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Fructose 1,6-Bisphosphate Aldolase Is a Heparin-Binding Protein

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Proteins with affinity to heparin under physiological conditions were isolated from bovine cerebral cortex. First, the extract of cerebral cortex was applied to a chondroitin polysulfate column under physiological conditions. Then, the pass-through fraction was applied to a heparin column. Among the bands on SDS polyacrylamide gel electrophoresis of the fraction bound to the heparin column, the major one was identified as fructose 1,6-bisphosphate aldolase (FPA), a cytosolic enzyme involved in the glycolytic pathway. The results indicated that FPA is a heparin-binding protein which exhibits no affinity to chondroitin polysulfate. The results of affinity chromatographies revealed that FPA binds to intact heparin and modified heparins desulfated at C2 OH of the iduronic acid residue or at C6 OH or C2 NH_2 of the glucosamine residue. When 6-*O*-desulfated heparin was employed as the affinity ligand, a single peak having FPA activity was isolated from the extract of bovine cerebral cortex. By further Mono Q chromatography and Superdex gel-filtration, five isoenzymes were purified with more than 50% recovery. These isoenzymes were identified as FPA A4, A3C1, A2C2, A1C3, and C4 by native electrophoresis with and without 4 M urea and subsequent amino acid sequence analysis. The use of 6-*O*-desulfated heparin affinity chromatography thus facilitated the purification of FPA.

Key words: bovine cerebral cortex, fructose 1,6-bisphosphate aldolase, heparin-binding protein, purification, regioselectively desulfated heparins.

Due to the negative charges of its sulfate groups and carboxyl groups, heparin (Hp) interacts with numerous proteins (*1*). The Hp structures essential for interaction with such proteins as antithrombin III have been identified precisely (*2*). As the sulfate groups participate significantly in Hp-protein interactions, the effect of sulfate groups on the interactions with several Hp-binding proteins such as acidic and basic fibroblast growth factors (*3, 4*), tissue factor pathway inhibitor (*5*), and midkine (*6*) has been investigated with the aid of a combination of regioselective desulfation methods.

Fructose 1,6-bisphosphate aldolase (FPA) [EC 4.1.2.13] is a key enzyme in the glycolytic pathway and catalyzes the reversible reaction of the cleavage of fructose 1,6-bisphosphate to form the two trioses, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. In mammals, FPAs have a tetragonal subunit structure comprised of three subunit isomers, A (muscle type), B (liver type), and C (brain type), with different catalytic properties and developmental expressions (*7-9*). FPA A4 and B4, consisting of the single subunit isomers A and B, can be easily purified from muscle and liver, respectively, by a simple procedure (*10*). The most significant common feature in purification is selective elution of the enzymes from a phosphocellulose column with the substrate, fructose 1,6-bisphosphate. FPA C4 is structurally distinct from FPA A4 and B4. In brain, it appears to be located in neuronal and glial cells (astrocytes) of the cerebral cortex (*11*). Brain FPA C4 is always accompanied by FPA A4 and the other hybrid isoforms, A1C3, A2C2, and A3C1 (*12*). Therefore, the purification of FPA C4 and its hybrid isoforms from brain is more complicated than that of FPA A4 or B4. FPA is known to bind to structural proteins of cells such as erythrocyte anion transporter (band 3) and particularly actin in muscle cells. This binding drastically reduces enzyme activity.

Here, we report the discovery, in the course of a survey of Hp-binding proteins occurring in brain, that FPA can bind to Hp under physiological ionic conditions. We further report the novel behavior of FPAs from bovine cerebral cortex on regioselectively desulfated derivatives of Hp.

