Proteoglycan Carrier of Human Platelet Factor 4

ISOLATION AND CHARACTERIZATION

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A large scale purification procedure for the human platelet factor 4 proteoglycan carrier molecule has been developed. A yield of 46% and a 33,000-fold purification have been achieved, using poly-L-lysine-Sepharose affinity column chromatography, PF4-agarose affinity column chromatography, and Bio-Gel A-0.5m gel filtration. The purified proteoglycan migrates as a single band during electrophoresis on cellulose acetate strips. A single symmetric peak was observed in sedimentation velocity analysis with an s value of 2.85. The molecular weight of the proteoglycan was determined to be 53,000 by sedimentation equilibrium. The purified proteoglycan contains 32% uronic acid, 31% galactosamine, 6.1% sulfate, and 9.9% protein. Aspartic acid, glutamic acid, leucine, glycine, and serine account for 55% of the total amino acids. The chondroitinase AC digest of the proteoglycan is sensitive to hydrolysis by chondro-4-sulfatase but not by chondro-6-sulfatase, indicating the presence of chondroitin 4-sulfate but not chondroitin 6-sulfate in the proteoglycan molecule. The interaction between this proteoglycan carrier of human PF4 and PF4 is strongly ionic strength-dependent. 0.3 M NaCl is required to dissociate the proteoglycan PF4 complex.

Platelet factor 4 is released from human platelets during normal blood coagulation or when platelets are exposed to damaged blood vessel walls (1-7). Early in coagulation, there also occurs a rise in the glycosaminoglycan content in human serum (8). Platelets exposed to thrombin release a specific glycosaminoglycan (9); subsequently, platelets were shown to contain two distinctly glycosaminoglycans (10). Barber et al. (11) showed that platelet factor 4 is released from platelets as a high molecular weight, proteoglycan-platelet factor 4 complex. The complex was dissociated by elution in high salt and the proteoglycan was purified and partially characterized.

We recently have purified platelet factor 4 and determined the complete amino acid sequence of this protein (12). The COOH-terminal is unusual and seemed a likely binding site for glycosaminoglycans. The COOH-terminal region of PF4 contains 4 lysine residues, occurring in pairs (-Lys-Lys-Ile-Ile-Lys-Lys-Leu-Glu-Ser-COOH). We further observed that the COOH-terminal tryptic peptide in high concentration partially reversed the prolonged thrombin time induced by heparin, suggesting heparin binding was localized to the COOH-terminal region of PF4. Support for the involvement of these lysines in the PF4-heparin binding activity was provided by the work of Handin and Cohen (13) who showed that guanidination of lysines in PF4 decreased heparin neutralizing activity, but that modification of arginines was without effect.

In an effort to investigate further the interactions of PF4 with glycosaminoglycans, we have purified the PF4-proteoglycan carrier molecule using affinity chromatography. This manuscript describes a novel procedure for the purification of the carrier molecule by PF4-agarose affinity chromatography, its characterization by physical and chemical criteria, and the interaction of the proteoglycan carrier molecule and PF4.

EXPERIMENTAL PROCEDURES

Materials

Human outdated frozen platelet-rich plasma was obtained from regional Red Cross Blood Banks. Alcian blue dye and Bio-Gel A-0.5m were purchased from Bio-Rad. Cellulose acetate plates were obtained from Helena Laboratories, Beaumont, TX. Cellulose thin layer plates were obtained from Eastman-Kodak. High resolution buffer (Tris(hydroxymethyl)aminomethane buffer, 32.133.7:54.2, w/w, pH 8.8) was obtained from Gelman Sciences, Inc. Poly-L-lysine (M. w. 6000), ω-aminobutyl agarose, chondroitinase AC, phenylmethylsulfonyl fluoride, benzamidine-HCl, soybean trypsin inhibitor, L-tosylamido-2-phenylchloromethyl ketone, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, chondro-4-sulfatase, chondro-6-sulfatase, and chondroitin sulfate types A and C were purchased from Sigma. Diisopropyl fluorophosphate was obtained from Calbiochem. Ponceau S staining solution was obtained from Beckman. Sepharose Cl-2B and 4B were purchased from Pharmacia. All other reagents were analytical grade.

