Human Platelet-derived Growth Factor: Radioimmunoassay and Discovery of a Specific Plasma-binding Protein

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ABSTRACT The platelet-derived growth factor (PDGF) is the principal mitogen in serum for cultured cells of mesenchymal origin. PDGF also is a potent chemotactic protein for inflammatory cells and for cells required for wound repair. Because activity levels of PDGF in biological fluids are difficult to measure, we attempted to develop a radioimmunoassay for PDGF. Rabbits were immunized with purified PDGF; the antiserum obtained was monospecific for PDGF in immunodiffusion analysis against concentrated platelet lysates, serum, and plasma. A radioimmunoassay for PDGF was developed with a sensitivity of ≃0.2 ng/ml. Levels of PDGF in plasma/serum were measured and compared with PDGF levels determined by a receptor-competition assay and by a standard biological assay measuring incorporation of [^{3}H]thymidine into 3T3 cells. Radioimmunoassay showed apparent PDGF levels of 50 ng/ml in human plasma and 103 ng/ml in serum. The 50 ng/ml PDGF in plasma was unexpected because the plasma samples contained little or no platelet release products as determined by very low levels of platelet factor 4. We therefore sought an immunologically reactive PDGF molecule in human plasma. No immunologically reactive protein was detected by immunodiffusion analysis or when plasma was treated with an immunoaffinity gel. Subsequently, a ^{125}I-PDGF-binding protein was identified; the ^{125}I-PDGF-plasma-binding protein complex was not reactive with anti-PDGF immunoglobulin. Correction for ^{125}I-PDGF bound by the plasma-binding protein established serum levels of PDGF of ≃50 ng/ml; ≃50 ng/ml PDGF was found in serum by radioreceptor-competition assays and by mitogenic assays as well. The plasma-binding protein may serve to clear PDGF released in the circulation, thereby limiting PDGF activity to its local interactions at the site of blood-vessel injury.

The platelet-derived growth factor (PDGF)[1] is a potent polypeptide growth factor released when platelets are activated during blood coagulation and at the site of blood-vessel injury. PDGF is strongly mitogenic for smooth muscle cells, skin fibroblasts, 3T3 cells, and cultured glial cells (1–9). PDGF recently was shown to have a second important biological activity in being a strong chemoattractant in vitro for human monocytes, neutrophils, fibroblasts, and for smooth muscles cells (10–13). While the precise roles of PDGF in vivo remain to be defined, these combined biological properties of PDGF as a potent mitogen and chemoattractant suggest a unique role for PDGF in inflammation and in wound healing. These properties also make PDGF an ideal agent to mediate several of the initial events in the pathological process of human atherosclerosis.

We have recently purified PDGF to homogeneity (14–16) and used the purified protein to immunize rabbits. The antiserum has been used to develop a radioimmunoassay for PDGF in biological fluids, and the results of radioimmunoassay have been compared with those obtained from a newly developed receptor competition assay and with the mitogenic assay of PDGF in vitro.

During the course of these experiments a plasma protein was discovered which bound ^{125}I-PDGF and which interfered with the radioimmunoassay developed to measure PDGF in plasma/serum.

MATERIALS AND METHODS

Materials: Swiss mouse 3T3 fibroblasts (CLL 92) were obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified

[1] *Abbreviations used in this paper:* PDGF, platelet-derived growth factor; DME medium, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; PF4, platelet factor 4.

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Eagle's medium was obtained from K. C. Biologicals (Lenexa, KS). Cell culture cluster plates (24 wells, 16-mm well diameter) were obtained from Costar (Cambridge, MA). Na^{125}I (17 Ci/mg) and [methyl-^{3}H]thymidine (79.4 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Protein A-sepharose CL-4B (lot EM 15161) was obtained from Pharmacia Fine Chemical (Piscataway, NJ). Human serum albumin (25%) was obtained from American Red Cross. Bio-Gel P-6 (50–100 mesh), Bio-Gel A 1.5 m (100–200 mesh), and DEAE-blue gel were obtained from Bio-Rad Laboratories (Richmond, CA). Glutaraldehyde (25%) was obtained from Sigma Chemical Co. (St. Louis, MO). Immunodiffusion plates were obtained from Hyland Diagnostics (Deerfield, IL). IgGsorb was obtained from the Enzyme Center, Inc. (Boston, MA). Keyhole limpet hemocyanin was the kind gift of Dr. Joseph Davie, (Washington University School of Medicine).