MATERIALS AND METHODS

Materials—Bovine intestinal Hp and Amino Cellulofine were products of Viobin (Waunakee, WI, USA) and Chisso (Tokyo), respectively. Bovine brains were purchased from Nihon Ham (Tokyo). Fructose 1,6-bisphosphate (sodium salt) and FPA A4 were purchased from Sigma Chemical

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Abbreviations: CP, chondroitin polysulfate; 6DS-Hp, 6-*O*-desulfated Hp; 2DS-Hp, 2-*O*-desulfated Hp; 2,6DS-Hp, 2 and 6-*O*-desulfated Hp; FPA, fructose 1,6-bisphosphate aldolase; GEM-TB (or TBS), TB (or TBS) containing 10% glycerol, 0.5 mM 2-mercaptoethanol, and 1 mM EDTA; Hp, heparin; NDS-Ac-Hp, *N*-desulfated and *N*-acetylated Hp; 6,NDS-NAc-Hp, 6-*O* and *N*-desulfated and *N*-acetylated Hp; 2,N-DS-NAc-Hp, 2-*O* and *N*-desulfated and *N*-acetylated Hp; SDS-PAGE, SDS polyacrylamide gel electrophoresis; TB, 10 mM Tris-HCl buffer, pH 7.4; TBS, TB containing 0.14 M NaCl.

(St. Louis, MO, USA). A mixed crystalline suspension of α-glycerophosphate dehydrogenase and triosephosphate isomerase was from Boehringer (Mannheim, Germany). Chondroitin polysulfate (CP) containing 3.0 sulfate groups per *N*-acetylgalactosamine residue was prepared by sulfation of chondroitin sulfate A (Seikagaku) as described by Whistler *et al.* (*13*). Other reagents used were of analytical grade.

General Methods—SDS polyacrylamide gel electrophoresis (SDS-PAGE, 10%) was used for analysis of proteins bound to CP- and Hp-immobilized columns (*14*). Protein samples were boiled for 2 min in Tris-HCl, pH 6.8, containing 5% 2-mercaptoethanol, 2.5% SDS, and 10% glycerol, then electrophoresed using bovine serum albumin (66.2 kDa), egg albumin (45 kDa), and trypsinogen (24 kDa) as the standard molecular mass markers. Native polyacrylamide gel electrophoresis was carried out using 7% gel at pH 8.8 under constant current of 10 mA for 3 h according to the method of Ornstein and Davis (*15, 16*). The subunits A and C of FPAs were separated under the conditions described above using a gel containing 4 M urea. Transblotting to PVDF membrane (Bio-Rad) was carried out using a transblotting apparatus Multiphor II (LKB Bromm) with a constant current of 0.8 mA/cm^2. Amino acid sequences were analyzed using a protein sequencer PPSQ-10 (Shimadzu, Kyoto). Homology of the amino acid sequences was surveyed in the SwissProt database. Preparative electrophoresis was performed using Rotofor Cell (Bio-Rad).

Preparation of Regioselectively Desulfated Hps—The method for preparation of regioselectively desulfated Hps, 2-*O*-desulfated (2DS), 6-*O*-desulfated (6DS), *N*-desulfated and *N*-acetylated (NDS-NAc), 6-*O*- and *N*-desulfated and *N*-acetylated (6,NDS-NAc), 2-*O*- and *N*-desulfated and *N*-acetylated (2,NDS-NAc), and 2,6-di-*O*-desulfated (2,6-DS) Hp, is described elsewhere (*17-19*).

Preparation of Affinity Resins—Heparin, regioselectively desulfated Hps, and CP were immobilized to Amino-Cellulofine by reductive amination between the amino group in the matrix and the reducing end of the polysaccharide (*20*). To estimate the amount of the immobilized polysaccharide, the dried affinity resin (5 mg) was hydrolyzed at 100°C for 6 h with 1 N HCl (200 μl) in an evacuated test tube. Then the supernatant was collected by centrifugation. An aliquot of the supernatant (100 μl) was dried, dissolved in water (100 μl), and the amount of sulfate ion was determined by ion chromatography using an IC-anion-PWXL, TSK gel column (Tosoh, Tokyo). The sulfate content of the polysaccharides was determined under the same conditions. The amount of the polysaccharide bound to the resin was estimated by comparison of the sulfate content of the polysaccharide and the amount of sulfate ion liberated from the affinity resin (Table I).

FPA Activity Assay—The FPA activity was determined as described by Blostein and Rutter (*21*). The assay is based on the oxidation of NADH in a coupled system containing triosephosphate isomerase and α-glycerophosphate dehydrogenase. The change in absorbance at 340 nm was measured at 30°C, and the molar extinction coefficient of NADH was taken as 6.22×10^3. One unit of the enzyme activity was defined as the activity to cleave 1 μmol/min of fructose 1,6-bisphosphate.

