3. D. Evered and J. Whelan (eds), Functions of the Proteoglycans, 1-299, Wiley, New York (1986).
4. L.-Å. Fransson, Trends Biochem. Sci. 12, 406-411 (1987).
5. E. Ruoslahti, J. Biol. Chem. 264, 13369-13372 (1989).
6. J.T. Gallagher, Current Opinion in Cell Biol. 1, 1201-1218 (1989).
7. K. Doege, M. Sasaki, E. Horigan, J.R. Hassell and Y. Yamada, J. Biol. Chem. 262, 17757-17767 (1987).
8. T. Krusius, K.R. Cehlsen and E. Ruoslahti, J. Biol. Chem. 262, 13120-13125 (1987).
9. A. Noro, K.Kimata, Y. Oike, T. Shinomura, N. Maeda, S. Yano, N. Takahashi and S. Suzuki, J. Biol. Chem. 258, 9323-9331 (1983).
10. L. Vaughan, K.H. Winterhalter and P. Bruckner, J. Biol. Chem. 260, 4758-4763 (1985).
11. H. Konomi, J.M. Seyer, Y. Ninomiya and B.R. Olsen, J. Biol. Chem. 261, 6742-6746 (1986).
12. T. Yada, S. Suzuki, K. Kobayashi, M. Kobayashi, T. Hoshino, K. Horie and K. Kimata, J. Biol. Chem. 265, 6992-6999 (1990).
13. S.R. Ledbetter, L.W. Fisher and J.R. Hassell, Biochemistry 26, 988-995 (1987).
14. U. Lindahal, Heparin, pp.159-189, Edward Arnord, London (1989).
15. A.D. Snow, J.P. Willmer and R. Kisilevsky, Human Pathol. 18, 506-510 (1987).
16. A.D. Snow, H. Mar, D. Nochlin, K. Kimata, M. Kato, S. Suzuki, J. Hassell and T.N. Wight, Amer. J. Pathol. 133, 456-463 (1988).
17. K.S. Giacolleto, A.J. Sant, C. Bono, J. Gorka, D.M. O'Sullivan, V. Quaranta and B.D. Schwartz, J. Exp. Med. 164 1422-1439 (1986).
18. S. Cheifetz and J. Massague, J. Biol. Chem. 264, 10205-12028 (1989).
19. L.A. Goldstein, D.F.H. Zhou, L.J. Picker, C.N. Minty, R.F. Bargatze, J.F. Ding and E.C. Butcher, Cell 56, 1063-1072 (1989).
20 I. Stamenkovic, M. Amiot, J.M. Pesando and B. Seed, Cell 56, 1057-1062 (1989).

21. T. Krusius and E. Ruoslahti, Proc. Natl. Acad. Sci. USA 83, 7983-7687 (1986).
22. K. Meyer, Amer. J. Med. 47, 664-672 (1969).
23. T. Yamagata, H. Saito, O. Habuchi and S. Suzuki, J. Biol. Chem. 243, 1523-1535 (1968).
24. S. Suzuki, J. Biol. Chem. 235, 3580-3588 (1960).
25. S. Suzuki, H. Saito, T. Yamagata, K. Anno, N. Seno, Y. Kawai and T. Furuhashi, J. Biol. Chem. 243, 1543-1550 (1968).
26. N. Seno, F. Akiyama and K. Anno, Biochim. Biophys. Acta 264, 229-233 (1972).
27. L.-Å. Fransson, J. Biol. Chem. 243, 1504-1510 (1968).
28. H. Habuchi, T. Yamagata, H. Iwata and S. Suzuki, J. Biol. Chem. 243, 6019-6028 (1973).
29. O. Habuchi, K. Sugiura, N. Kawai and S. Suzuki, J. Biol. Chem. 252, 4570-4576 (1977).
30. K. Otsu, H. Inoue, Y. Tsuzuki, H. Yonekura, Y. Nakanishi and S. Suzuki, Biochem. J. 227, 37-48.
31. H. Inoue, K. Otsu, S. Suzuki and Y. Nakanishi, J. Biol. Chem. 261, 4470-4475 (1986).
32. J.T. Gallagher and M. Lyon, Heparin pp.135-158, Edward Arnord, London (1989).
33. U. Lindahl, D. Feingold and L. Rodén, Trends Biochem. Sci. 11, 221-225 (1986).
34. J.E. Christner, B. Caterson and J.R. Baker, J. Biol. Chem. 255, 7102-7105 (1980).
35. Z. Avnur and B. Geiger, Cell 38, 811-822 (1984).
36. M. Yamagata, K. Kimata, Y. Oike, K. Tani, N. Maeda, K. Yoshida, Y. Shimomura, M. Yoneda and S. Suzuki, J. Biol. Chem. 262, 4146-4152 (1987).
37. J.M. Sorrell, E. Mahmoodian, I.A. Schafer, B. Davis and B. Caterson, J. Histchem. Cytochem. 38, 393-402 (1990).
38. M. Höök, I. Björk, J. Hopwood and U. Lindahl, FEBS Lett. 66, 90-93 (1976).
39. L.H. Lam, J.E. Silbert and R.D. Rosenberg, Biochem. Biophys. Res. Commun. 69, 570-577 (1976).
40. L.-O. Anderson, T.W. Barowcliffe, E. Holmer, E.A. Johnson and G.E.C. Sims, Thromb. Res. 9, 575-583 (1976).
41. J.A.Marcum, D.H. Atha, L.M.S. Fritze, P. Nawroth, D. Stern and R.D. Rosenberg, J. Biol. Chem. 261, 7507-7517 (1986).
42. G. Pejler, G. Backstrom, U. Lindahl, M. Paulsson, M. Dziadek, S. Fujiwara and R. Timpl, J. Biol. Chem. 262, 5036-5043 (1987).
43. Y. Koike, K. Kimata, M. Kato and S. Suzuki, Seikagaku 58, 785 (1986).
44. S. Kure and O. Yoshie, Seikagaku 58, 915 (1986).
45. S. Kure and O. Yoshie, J. Immunol. 137, 3900-3908 (1986).