PDGF: PDGF was purified from human platelet-rich plasma as described previously and was separated into two homogeneous, equally mitogenic proteins, PDGF I and PDGF II (14–16). The homogeneity of individual preparations of PDGF I and PDGF II was established by analytical SDS PAGE and by isoelectric focusing (15). The specific activity of PDGF II was $\simeq 6 \times 10^5$ U/mg. PDGF was measured by use of the Bio-Rad protein assay (Bio-Rad Laboratories). The assay was correlated with the true protein concentration after measurements of dry weight and by the method of Lowry.

Iodination of PDGF: PDGF II was radiolabeled with Na^{125}I as previously described and was stored in 0.1 M acetic acid containing human serum albumin (1 mg/ml) at −20°C (17). Radiolabeled PDGF II retained full biological activity as measured in a standard [^{3}H]thymidine incorporation assay (15, 16). Nearly 0.7 mol of iodide was incorporated per mole of PDGF with a specific radioactivity of 16 μCi/μg ^{125}I-PDGF.

Coupling of PDGF to Keyhold Limpet Hemocyanin: 5 mg of keyhole limpet hemocyanin was dissolved in 0.45 ml of 0.1 M $NaHCO_3$ (pH 9.0) and reacted with 0.05 ml of 2.5% glutaraldehyde. After 2 h at 22°C, free glutaraldehyde was removed by Bio-Gel P-6 (50–100 mesh, 0.9 × 30 cm) gel permeation. 2 mg of glutaraldehyde-treated hemocyanin was subsequently reacted with 212 μg of PDGF II at room temperature for 18 h, dialyzed against 0.1 M acetic acid, and lyophilized. After coupling, ≃10% of the weight of the conjugate was estimated to be PDGF. 1.5 mg of PDGF/hemocyanin conjugate dissolved in 0.25 ml of 0.1 M $NaHCO_3$ (pH 9.0) was mixed with an equal volume of complete Freund's adjuvant and injected into the footpads of a 2-kg male New Zealand rabbit. At three subsequent 3-wk intervals, 20 μg of PDGF in complete Freund's adjuvant was also given. Blood for analysis was obtained from the central ear artery 1 d before each injection, and serum was removed after 2 h of allowing the blood to clot.

Purification of Anti-PDGF: The immune serum was partially purified with a DEAE blue gel column (18). Endogenous PDGF in rabbit serum was retained on the column, whereas rabbit IgG appeared in the flow-through fractions.

Immunodiffusion: Immunodiffusion plates (Hyland Diagnostic) were incubated overnight at room temperature in a moist chamber. For staining with Coomassie Brilliant Blue, the immunodiffusion plates were kept in 0.9% NaCl to remove nonimmunoprecipitable substances, fixed in 25% isopropyl alcohol–10% acetic acid, and stained for ½ h with 0.25% Coomassie Brilliant Blue in 50% methanol. The immunodiffusion plates were then destained with multiple changes of 10% isopropyl alcohol–10% acetic acid.

Concentration of Human Platelet Lysates: Human platelets obtained from 1 U of blood were suspended in 2 ml of PBS solution and frozen-thawed twice, concentrated to 0.1 ml in dialysis tubing with dextran powder, and, after centrifugation, the supernate was tested in immunodiffusion analysis.

Human Plasma and Serum: 12 samples of human plasma were kindly provided by Dr. Shirley Levine, (University of Texas Health Science Center, San Antonio), with PF4 levels ranging from 14 ng/ml to 52 ng/ml (average of 37 ± 15 ng/ml). Other plasma samples were prepared according to procedures as previously described by Dr. Levine (19). Human sera were obtained from normal healthy volunteers according to standard procedures of the clinical laboratory. The serum and plasma samples were stored at −20°C before assay.