Identification of Hp-Binding Proteins from Bovine Cerebral Cortex—Cerebral cortex of calf brain (15 g) was cut into small pieces and homogenized in 40 ml of GEM-TBS with a Waring blender at 10,000 rpm for 5 min and further with a Teflon homogenizer at below 4°C. The homogenate was centrifuged at 15,000 rpm for 20 min. An aliquot of the supernatant (1 ml) was subjected to affinity chromatography on serially connected columns of immobilized CP and Hp (1 ml each) equilibrated with the same buffer. After washing with GEM-TBS (10 ml), both columns were eluted individually with GEM-TBS containing 3 M NaCl. After dialysis and lyophilization, the eluates from the CP and Hp columns were analyzed by SDS-PAGE. To identify the resulting bands of proteins, the amino acid sequences were analyzed after blotting to PVDF membrane. The identification of each band was based on a sequence homology search in the database of SwissProt. The eluate from the Hp column was also subjected to preparative isoelectric electrophoresis, and the resulting fractions were further analyzed by SDS-PAGE as described above.

TABLE I. **Sulfate contents and amounts of native or desulfated heparins immobilized as ligand in affinity matrices.**

Ligand	Sulfate content of Hp derivatives (wt% as SO_4^-)	Sulfate content of affinity matrix (wt% as SO_4^-)	Amount of immobilized ligand in 1 mg of dry matrix (μg)	Amount of immobilized ligand in 1 ml of swollen matrix (μg)
Native Hp	31.64	0.80	25.4	1,890
6DS-Hp	20.18	0.56	27.8	2,100
2DS-Hp	24.26	0.49	20.2	1,500
NDS-NAc-Hp	21.33	0.43	20.2	1,500
2,6DS-Hp	17.30	0.28	16.4	1,220
6,NDS-NAc-Hp	11.23	0.22	19.7	1,500
2,NDS-NAc-Hp	13.15	0.32	24.3	1,810

The sulfate contents were determined by hydrolysis with hydrochloric acid before ion chromatography. The amount of immobilized ligand was calculated from sulfate content of the ligand and that of the affinity matrix.

Affinity of FPA to Modified Hps—To estimate the affinity of FPA to modified Hps (6DS, 2DS, NDS-NAc, 2,6DS, 6,NDS-NAc, and 2,NDS-NAc), the crude extract of cerebral cortex (1 ml) described above was mixed with the modified Hp resins (1 ml) equilibrated with GEM-TBS, and the mixture was shaken for 30 min. Each resin was washed 5 times with GEM-TBS and packed in a small column. The column was eluted with GEM-TB containing 3 M NaCl, and the eluate was subjected to FPA activity assay.

Purification of FPA Isomers—The extract of bovine cerebral cortex (15 ml) was applied to a CP-immobilized column (0.7×8 cm), and the column was washed thoroughly with GEM-TBS. After dialysis, the pass-through fraction was applied to a 6DS-Hp-immobilized column (0.7×8 cm), and the column was eluted with a 30-min linear gradient of 0 to 1.5 M NaCl in GEM-TBS using an FPLC system (Pharmacia Biotech., Sweden) at a flow rate of 1 ml/min after washing with the same buffer without NaCl. The protein fraction detected by monitoring absorbance at 280 nm was further subjected to anion-exchange chromatography with a Mono Q (HR 5/5) (Pharmacia Biotech.) column using an FPLC system. The elution was carried out with a linear NaCl concentration gradient from 0 to 0.5 M in TB. The resulting protein fractions were further purified by Superdex 200 HR 10/30 (Pharmacia Biotech.) gel filtration with TBS (flow rate, 1 ml/min) using an FPLC system, and analyzed by electrophoresis with and without 4 M urea.

RESULTS

Hp-Binding Proteins of Bovine Cerebral Cortex—To identify proteins binding Hp in brain, the extract of bovine cerebral cortex was chromatographed on the Hp column after removing CP-binding proteins. The extract of cerebral cortex of calf brain was subjected to affinity chromatography on serially connected columns of immobilized CP and Hp. After washing with GEM-TBS, the protein fractions bound to the CP- and Hp-immobilized columns were obtained by elution with GEM-TBS containing 3 M NaCl. The results of SDS-PAGE of the CP- and Hp-binding fractions are shown in Fig. 1. The different SDS-PAGE patterns of the CP-binding fraction (lanes 1 and 2) and the Hp-binding fraction (lanes 3 and 4) indicate the difference in the protein-binding property between CP and Hp.

