Glycosaminoglycan Binding Properties of Annexin IV, V, and VI*

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Reiko Ishitsuka, Kyoko Kojima, Hideko Utsumi, Haruko Ogawa, and Isamu Matsumoto‡

From the Department of Chemistry, Faculty of Science, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan

We have previously demonstrated that annexin IV, one of the calcium/phospholipid-binding annexin family proteins, binds to glycosaminoglycans (GAGs) in a calcium-dependent manner (Kojima, K., Yamamoto, K., Irimura, T., Osawa, T., Ogawa, H., and Matsumoto, I. (1996) J. Biol. Chem. 271, 7679–7685). In this study, we investigated the GAG binding specificities of annexins IV, V, and VI by affinity chromatography and solid phase assays. Annexin IV was found to bind to a calcium-dependent manner to all the GAG columns tested. Annexin V bound to heparin and heparan sulfate columns but not to chondroitin sulfates columns. Annexin VI was adsorbed to heparin and heparan sulfate columns in a calcium-independent manner, and to chondroitin sulfate columns in a calcium-dependent manner. An N-terminal half fragment (A6NH) and a C-terminal half fragment (A6CH) of annexin VI, each containing four units, were prepared by digestion with V8 protease and examined for GAG binding activities. A6NH bound to heparin in the presence of calcium but not to chondroitin sulfate C, whereas A6CH bound to heparin calcium-independently and to chondroitin sulfate C calcium-dependently. The results showed that annexin IV, V, and VI have different GAG binding properties. Some annexins have been reported to be detected not only in the cytoplasm but also on the cell surface or in extracellular components. The findings suggest that the some annexins function as recognition elements for GAGs in extracellular space.

Annexins are a family of about 13 structurally related calcium-dependent phospholipid-binding proteins. They consist of four or eight conserved repeating structures of approximately 70 amino acid residues and with an N-terminal domain that is highly variable in both sequence and length, which distinguishes different family members. The N-terminal domain is thought to confer functional diversity, whereas this family’s common calcium and phospholipid binding activities probably reside in each repeating unit. Several functions proposed for annexins based on their membrane and calcium binding activities include inhibition of phospholipase A₂ and blood coagulation, regulation of membrane traffic and exocytosis, binding to cytoskeletal proteins, transmembrane channel activity, and intracellular signaling as a kinase substrate (1–3). However, the complete range of their physiological functions has not been elucidated.

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‡ To whom correspondence should be addressed. Tel.: 81-3-5978-5342; Fax: 81-3-5978-5344; E-mail: isamu@hosooipc.chem.ocha.ac.jp.

* The abbreviations used are: CRD, carbohydrate recognition domain; GAG, glycosaminoglycan; PAGE, polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; PVDF, polyvinylidene difluoride; AN, annexin; GST, glutathione S-transferase; HPLC, high performance liquid chromatography; UTI, urinary trypsin inhibitor; MTBS, Tris-buffered saline containing 4 mM 2-mercaptoethanol; BSA, bovine serum albumin.

We have previously purified a calcium-dependent carbohydrate-binding protein, p33/41, from bovine kidney by two-step affinity chromatography on heparin and fetuin columns (4). Amino acid sequence analyses and cDNA cloning (5, 6) revealed that p33/41 is identical to annexin IV and is a newly identified carbohydrate-binding protein having neither known conserved amino acid sequences in carbohydrate recognition domains (CRDs) of animal lectins (7, 8) nor heparin-binding motifs reported for a number of heparin-binding proteins such as growth factors and extracellular matrix molecules (9, 10). Proteoglycans (macromolecules consisting of a protein core and GAG side chains, which are found in the extracellular matrix, on the cell surface, and in secretory granules) are involved in a broad range of activities. Some of their functions are likely to depend on the direct interactions between GAGs and the other molecules (11–13). Therefore, annexin IV was assumed to be one kind of lectin that recognizes these GAGs of proteoglycans. In this study, we investigated the GAG binding activities of annexin IV, V, and VI and their binding specificities to GAGs.