Methods

Human platelet factor 4 was purified by previously described methods (12), including heparin-agarose chromatography and gel filtration with Sephadex G-100. The purified PF4 appeared as a single band in SDS-polyacrylamide gel electrophoresis as reported previously (12).

Preparation of Poly-L-lysine-Sepharose 4B — One g of poly-L-lysine was dissolved in 100 ml of 0.1 M NaHCO3 (pH 8.0) and added to 900 g (wet weight) of cyanogen bromide-activated Sepharose 4B in 900 ml of 0.1 M NaHCO3 (pH 8.0). After mixing at 4 °C overnight, the gel was sequentially washed with 2 liters of 0.1 M NaHCO3 (pH 8.0), 2 liters of 1.0 M NaCl, and 4 liters of distilled water.

Preparation of PF4-ω-Aminobutyl-Agarose — Ten mmol of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was added to a gel suspension of ω-aminobutyl-agarose (50 g wet weight of gel in 50 ml of water, pH 4.5). Twenty mg of PF4 in 4 ml of 1 M NaCl was added drop by drop. The gel suspension was stirred for 4 h and the pH was maintained at 4.8, incubated at room temperature overnight, and washed with 2 liters of 1 M NaCl and extensively with water. More than 95% of the PF4 was added and coupled under these conditions.
**Platelet Factor 4 Proteoglycan Carrier Molecule**

**Cellulose Acetate Electrophoresis**—Proteoglycan samples (1 mg/ml) were applied to cellulose acetate plates for electrophoresis with zinc acetate buffer (pH 3.5) at a constant current 0.5 mA/cm for 2 h (11) or with Trisboric acid buffer (pH 8.8) for electrophoresis of PF4 and PF4-proteoglycan complex at 160 V for 15 min. Duplicate plates were analyzed. The proteoglycan was stained with 0.05% alcian blue in 1% acetic acid and proteins were stained with ponceau S staining solution and destained in 1% acetic acid. SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (14) using 5% gels, and the gels were stained with Coomassie brilliant R-250 for protein or with alcian blue for proteoglycans.

**Chondroitinase AC Digestion of Proteoglycan**—Proteoglycan (0.6 mg) in 1 ml of 0.1 M Trisodium acetate buffer (pH 7.3) was digested with chondroitinase AC (0.25 unit/mg) at 37 °C for 3 h. Aliquots were spotted on cellulose thin layer plates (10 × 3 cm) and developed (ascending chromatography) using butanol-acetic acid:1 M ammonia, 2:3:1 (v/v/v), for 30 min. The disaccharide product was visualized using a short wavelength mineral UV light (15) or stained with alcian silver nitrate reagent (16). One mg of chondroitin sulfate types A and C was digested by chondroitinase AC as a control.

**Chondroitin Sulfatase Digestion**—The chondroitin AC digests of proteoglycan and chondroitin sulfate types A and C were further digested with chondroitin-4-sulfatase or chondroitin-6-sulfatase at 37 °C for 30 min (15). An aliquot of the digest was spotted on cellulose thin layer plates (Kodak) and subjected to electrophoresis with 0.05 M sodium citrate-citric acid buffer (pH 5.0) at 45 V/cm for 30 min. The digested product was visualized under a short UV light.

**Analytical Methods**—Protein was measured by the method of Lowry et al. (17). Galactosamine was determined in a Beckman amino acid analyzer according to the method of Spackman et al. (20). The proteoglycan sample was hydrolyzed with 6 N HCl under vacuum at 100 °C for 18.5 h and a speed of 52,000 rpm. The proteoglycan concentration was 4 mg/ml, in 1 M NaCl. Sedimentation velocity studies were performed at a rotor temperature of 18.5 °C and a speed of 52,000 rpm. The proteoglycan concentration was 4 mg/ml in 1 NaCl.