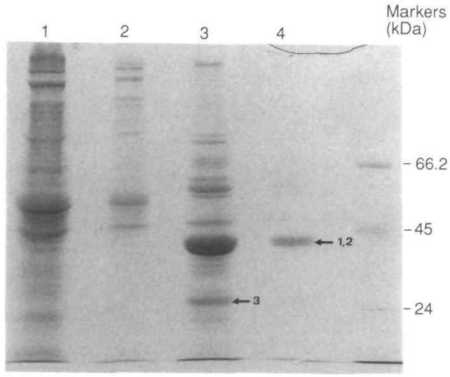

Fig. 1. **SDS-PAGE of CP- and Hp-binding proteins from bovine cerebral cortex.** The extract of bovine cerebral cortex was applied to serially connected columns of CP- and Hp-immobilized resin. After washing with GEM-TBS, each column was eluted with GEM-TBS containing 3 M NaCl to afford the fractions of CP- and Hp-binding proteins. The proteins were visualized by staining with Coomassie Brilliant Blue. Lane 1, CP-binding proteins, 25 μg; lane 2, CP-binding proteins, 5 μg; lane 3, Hp-binding proteins, 25 μg; lane 4, Hp-binding proteins, 5 μg. The horizontal arrows 1, 2, and 3 indicate FPA, glyceraldehyde 3-phosphate dehydrogenase, and heat shock protein 70, respectively. Identification of each band was based on the mobility and the N-terminal amino acid sequence.

TABLE II. **Binding ability of FPA to immobilized Hp derivatives and CP.**

Ligand	A_{280}	Recovered FPA activity (unit)	Recovered FPA activity relative to native Hp (%)	Specific activity
Native Hp	0.91	1.10	100	1.20
6DS-Hp	0.33	0.92	84	2.79
2DS-Hp	0.40	1.06	96	2.62
NDS-NAc-Hp	0.44	0.50	46	1.15
2,6DS-Hp	0.03	0.11	10	3.67
6,NDS-NAc-Hp	0.12	0.03	3	2.50
2,NDS-NAc-Hp	0.10	0.04	4	0.41
2,6,NDS-NAc-Hp	0.004	0	0	0
CP	0.39	0	0	0

The extract of cerebral cortex was shaken with the desulfated Hp- or CP-immobilized resin. The amount of FPA bound to the resin was estimated from the FPA activity recovered from the resin by elution with GEM-TB containing 3 M NaCl. One unit of FPA activity was defined as the activity cleaving 1 μmol/min of fructose 1,6-bisphosphate.

Among the bands observed in Hp-binding fraction, heat shock protein 70, FPA, and a small amount of glyceraldehyde 3-phosphate dehydrogenase were identified on the bases of the estimated molecular weight and the results of amino acid sequence analysis after blotting to a PVDF membrane by comparing the sequence data with the SwissProt database. Among the bands identified, the major Hp-binding protein was FPA (Fig. 1). Authentic FPA A4 showed the identical behavior on the chromatographies and SDS-PAGE. Since the mobility of glyceraldehyde 3-phosphate dehydrogenase was quite close to that of FPA, the eluate from the Hp column was also subjected to preparative isoelectric electrophoresis, and a fraction from pH range of 1.0-1.2 was further analyzed by SDS-PAGE as described above. The N-terminal amino acid sequence from the resulting band, which had similar mobility to that of FPA, was identical to that of glyceraldehyde 3-phosphate dehydrogenase.