EXPERIMENTAL PROCEDURES

Materials—Bovine brain and liver were obtained from a local slaughterhouse and used fresh or stored at −80 °C. Heparin (porcine intestinal mucosa) was purchased from Wako Pure Chemicals (Osaka, Japan). Heparan sulfate was prepared from porcine kidney in our laboratory (14). Other GAGs (chondroitin, chondroitin sulfate A (whale cartilage, super-special grade), chondroitin sulfate C (shark cartilage, super-special grade), and dermatan sulfate (porcine skin, super-special grade)) were purchased from Seikagaku Kogyo (Tokyo, Japan). Desulfated heparin was prepared by solvolysis of heparin according to Inoue (15) and Ogamo (16), and desulfation was confirmed by measuring the sulfate content as reported previously (17). Affinity gels coupled with GAGs were prepared by use of epoxy-activated Sepharose 4B (18, 19). Proteases, endoproteinase Lys-C (Lysobacter enzymogenes, sequencing grade), and V8 protease were purchased from Boehringer Mannheim (Mannheim, Germany). Rabbit polyclonal antibodies to annexin IV, V, and VI were prepared in our laboratory (20). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody was purchased from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Bovine annexin IV and V cDNA were inserted into plasmid pGEX-3X (Amer- sham Pharmacia Biotech, Uppsala, Sweden), and recombinant proteins were produced as GST fusion proteins as described previously (6).

Purification of Annexin V—Annexin V was purified from bovine brain extract. Bovine brain was homogenized in 4.5 volumes (v/w) of 2 mM EDTA in MTBS (150 mM NaCl, 4 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM Tris-HCl, pH 7.5). The homogenate was shaken for 30 min and centrifuged at 40,000 × g for 30 min. CaCl₂ was added to the supernatant to a final concentration of 7 mM. The pellet was washed twice with 5 mM CaCl₂-MTBS. The pellet was then resuspended in 2 mM EDTA-MTBS and centrifuged at
100,000 × g for 1 h. The final supernatant was applied to a heparin column which had been equilibrated with 2 mM EDTA-MTBS, and the flow-through fraction was recovered; CaCl\(_2\) was added to a final concentration of 5 mM excess. After 30 min on ice, it was applied to another heparin column equilibrated with 5 mM CaCl\(_2\)-MTBS. After the column had been washed with 1.5 column volumes of buffer, the column was washed with 0.75 column volumes of buffer, and CaCl\(_2\) was added to the supernatant to a final concentration of 7 mM. The extract was centrifuged at 27,000 × g for 30 min. The supernatant was discarded, and the pellet fraction resuspended in 200 ml of buffer B (150 mM NaCl, 2 mM CaCl\(_2\), 2 mM-mercaptoethanol, 0.5 mM phenylmethanesulfonyl fluoride, 50 mM HEPES-NaOH, pH 7.3). After the homogenate was centrifuged at 50,000 × g for 10 min, the pellet fraction was discarded, and CaCl\(_2\) was added to the supernatant to a final concentration of 7 mM. The column was eluted using a linear NaCl gradient (0–1 M).

**Purification of Annexin VI**—Annexin VI was purified from bovine liver extract by the method of Creutz et al. (22) with some modifications. 150 mg of bovine liver was homogenized in 400 ml of buffer A (150 mM NaCl, 5 mM EDTA, 4 mM 2-mercaptoethanol, 0.5 mM phenylmethanesulfonyl fluoride, 50 mM HEPES-NaOH, pH 7.3). After the homogenate was centrifuged at 50,000 × g for 30 min, the pellet fraction was discarded, and CaCl\(_2\) was added to the supernatant to a final concentration of 7 mM. The extract was centrifuged at 27,000 × g for 30 min. The supernatant was discarded, and the pellet fraction resuspended in 200 ml of buffer B (150 mM NaCl, 2 mM CaCl\(_2\), 2 mM-mercaptoethanol, 0.5 mM phenylmethanesulfonyl fluoride, 50 mM HEPES-NaOH, pH 7.3). This fraction was then centrifuged at 27,000 × g for 30 min, resuspended in 200 ml of buffer B, and centrifuged again at 27,000 × g for 30 min to complete the washing of the particulate fraction in Ca\(^{2+}\)-containing buffer. The final pellet was resuspended in 60 ml of buffer A and centrifuged at 27,000 × g for 30 min. The supernatant was centrifuged at 100,000 × g for 60 min. The supernatant containing the proteins extracted in EGTA was applied directly to a 1.6 × 20-cm column of phenyl-Sepharose (Amersham Pharmacia Biotech) equilibrated with buffer A. Ammonium sulfate was added to the flow-through fractions (66%) to concentrate the proteins by precipitation. The protein precipitate was collected by centrifugation at 27,000 × g for 45 min, resuspended in 5 ml of 0.3 M sucrose, 25 mM HEPES-NaOH, pH 7.3, and then desalted by passage through a Sephadex G-25 column (Amersham Pharmacia Biotech) equilibrated in the same buffer. The protein fraction was then applied to a DEAE-cellulose column equilibrated in the same buffer, and adsorbed proteins were eluted with a salt gradient formed by mixing with 1 M KCl, 0.3 M sucrose, 25 mM HEPES-NaOH, pH 7.3. In the final purification step, the fraction eluted with 240 mM KCl from a DEAE-cellulose column was applied to a heparin-Sepharose 4B column (inner diameter 0.8 × 6 cm) equilibrated with 150 mM NaCl, 5 mM CaCl\(_2\), and 10 mM Tris-HCl (pH 7.5). The column was washed with the equilibration buffer, and the adsorbed protein was eluted with 2 mM EDTA and 300 mM NaCl by stepwise elution.