**RESULTS**

The large scale purification of the proteoglycan carrier of PF4 involves two affinity columns, poly-L-lysine-Sepharose 4B and PF4-ω-aminobutyyl agarose, and gel filtration on Bio-Gel A-0.5m. A purification scheme was summarized in Table I. About 100 units (volume ~ 4000 ml) of outdated, frozen human platelet-rich plasma were thawed and centrifuged at 13,000 X g for 30 min to remove cell debris and other insoluble materials. Protease inhibitors including 1 mM diisopropyl fluorophosphate, 0.05 mM phenylmethylsulfonyl fluoride, 10 mM benzamidine-HCl, 0.03 mM L-tosylamido-2-phenylthylole chloride, 10 mM EDTA, and 10 mg/liter of soybean trypsin inhibitor were added during the thawing procedure. The clear plasma solution was then mixed at a ratio of 20 g (wet weight) of poly-L-lysine-Sepharose 4B gel per 500 ml of plasma solution. After mixing overnight at 4 °C, the gel suspension was packed onto a column (5 × 45 cm). The column was extensively washed with 0.15 M NaCl (absorbance at 280 nm < 0.1). The proteoglycan carrier eluted at an ionic strength of ~0.45 M NaCl in a linear gradient from 0.15 M NaCl to 1 M NaCl (Fig. 1). About a 560-fold purification with 85% yield was achieved. The starting large volume of plasma (4000 ml) was reduced to 100 ml of column eluent. The proteoglycan-containing fractions were collected, dialyzed against 0.15 M NaCl, and applied onto a column of PF4-ω-aminobutyyl-agaro-

![Table 1](image)

**Purification of the proteoglycan from human platelet-rich plasma**

| Step | Total protein (mg) | Total hexuronic acid (μmol) | Specific activity | Yield (%) | Purification fold |
|------|-------------------|----------------------------|------------------|----------|------------------|
| I. Platelet-rich plasma | 261.45 | 117.9 | 0.00045 | 100 | 1 |
| II. Poly-L-lysine-Sepharose | 389.5 | 99.7 | 0.253 | 85 | 562 |
| III. PF4-ω-aminobutyyl-agarose | 7.1 | 83.1 | 11.7 | 71 | 26,000 |
| IV. Bio-Gel A-0.5m | 3.6 | 54.2 | 15.05 | 46 | 33,444 |

*Since human platelet-rich plasma contains a substantial quantity of proteins/glycoproteins which produce an intense brown color during sulfuric acid treatment in the carbazole reaction, interfering with the assay of uronic acid, it was not possible to measure directly the PF4 proteoglycan carrier-specific uronic acid content in human platelet-rich plasma. To estimate the concentration of the PF4 proteoglycan carrier-specific uronic acid, an internal standard of the purified PF4 proteoglycan carrier was added to the plasma for measuring the recovery after poly-L-lysine column chromatography. Based on the 85% recovery from the poly-L-lysine column, the concentration of PF4-proteoglycan carrier-specific uronic acid in human platelet-rich plasma was calculated at 117.2 μmol/4000 ml of plasma.

![Fig. 1](image)

**Fig. 1. Poly-L-lysine-Sepharose column chromatography of human platelet-rich plasma.** Four liters of frozen and thawed human platelet-rich plasma were mixed with 160 g (wet weight) of poly-L-lysine-Sepharose and packed into a column (5 × 45 cm). After being washed extensively with 0.15 M NaCl, the bound proteoglycan was eluted with a linear gradient (volume, 600 ml) of 0.15 M NaCl to 1 M NaCl. Fraction volumes of 0.8 ml were collected. Each fraction was analyzed for A280 (■) and uronic acid content measured at A330 (○) by carbazole reaction. Fractions were pooled as indicated.
Platelet Factor 4 Proteoglycan Carrier Molecule

FIG. 2. Affinity column chromatography of PF4-ω-aminobutyl-agarose. The proteoglycan-containing fractions obtained from poly-L-lysine-Sepharose column were collected, dialyzed against 0.15 M NaCl, and then applied to the PF4-ω-aminobutyl-agarose column (volume, 50 ml) which was previously equilibrated with 0.15 M NaCl. The proteoglycan was eluted with a linear gradient (300 ml) of 0.15 M to 1 M NaCl. The fraction volumes were 2.6 ml. The proteoglycan was pooled as indicated, dialyzed against water, and lyophilized.

FIG. 3. Gel filtration on Bio-Gel A-0.5m of the proteoglycan from PF4-ω-aminobutyl-agarose column chromatography. The lyophilized proteoglycan from PF4-ω-aminobutyl-agarose column was dissolved in 5 ml in 30 mM Tris-HCl, pH 7.4, containing 0.12 M NaCl and applied to a column of Bio-Gel A-0.5m (2.5 X 90 cm) previously equilibrated with the same buffer. Fractions of 5.8 ml were collected and analyzed for A280 nm (●—●) and uronic acid content was measured at A530 nm (○—○) by carbazole reaction.

obtained from 4 liters of platelet-rich plasma with a recovery of 46%.