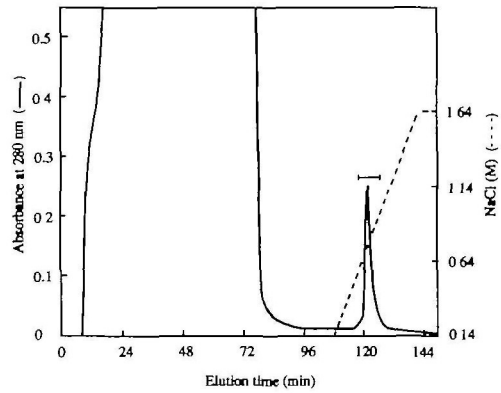

Fig. 2. **6DS-Hp affinity chromatography of bovine brain FPA.** The extract of bovine cerebral cortex was applied to a CP column. The pass-through fraction was desalted and applied to a 6DS-Hp column equilibrated with GEM-TBS. Elution was carried out with a linear gradient of NaCl from 0 to 1.5 M in the same buffer. Proteins were monitored by A_{280}. FPA activity is indicated by the bar.

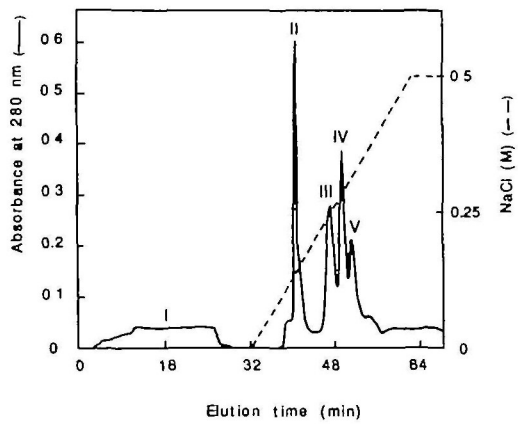

Fig. 3. **Anion exchange chromatography of FPA isoforms from bovine cerebral cortex.** The 6DS-Hp-binding fraction (Fig. 2) was desalted and applied to a Mono Q HR 5/5 column equilibrated with GEM-TB. Elution was carried out with a linear gradient of NaCl from 0 to 0.5 M in the same buffer. Proteins were monitored by A_{280}. Five protein peaks (I, II, III, IV, and V) with FPA activity were observed.

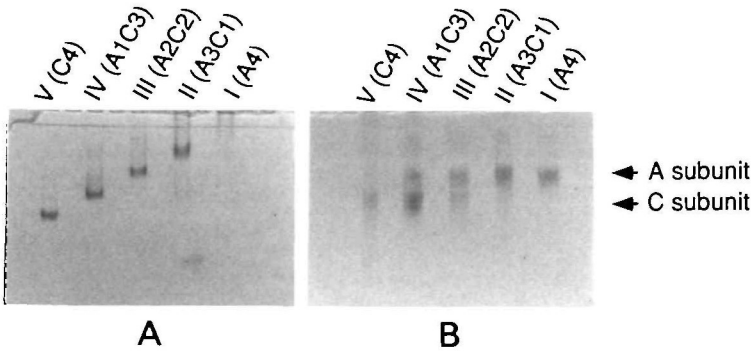

Fig. 4. **Denatured and non-denatured PAGE of purified FPA isoforms.** Each isofrom of FPA obtained from Mono Q chromatography (Fig. 3) was subjected to slab gel electrophoresis using 7% gels at pH 8.8 with (B) or without 4 M urea (A). The gel was stained with Coomassie Brilliant Blue. Bands of the five FPA isozymes A4, A3C1, A2C2, A1C3, and C4, respectively, were detected from the fractions I, II, III, IV, and V (A), and their subunits A and C were separately detected (B). Identification of each band was based on the literature (*10*) and the N-terminal amino acid sequence.

TABLE III. **Purification of the FPA isomers from bovine cerebral cortex.**

Purification step		Total protein (mg)	Total FPA activity (unit)	Specific activity (unit/mg)	Yield (%)	Purification (fold)
Crude extract		544	48.3	0.09	100	1
Pass-through fraction from CP column		429.6	45.6	0.11	94	1.2
6DS-Hp-binding fraction		19.6	42.3	2.2	88	24
MonoQ	I	0.3	2.4	8.0	5	89
	II	1.4	13.0	9.3	27	103
	III	0.6	8.4	14.0	17	156
	IV	0.6	5.1	8.5	11	94
	V	0.8	6.6	8.3	14	92
Superdex 200	I	0.2	1.6	8.0	3	89
	II	1.0	11.8	11.8	24	131
	III	0.6	7.0	11.7	14	130
	IV	0.3	3.7	12.3	8	137
	V	0.4	5.2	13.0	11	144

Crude extract from bovine cerebral cortex was applied to the CP column. The pass-through fraction was then applied to the 6DS-Hp column. The 6DS-Hp-binding fraction was subjected to Mono Q chromatography to afford five fractions, I to V. Each fraction was further purified by gel filtration with Superdex 200. One unit of the FPA activity was defined as the activity cleaving 1 μmol/min of fructose 1,6-bisphosphate.