**Preparation of Annexin VI Fragments**—Annexin VI was digested with V8 protease at a ratio of 5:1 (v/w) for 1.5 h at 37 °C in 200 mM NaCl, 5 mM CaCl\(_2\), 1 mM dithiothreitol, and 20 mM Tris-HCl (pH 7.5) (23). The size of the fragments obtained was determined by gel filtration HPLC using a Shodex Protein KW-803 column (inner diameter 0.8 × 6 cm; Showa Denko, Tokyo, Japan). SDS-Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting—One-dimensional SDS-PAGE was carried out by the method of Laemmli (24). The electrophoresed proteins were transferred to a polyvinylidene difluoride (PVDF) membrane and then probed with rabbit polyclonal antibodies, and the bound antibodies were detected with HRP-conjugated anti-rabbit IgG antibody and 4-chloro-1-naphthol.

**Amino Acid Sequence Analysis**—Annexin V and VI were digested with proteases, and peptide fragments produced were purified by reverse-phase HPLC using a C\(_18\) column (Waters, Burlington, MA) or by affinity chromatography. Amino acid sequencing was performed with a pulse-liquid protein sequencer 476A (Applied Biosystems Japan, Tokyo, Japan).

**Immobilization of GAGs to Sepharose**—All GAG-Sepharose gels were prepared by the methods described previously (18, 19). 100 mg of GAG was incubated with 5 g of amino-Sepharose and 100 mg of N-ethylmaleimide in 2-ethyl-2-dihydroxypropane/5 ml of methanol at 40 °C overnight. The gel was acetylated to block the remaining amino groups. The amounts of immobilized GAGs were calculated from the hexosamine values of each GAG reported previously (14, 16) and from intact hexosamine contents of the gels obtained by the methods of Blix (25) and Gardell (26).

**GAG Affinity Chromatography**—GAG binding activity was assayed by affinity column chromatography. Annexins were applied to a heparin-, heparan sulfate-, N-desulfated heparin-, N,O-desulfated heparin-, chondroitin sulfate A-, chondroitin sulfate B-, chondroitin sulfate C-, or chondroitin-Sepharose column (inner diameter, 0.3 × 7 cm) in the presence of 5 mM CaCl\(_2\) and the bound protein was eluted by stepwise elution with 2 mM EDTA and then 300 mM NaCl. The amount of protein in each fraction was monitored by enzyme-linked immunosorbent assay (ELISA) using rabbit anti-annexin polyclonal antibodies, HRP-conjugated anti-rabbit IgG antibodies, and o-phenylene diamine. GAG binding activity of peptide fragments produced from annexin VI were assayed using chondroitin sulfate C- and heparin-Sepharose 4B columns under the same conditions as those for intact annexin VI except for concentrations of 5 mM excess. The amounts of GAG affinity chromatography are shown in Figs. 1–3. Annexin IV bound to heparin in the presence of 5 mM CaCl\(_2\) (Fig. 2b) and was not dissociated by 2 mM EDTA but was with 300 mM NaCl (Fig. 3a). In addition, annexin VI bound to heparin even in the absence of calcium (data not shown). Therefore, annexin VI binds to heparin and heparan sulfate in a calcium-independent
manner. Annexin VI bound to N-desulfated heparin in a calcium-independent manner, but the protein adsorbed to N, O-desulfated heparin, and N,O-desulfated heparin was eluted with 2 mM EDTA. Annexin VI was adsorbed onto the chondroitin sulfate A, B, and C and chondroitin columns in the presence of 5 mM CaCl2 and eluted with 2 mM EDTA (Fig. 3b). The binding of annexin VI to chondroitin sulfate-related GAGs required calcium and differed from that to heparin/heparan sulfate GAGs.