The purity of the purified proteoglycan has been assessed by cellulose acetate electrophoresis, SDS-gel electrophoresis, and ultracentrifugation. The proteoglycan appears as a single positively alcian blue-stained band after electrophoresis on cellulose acetate. No protein bands were observed when the strip was stained for protein. SDS-polyacrylamide gel electrophoresis was also used to analyze the purity of the proteoglycan. One hundred μg of the proteoglycan was subjected to SDS-polyacrylamide gel electrophoresis. No contaminating protein bands were detected by Coomassie blue staining although less than 1 μg of protein is detectable in our gel system. Alcian blue staining demonstrated a single stained band of the proteoglycan at the junction of stacking gel and separating gels. It is likely that aggregation of the proteoglycan accounted for the failure of the material to enter the 5% gel.

Further analyses of purity was attempted using sedimentation velocity. The proteoglycan carrier molecule sediments as a single sharp symmetrical peak in the analytical ultracentrifuge (Fig. 4) and has an uncorrected sedimentation coefficient of 2.85 S. No evidence of impurities was found. The molecular weight of the proteoglycan was measured by sedimentation equilibrium analysis. The calculated molecular weight in this experiment is 53,000. High concentrations of salt were needed to prevent self-association during centrifugation; 1 M NaCl was used in all centrifugation analyses. Gel filtration (Sephacryl CL-2B, 90 X 0.9 cm, 1 M NaCl) analysis was done; the platelet factor 4 proteoglycan carrier molecule eluted with a Kav of 0.76. By comparison with the Kav obtained with three other reference proteoglycans used by Silvestri et al. (23), the platelet factor 4 proteoglycan carrier had a Mr ~ 56,000 estimated in this analysis.

Chemical analyses were also done. Two mg of the purified proteoglycan were subjected to NH2-terminal amino acid analysis using Edman degradation in a Beckman Sequencer. No phenylthiohydantoin-amino acid residues were released after Edman degradation of the purified proteoglycan, consistent with previous results indicating no significant contamination by other peptides or proteins. It is likely that the high carbohydrate content of the proteoglycan molecule hinders detection by Edman degradation of NH2-terminal amino acid residues in the peptide moiety.

The chemical composition of the proteoglycan is shown in Table II. The proteoglycan contains 10% protein, 32% uronic acid, 31% galactosamine, and 6.1% sulfate. No glucosamine was found in the proteoglycan molecule. The amino acid composition of the peptide moiety in the proteoglycan is shown in Table III. Since the proteoglycan contains ~10% protein, the molecular weight of the peptide moiety was calculated as ~5300. A total of 43 amino acid residues were present in 1 mol of the proteoglycan. Aspartic acid, glutamic acid, leucine, glycine, and serine account for ~55% of the total amino acid content of the peptide portion of the PF4-proteoglycan carrier molecule. No methionine residues were found. The proteoglycan shows the characteristics of chondroitin sulfate containing glucuronic acid, galactosamine, and sulfate.

The identity of chondroitin 4-sulfate or 6-sulfate in the proteoglycan was established by a combination of digestion with chondroitinase AC and chondro-4- or -6-sulfatase (15).

Fig. 4. Sedimentation velocity pattern of proteoglycan at 4 mg/ml in 1 M NaCl. The direction of sedimentation is from left to right. Centrifugation was performed at 18.5°C and 52,000 rpm. The picture was taken 30 min after reaching full speed.

TABLE II
Chemical composition of proteoglycan from platelet-rich plasma

| Composition | g |
|-------------|---|
| Protein*    | 9.9 |
| Uronic acid | 32 |
| Galactosamine | 31 |
| Sulfate    | 6.1 |

* The data were taken from direct analysis of amino acid composition with a Beckman amino acid Auto-analyzer.
The chondroitinase AC digest of proteoglycan yielded one product, with the same mobility as that of the standard chondroitin 4-sulfate treated with chondroitinase on paper chromatography (data not shown). For confirmation of the chondroitin 4-sulfate treated with chondroitinase on paper product, with the same mobility as that of the standard chondroitin 4-sulfate configuration, the chondroitinase AC digest was further hydrolyzed with chondro-4- or -6-sulfatase product was found when the product was digested with chondroitinase AC and then with chondro-4-sulfatase. It is concluded therefore that the glycosaminoglycan chains of proteoglycan carrier consist of chondroitin 4-sulfate. The data (Fig. 5) also provide evidence that the isolated chains are homogeneous and, thus, additional evidence that the intact isolated proteoglycan has been purified to homogeneity as well.