Binding Ability of FPA to Modified Hps—Binding of FPA to a series of the desulfated Hps (6DS, 2DS, NDS-NAc, 2,6DS, 6,NDS-NAc, and 2,NDS-NAc) was investigated. The extract of cerebral cortex was applied to the regioselectively desulfated Hp resins and the fractions bound to the desulfated Hp resins were subjected to FPA activity assay (Table II). Although FPA activity hardly bound to the modified Hps of which two of three kinds of sulfate groups were removed, it bound to Hps desulfated at only C2 OH of the iduronic acid residue or C6 OH or C2 NH_2 of the glucosamine residues.

Isolation of FPA Isoforms—In brain, FPA is composed of tetragonal subunits that are present as five isoforms, A4, A3C1, A2C2, A1C3, and C4. These isoforms were isolated as follows. The extract of cerebral cortex was applied to the CP column, and the pass-through fraction was applied to the 6DS-Hp column. The fraction bound to the column was eluted with a gradient of NaCl (0 to 1.5 M) in GEM-TBS. As shown in Fig. 2, a single peak of absorbance at 280 nm was observed. This peak was applied to a Mono Q column, and five fractions (I-V) possessing FPA activity were observed (Fig. 3). Each fraction was further gel-filtered on a Superdex 200 column. Since the isoforms of FPA afford the identical SDS-PAGE pattern, the five purified fractions were characterized by native PAGE with and without 4 M urea for the analyses of their subunits (Fig. 4A without 4 M urea, and Fig. 4B with 4 M urea). Comparison with data in the literature (*10*) indicated that the fractions I, II, III, IV, and V were FPAs A4, A3C1, A2C2, A1C3, and C4, respectively. In the absence of urea, the order of the mobility of the isoforms was A4<A3C1<A2C2<A1C3<C4; and in the presence of urea, the subunits A and C were separated and detected with intensities depending on their contents. In addition, the N-terminal amino acid sequences from each band obtained after blotting to PVDF membrane agreed with those of FPA subunits available from the SwissProt database. The purification of the FPA isoforms by this method is summarized in Table III. More than 50% of total activity was recovered.

DISCUSSION

In mammals, FPAs are tetramers comprising combinations of three subunits, A, B, and C. The homogeneous tetramers, A4 and B4 can be easily purified from muscle and liver, respectively, by a simple procedure. Heterogeneous tetramers composed of subunits A and B, and A and C, have been also purified from liver and brain, respectively. Since the A-C sets in the tetragonal subunit structure are dominant in brain as well as heart and spleen (*7*), these isoforms cannot be purified simply by elution with fructose 1,6-bisphosphate from phosphocellulose. The separation of these isoforms requires further anion exchange chromatography.

Both CP and Hp are sulfated polysaccharides, which carry *ca.* 3 mol of sulfate groups per repeating disaccharide unit. However, the backbones of the two glycosaminoglycans are quite different: CP consists of the alternating repeat of the (1→4)-linked glucuronic acid residue and the (1→3)-linked *N*-acetylgalactosamine residue, while the