The results of affinity chromatography were summarized in Table I. It is noteworthy that there are two modes of binding, one calcium-dependent and the other calcium-independent, and that the three annexins showed different binding specificities toward GAGs.

Semi-quantitative binding assays on the annexins were also performed using heparin and chondroitin sulfate conjugated with BSA. As shown in Fig. 4, annexin IV, V, and VI bound to heparin immobilized on a microtiter plate dose-dependently. In the case of chondroitin sulfate, annexin IV and VI showed calcium-dependent specific binding. On the other hand, the interaction of annexin V with chondroitin sulfate was observed only at high concentrations of annexin.

**Binding of Proteolytic Peptides of Annexin VI to GAG-Sepharose**—Annexin VI is unique structurally, containing eight conserved repeating units, in contrast to all other annexins, which have four repeating units. To investigate the domains of annexin VI responsible for the GAG binding activities, we prepared the annexin VI fragments by a limited proteolytic digestion and examined the binding activities of the fragments by affinity chromatography. Annexin VI was partially digested with V8 protease as described under "Experimental Procedures." The fragments produced were analyzed by SDS-PAGE and subsequent staining with Coomassie Brilliant Blue. As shown in Fig. 5, three peptide fragments with molecular masses of 33, 19, and 14 kDa were observed. The assignment of each peptide fragment was performed by N-terminal amino acid sequencing after separation by SDS-PAGE and subsequent electroblotting onto a PVDF membrane. The N-terminal amino acid sequence of the 19-kDa fragment was LKGTVR...... The sequence is identical to an amino acid sequence of the C-terminal half of annexin VI starting from leucine 352 in the linker region between repeat 4 and 5 (28, 29) (Fig. 6). The 14-kDa fragment gave the N-terminal sequence IADTTSGD......, which is identical to that starting from isoleucine 531. Therefore, both low molecular mass fragments were derived from N-terminal amino acid sequences.

**Fig. 1. Affinity chromatography of annexin IV on GAG-Sepharose gels.** Annexin IV was applied to heparin-related GAG columns of heparin (●), heparan sulfate (○), N-desulfated heparin (■), and N,O-desulfated heparin (□) (a) and to chondroitin sulfate-related GAG columns of chondroitin sulfate A (●), B (○), C (■), and chondroitin (□) (b) in the presence of 5 mM CaCl2 and eluted with 2 mM EDTA. The detection of annexin IV was performed by ELISA with anti-annexin IV antibodies and HRP-conjugated secondary antibodies, measuring absorbance at 490 nm.

**Fig. 2. Affinity chromatography of annexin V on GAG-Sepharose gels.** Annexin V was applied to heparin-related GAG columns of heparin (●), heparan sulfate (○), N-desulfated heparin (■), and N,O-desulfated heparin (□) (a) and to chondroitin sulfate-related GAG columns of chondroitin sulfate A (●), B (○), C (■), and chondroitin (□) (b) in the presence of 5 mM CaCl2 and eluted with 2 mM EDTA. The detection of annexin V was performed by ELISA with anti-annexin V antibodies and HRP-conjugated secondary antibodies.
showed that the 14- and 19-kDa fragments of annexin VI exist as an associated form with an apparent molecular mass of 33 kDa (data not shown), which we hereafter call the C-terminal half.

The digestion mixtures were directly applied onto the heparin-Sepharose column in the presence of 5 mM CaCl₂ and eluted with 2 mM EDTA and 300 mM NaCl. The fractions were collected, and the amount of the annexin VI fragments in each fraction was monitored by ELISA using anti-annexin VI antibodies. Peaks were observed from 2 mM EDTA- and 300 mM NaCl-eluted fractions (Fig. 7a). SDS-PAGE detected a single band corresponding to the 33-kDa fragment in the 2 mM EDTA-eluted fraction and two bands corresponding to the 19- and 14-kDa fragments in the 300 mM NaCl-eluted fraction (Fig. 8).

In the end, the N- and C-terminal halves bound to the heparin column, and the N-terminal half was eluted with EDTA, whereas the C-terminal half was eluted with 300 mM NaCl.