The use of PF4-ω-aminobutyl-agarose to purify proteoglycan from platelet-rich plasma clearly demonstrates the affinity of the proteoglycan for PF4. The dissociation of proteoglycan molecule from PF4 affinity gel at high ionic strength (~0.3 M NaCl) suggested that the interaction between PF4 and the proteoglycan molecule is mainly through ionic interactions. The complex formation between PF4 and proteoglycan was further confirmed by cellulose acetate electrophoresis studies. Under the conditions shown (Fig. 6), PF4 remained at the origin and showed a positive reaction to ponceau S staining while proteoglycan migrated rapidly toward the anode and showed a positive reaction with alcin blue staining. The proteoglycan-PF4 complex migrated more slowly toward anode than the proteoglycan alone and showed a positive reaction to both protein and proteoglycan staining. The data clearly demonstrated that a complex of PF4 and proteoglycan was formed which has different electrophoretic mobility from PF4 and the proteoglycan alone. The binding of the proteoglycan to PF4 has not been studied in further detail.

We have used purified human platelet-derived growth factor (24) and concentrated human platelet lysates to seek other proteins binding to the proteoglycan carrier molecule. In preliminary experiments, no evidence of significant binding of other proteins has been found, suggesting the proteoglycan has a high degree of specificity for PF4. PF4 is a chemotactic protein for human monocytes and for human neutrophils (25).

### Table III

| Amino acid residue | Proteoglycan residues/mol | Proteoglycan residues/1000 residues |
|-------------------|--------------------------|-----------------------------------|
| Lysine            | 2.0 (2)                  | 45.8                              |
| Histidine         | 1.1 (1)                  | 25.6                              |
| Arginine          | 1.9 (2)                  | 44.5                              |
| Aspartic acid     | 6.4 (6)                  | 146.5                             |
| Threonine         | 2.2 (2)                  | 49.5                              |
| Serine            | 3.5 (4)                  | 89.9                              |
| Glutamic acid     | 5.8 (6)                  | 134.2                             |
| Proline           | 1.9 (2)                  | 43.3                              |
| Glycine           | 4.0 (4)                  | 92.5                              |
| Alanine           | 1.8 (2)                  | 41.8                              |
| Half-cystine      | 1.1 (1)                  | 25.3                              |
| Methionine        | 6 (0)                    | 0                                 |
| Isoleucine        | 1.7 (2)                  | 39.6                              |
| Leucine           | 4.2 (4)                  | 96.6                              |
| Tyrosine          | 1.3 (1)                  | 29.1                              |
| Phenylalanine     | 2.4 (2)                  | 55.6                              |
| Tryptophan        | N.D.                     | N.D.                              |
| Total             | 43                       |                                   |

* The data were the average values from the analyses of three separate samples.
* The data shown are the nearest integers.
* The values of serine and threonine residues were corrected for decomposition by extrapolation of values to zero time hydrolysis.
* Determined after performic acid oxidation and hydrolysis.
* N.D., not determined.

![Fig. 5. Electrophoretic analysis of chondro-4- and -6-sulfatase digest of proteoglycan on cellulose thin layer plate.](image)

Fig. 5. Electrophoretic analysis of chondro-4- and -6-sulfatase digest of proteoglycan on cellulose thin layer plate. The digest product of chondro-4- or -6-sulfatase was applied to cellulose plate and run with 0.05 M sodium citrate/citric acid buffer (pH 5.0) for 30 min. The plate was visualized under a UV light. a, proteoglycan was first digested with chondroitinase AC and then with chondroitinase AC digestion product of chondro-4-sulfatase. b, proteoglycan was digested only with chondroitinase AC. c, proteoglycan was digested with chondroitinase AC and then with chondroitinase AC but not with chondroitinase AC.