main repeating disaccharide unit of heparin contains the (1→4)-linked iduronic acid residue and the (1→4)-linked *N*-sulfated glucosamine residue. As indicated in Fig. 1, CP bound many proteins under physiological conditions, whereas many other proteins were not trapped on the CP column but on the Hp column. Among the proteins bound to Hp, glyceraldehyde 3-phosphate dehydrogenase, heat shock protein 70, and FPA were identified on the bases of their amino acid sequences and the molecular weights estimated from the mobility. Heat shock protein 70 was already known as a Hp-binding protein, while FPA and glyceraldehyde 3-phosphate dehydrogenase have not generally been recognized as Hp-binding proteins. Our results revealed that Hp can bind FPA and glyceraldehyde 3-phosphate dehydrogenase under physiological conditions, while CP sulfated as highly as Hp cannot. This strongly indicates that FPA is a new member of the Hp-binding proteins, and suggests that the proteins bound to Hp recognize different backbone structures and sulfation patterns within the glycosaminoglycan chains from those bound to CP. In addition, the use of the CP column was effective for the isolation of the Hp-binding proteins, because the direct application of the brain extract to the Hp column resulted in a much more complicated SDS-PAGE pattern of the fraction bound to the Hp column (data not shown).

We investigated further the mechanism of the interaction between FPA and Hp, especially effects of the location of sulfate groups in Hp, with the aid of affinity chromatography using regioselectively desulfated Hps. The results in Table II showed that the native Hp and the desulfated Hps in which a sulfate group at C2 OH of the uronic acid residue or at C6 OH or C2 NH_2 of the glucosamine residue was regioselectively removed, could bind FPA. On the other hand, FPA hardly bound to the desulfated Hps in which sulfate groups at any two positions were removed, suggesting that at least two sulfate groups of the repeating disaccharide unit were required for the binding of FPA under physiological conditions. That the binding of FPA and Hp is in some way structure-dependent manner was also suggested by the fact that CP carrying no *N*-sulfate group did not bind FPA, while NDS-Ac-Hp also carrying no *N*-sulfate group exhibited a moderate binding capacity (46% of the intact Hp).

Since the specific FPA activity of the fraction bound to the 6DS-Hp column was higher than that bound to the other desulfated Hp columns, use of the 6DS Hp-column instead of the native Hp column was expected to be effective for the isolation of FPA from other proteins. By employing the 6DS-Hp column, a single peak of FPA activity was eluted (Fig. 2). This fraction was further separated on a Mono Q column into five peaks (I, II, III, IV, and V in Fig. 3), which after further purification by gel filtration were identified as the five A-C isoforms A4, A3C1, A2C2, A1C3, and C4, respectively (Fig. 4A). The molar ratio of the subunits in each FPA isomer estimated from SDS-PAGE with 4 M urea (Fig. 4B) using NIH image version 1.58 agreed with that expected from the subunit composition (data not shown). Overall yields of each isoform as listed in Table III are totally more than 50% of the content of the extract of cerebral cortex. The use of the 6DS-Hp column instead of the conventional phosphocellulose column is thus more effective for the purification of FPA. The A-C isoforms isolated in this study have higher specific activities and yields. Besides, 6DS-Hp affinity chromatography is more economical than phosphocellulose chromatography because NaCl can be used instead of fructose 1,6-bisphosphate as the eluant.

The molecular bases for the interactions of several Hp-binding proteins and Hp, including the location of sulfate groups, have been investigated; the minimal structures of Hp required for binding of antithrombin III and factor Xa are now well known (*2*); the effects of Hp sulfate groups on interaction with fibroblast growth factor 1 and 2 have been revealed by *in vitro* cell growth assay (*3, 4*); and we have recently reported the structural requirements of Hp for tissue factor pathway inhibitor using regioselectively desulfated Hps as ligands for affinity chromatography (*5*). These instances indicate the importance of the particular sulfation patterns of the heparin chain in determining the interaction of heparin with a particular protein. The affinities of FPA to the regioselectively desulfated Hps indicate that the *N*-sulfate group contributes most to its interaction with Hp (Table II), while the *O*-sulfate groups contribute less. We thus conclude that FPA is a Hp-binding protein which requires a new type of sulfation pattern for the interaction. Since FPA is cytosolic, while Hp/heparan sulfate occurs in extracellular matrices or the cell surface, it is difficult to conclude that Hp/heparan sulfate and FPA really interact *in vivo*. However, to the extent that heparan sulfate can penetrate the membrane and migrate to the nucleus (*22*) and that cell membrane heparan sulfate may be incorporated into the cytosol as a result of endocytosis (*23*), it is not inconceivable that the Hp-related polymer would interact with FPA if the two biomolecules came into contact.

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