The results of chondroitin sulfate C-Sepharose affinity chromatography of the annexin VI fragments are shown in Fig. 7b. The N-terminal half (the 33-kDa fragment) passed through the fraction was monitored by ELISA using anti-annexin VI antibodies. Peaks were observed from 2 mM EDTA- and 300 mM NaCl-eluted fractions (Fig. 7a). SDS-PAGE detected a single band corresponding to the 33-kDa fragment in the 2 mM EDTA-eluted fraction and two bands corresponding to the 19- and 14-kDa fragments in the 300 mM NaCl-eluted fraction (Fig. 8).

In the end, the N- and C-terminal halves bound to the heparin column, and the N-terminal half was eluted with EDTA, whereas the C-terminal half was eluted with 300 mM NaCl.

The results of chondroitin sulfate C-Sepharose affinity chromatography of the annexin VI fragments are shown in Fig. 7b. The N-terminal half (the 33-kDa fragment) passed through the

![Fig. 3. Affinity chromatography of annexin VI on GAG-Sepharose gels.](image)

Annexin VI was applied to heparin-related GAG columns of heparin (●), heparan sulfate (○), N-desulfated heparin (■), and N,O-desulfated heparin (□) (a), and to chondroitin sulfate-related GAG columns of chondroitin sulfate A (●), B (○), C (■), and chondroitin (□) (b) in the presence of 5 mM CaCl₂ and eluted with 2 mM EDTA and 300 mM NaCl. The detection of annexin VI was performed by ELISA with anti-annexin VI antibodies and HRP-conjugated secondary antibodies.

![Fig. 4. BSA-conjugated GAG binding assay of annexins.](image)

Annexin IV (●), annexin V (■), and annexin VI (△) were incubated with BSA-heparin (a) and BSA-chondroitin sulfate A (b) immobilized on plates in the presence of 5 mM CaCl₂. The detection of annexins adsorbed on BSA-GAG was performed by using anti-GST antibodies for GST fusion proteins of annexin IV and V and anti-annexin VI antibodies.

![Fig. 5. SDS-PAGE analysis of annexin VI fragments.](image)

Annexin VI (160 µg) was treated with V8 protease (32 µg) as described under "Experimental Procedures." The peptides obtained were analyzed on 13% SDS-PAGE and stained with Coomassie Brilliant Blue. The N-terminal amino acid sequences of the protein bands were analyzed as described under "Results."
The 19- and 14-kDa fragments was adsorbed onto the column and eluted with EDTA (Fig. 8). Western blotting analysis showed that the N-terminal half of annexin VI was more sensitive to the antibodies than the C-terminal half (data not shown). The results indicate that the two halves of annexin VI have completely different binding properties for heparin and chondroitin sulfate C and, therefore, annexin VI has at least two distinct GAG-binding sites located in the N- and C-terminal halves of the annexin VI molecule.

**DISCUSSION**

The finding that each annexin has distinct binding specificities supports the theory that the GAG binding activities are involved in the functional diversities of each annexin. On solid phase binding assay, annexin IV, V, and VI were found to bind dose-dependently to heparin, and annexin IV and VI to chondroitin sulfate. Complete chemical desulfation of heparin significantly reduced its binding to annexin V but not to annexin IV. Therefore, sulfate groups of heparin appear to be important for the calcium-dependent interaction between annexin V and heparin, and the calcium-independent interaction between annexin VI and heparin. Annexin VI shows calcium-dependent binding to N,O-desulfated heparin, but calcium-independent binding to heparin and N-desulfated heparin; however, the mechanism remains to be elucidated. In the case of antithrombin III (31, 32), not only the concentration of anionic groups in GAGs but also specific sugar sequences in the GAG are required for the interaction with the proteins. Further experiments are required to elucidate the most complementary oligosaccharide structure in GAG chains involved in the interaction with each annexin.