![Fig. 6. Cellulose acetate electrophoresis patterns of proteoglycan, PF4, and the proteoglycan-PF4 complex.](image)

Fig. 6. Cellulose acetate electrophoresis patterns of proteoglycan, PF4, and the proteoglycan-PF4 complex. Five μl of the complex (e, 1 mg/ml of proteoglycan and PF4), PF4 (b, 1 mg/ml), and proteoglycan (c and d, 1 mg/ml), was applied to two separate cellulose acetate plates. After electrophoresis at pH 8.8 at 160 V for 15 min, one plate was stained for proteoglycan with alcin blue and the other were stained for protein (PF4) with ponceau S. The two plates were superimposed and photographs were taken to demonstrate the precise relationships of the alcin blue/ponceau S staining materials.
The purification procedure for the proteoglycan carrier of PF4 described in this communication is relatively simple and results in a high recovery (46%), with an ~33,000-fold purification achieved. PF4 coupled to cyanogen bromide-activated agarose through the amino groups of PF4 was not effective in binding the proteoglycan to the affinity gel, suggesting that the amino groups of the lysine residues in PF4 are important in the interaction between PF4 and proteoglycan. It is believed that the two pairs of lysine in the carboxyl-terminal end of the PF4 molecule are the binding sites for heparin (12, 13). It is likely that the proteoglycan binds poly-L-lysine chains through a similar interaction with the multiple charged groups of the polylysine ligand.

The proteoglycan contains 10% protein and is rich in uronic acid and galactosamine. The lack of glucosamine in the proteoglycan rules out contamination with heparin. Recently, Silvestri et al. (23) isolated a proteoglycan inhibitor for Clq from human serum. Although it was shown to contain chondroitin 4-sulfate, its chemical composition and molecular size are quite different from our PF4 carrier proteoglycan, suggesting that these molecules are distinct proteoglycan molecules. The molecular weight (53,000) of the PF4-proteoglycan carrier is smaller than that of the Clq proteoglycan inhibitor and proteoglycans isolated from other sources (26, 27).

A question arises of whether the \( M_r \) = 53,000 proteoglycan is the proteolytic product of a larger proteoglycan. In order to minimize proteolysis during isolation and purification, we included multiple protease inhibitors (diisopropyl fluorophosphate, phenylmethylsulfonfluoride, L-tosylamide-2-phenylethyl chloromethyl ketone, benzamidine-\( \cdot \)HCl, EDTA, and soybean trypsin inhibitor) in preparations of human platelet-rich plasma as they were thawed. The separate isolations of the PF4-proteoglycan carrier consistently resulted in a \( M_r \) = 53,000 product. In the preparation of Silvestri et al. (23), protease inhibitors were not used, calcium was present, and two 37°C incubations were included in the purification. If the two molecules are related, it seems likely that cleavage of proteoglycan to Clq inhibitor to a \( M_r \) = 53,000 species would have occurred and been detected. It is not clear why the proteoglycan does not enter 5% SDS-polyacrylamide gels, but, under the conditions of electrophoresis, it seems likely that the proteoglycan molecule aggregates at the top of the gel.

Several experiments suggest that the proteoglycan isolated is the specific proteoglycan released by platelets in association with PF4 (11). The proteoglycan is present in high concentrations in platelet-rich plasma. It binds to PF4-agaro and to PF4 during cellulose acetate electrophoresis. No other proteoglycan was found to bind to PF4-agaro; high ionic strength (0.3 M NaCl) is required to release the proteoglycan from the column. No other proteins in platelet lysates were found to bind to the proteoglycan. The role of the proteoglycan carrier molecule in the platelet is not clear, however. One possible role for the proteoglycan carrier molecule is in the "packaging" of PF4 in platelet α-granules. PF4 is relatively insoluble in physiological ionic strength (11), whereas the PF4-proteoglycan complex is highly soluble at this ionic strength. PF4 binds heparin with high affinity. PF4 recently has been shown to be a powerful chemotactrant protein for human monocytes and for human polymorphonuclear leucocytes (25). The proteoglycan carrier molecule may serve to maintain PF4 in soluble form for interactions in the circulation or for delivery to sites of blood vessel injury. Investigations presently are in progress to further characterize the structure and function of this potentially important molecule.

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