Animal lectins have been classified into several families such as C-type lectin, S-type lectin or galectin, P-type lectin, and I-type lectin based on shared amino acid sequences and carbohydrate binding properties. The lectin families contain CRDs with a distinct CRD motif as the active segment of carbohydrate binding activity (7, 8). Annexin IV, V, and VI have none of the consensus sequences conserved in the CRDs of those animal lectins. Furthermore, many heparin-binding proteins such as extracellular matrix proteins, growth factors, and en-
zymes share consensus sequences rich in basic amino acids. Two consensus sequences, XBBXBX and XBBXXBX, where B designates a basic amino acid and X any other amino acid, were identified by Cardin and Weintraub (9, 33). Another heparin-binding motif containing tryptophan residues has been identified in other heparin-binding proteins (10). Recently, Kassam et al. have shown that annexin II contains a Cardin-Weintraub sequence in the repeating domain 4: 306-FKKKYGKS314, and actually binds to heparin (34). Annexins IV, V, and VI do not have such complete consensus heparin-binding motifs, but they have some basic amino acid residues in the corresponding region (repeating domain 4 and 8). Chemical modification of lysine residues of annexin VI reduced binding activity of annexin VI to heparin, so basic amino acid residues could contribute to the binding. However, other amino acids may be involved in the calcium-dependent interaction between annexins and GAGs. Several examples of x-ray crystal structure for CRD of C-type animal lectin, which binds sugar calcium-dependently and has a common sequence motif, suggested that coordination bonds between calcium ions and glycoligands are formed (35). Crystallization of most annexins in the presence of calcium showed that calcium-binding sites of annexins are located within endonexin folds (36). Interestingly, calcium binding to annexin induces drastic conformational changes in annexin where a new calcium binding site is formed within the N-terminal half (23). The third repeat of the C-terminal half has the lower homology than other annexins, and this may be responsible for the distinct binding property of the C-terminal half. However, the present data on pIs, calcium binding properties, and homology analyses are not sufficient for complete understanding the GAG-binding sites of the annexins.

Annexins are expressed in various tissues and cells, but individual annexins show selective expression patterns. Distribution of each annexin in cells in which various annexins were expressed varies (44–47). The phospholipids and membrane binding properties of annexins are not sufficient to explain the selective expression of each annexin and the complicated mechanism of its functions. N-terminal domains specific to each type of annexin or properties of repeating domains other than lipid binding activity seem to be involved in their own specific expression or localization. This study shows that annexin IV, V, and VI each have their own specific binding activities for GAGs. Various cells in tissues have specific glycoconjugates depending on the type of cell and the stage of development so the carbohydrate binding properties of annexins may be an important key to clarifying the biological functions of annexins.

For annexins to play a role as ligands for proteoglycans, they must be localized where they can encounter GAG chains of proteoglycans, i.e. on the cell surface, in the extracellular matrix, or in secretory granules. Actually, some annexins have been shown to be localized not only in the cytoplasm but also on the cell surface or in extracellular components.

Several annexins are distributed on various cell surface membranes, such as endothelial cells, chondrocytes, lymphocytes, etc. (48–51). Proteoglycans, too, have been found on the cell surface membrane, and are involved in cell-cell interactions. Yeatman et al. (52, 53) demonstrated the presence of annexins I–VI on the external surface of higher metastatic cells as a cell adhesive molecule, and we have reported that annexin IV is located on the cell surface of a human tumor cell line (54). The adhesive activities of annexins, which are probably responsible for the metastasis of cancer cells, can be explained by our findings that the annexins bind to GAGs.

Furthermore, the extracellular matrix contains various proteoglycans, which are involved not only in mechanical support functions but also various cellular processes such as cell adhesion, proliferation, and differentiation. Annexins, too, on the cell surface may interact with proteoglycans in the extracellular matrix, as the case in which annexin VI binds to chondroitin sulfate as a cell surface receptor and may be responsible for anti-adhesion during chick embryonic development (55).

Annexin IV is highly concentrated in the apical plasma membrane of the epithelial cells in renal proximal tubules (56). Urinary trypsin inhibitor (UTI) having a multipotent inhibitory effect on proteases such as trypsin, chymotrypsin, plasmin, human leukocyte esterase, or hyaluronidase is glycosylated with a disialo-biantennary N-linked oligosaccharide and a low sulfated chondroitin sulfate chain (57, 58). When UTI is intravenously injected, it accumulates predominantly in kidney. In vitro experiment showed that annexin IV binds UTI calcium-dependently. The renal annexin IV may be, therefore, responsible for the accumulation by recognizing a chondroitin sulfate chain of UTI. Although our present data support the hypothesis that some annexins are recognition elements for GAGs, identification of the endogenous ligands for annexins will provide a basis for understanding the mechanism of action and physiological importance of annexins as carbohydrate-binding receptors.

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2 R. Ishitsuka, K. Kojima, H. Ogawa, and I. Matsumoto, unpublished data.

3 K. Kojima, Y. Kato, T. Shinkawa, H. Ogawa, and I. Matsumoto, unpublished data.