Radioimmunoassay Procedure: The reaction mixture (0.2 ml) contained 10 μl of antiserum (1:150 dilution), 10 μl of the test sample or standard PDGF (0–20 ng) in 10 mM sodium phosphate buffer (pH 7.4), 0.1% Tween 80, 0.5 M NaCl, 0.01% sodium azide, and 0.5% human serum albumin. After 19 h at 22°C, 5 ng of ^{125}I-PDGF in 0.1 ml of the above phosphate buffer was added. After 2 h at 22°C, 50 μl of 10% IgGsorb was added, the incubation was continued for another 2 h, and the mixture was centrifuged. The immunoprecipitate was washed three times with the above phosphate buffer and radioactivity measured with a Beckman gamma counter (Beckman Instruments, Inc., Palo Alto, CA). An internal standard of PDGF (0.2 ng) was also included in the test samples. Nonspecific binding was measured with a 100 molar excess unlabeled PDGF and with nonimmune serum substituted for the anti-PDGF antisera.

Receptor-Competition Assay: The assay conditions were essentially identical to those previously described in binding assays for ^{125}I-PDGF (17). 1 ml of the assay medium consisted of 5 ng ^{125}I-PDGF, test samples or standard PDGF, 0 to 20 ng/ml, and 5% plasma-derived serum in 5 mM HEPES, 0.15 M NaCl (pH 7.4) buffer solution. Two concentrations of serum/plasma (10 μl, 20 μl) with or without 2 ng of PDGF (internal standard) were assayed. All assays were carried out in duplicate.

Mitogenic Activity Assay: The mitogenic activity of PDGF was measured by assaying the PDGF-dependent incorporation of [^{3}H-methyl]thymidine into a trichloroacetic acid precipitate after incubation with confluent Swiss mouse 3T3 fibroblasts in MEM and 1% plasma-derived serum (16). The dose responses of assays were carried out within the linear range of added PDGF (0–20 ng); epidermal growth factor and fibroblast growth factor (0–100 ng/ml) do not elicit a linear dose response curve under the conditions of our assay system. Sera and plasma (5, 10, and 20 μl) were measured in the presence and absence of 2 ng of PDGF (internal standard). Under standard assay conditions, a linear dose response was found with either standard PDGF or sera; nondetectable PDGFlike mitogenic activity was present in plasma.

Bio-Gel A–1.5 m Gel Permeation Chromatography: 200 μl of human plasma or 10 mM sodium phosphate buffer, pH 7.4, 0.15 M NaCl containing 100 ng of ^{125}I-PDGF was applied to a column of Bio-Gel A–1.5 m (0.9 × 52 cm) previously equilibrated with 10 mM sodium phosphate (pH 7.4) containing 0.15 M NaCl, 0.01% sodium azide, and human serum albumin (1 mg/ml) and eluted with the same buffer. Fractions of 0.7 ml were collected and counted for radioactivity.

SDS Gel Electrophoresis and Autoradiography: 5 and 15% PAGE were carried out according to the procedure described by Laemmli (20). Gels were stained with Coomassie Brilliant Blue, destained with 10% 2-propanol/10% acetic acid, and dried on filter paper (Bio-Rad filter paper backing). Radiolabeled proteins were visualized by exposure of the dried gels to Kodak XAR-5 films with DuPont lightning-plus intensifying screens (DuPont Instruments, Wilmington, DE).

RESULTS

The specificity of rabbit anti-human PDGF antisera was tested by immunodiffusion analysis. PDGF II and a highly concentrated platelet lysate (see Materials and Methods) were tested against anti-human PDGF antisera (Fig. 1 *a*). A single immunoprecipitin line is observed between antisera and each antigen; the precipitin lines are smoothly fused between PDGF and platelet lysates. The absence of spur formation between immunoprecipitin lines and the absence of additional immunoprecipitin lines suggest that the antiserum recognizes purified PDGF and only PDGF in lysates of human platelets. When PDGF I and PDGF II are tested against each other, immunological identity of the two proteins was found (Fig. 1 *b*). Immunocross-reactive protein was not found in plasma samples tested (data not shown). The immunological identity of PDGF I and PDGF II is consistent with the identity of these proteins in direct binding analyses (17) and in the mitogenic responsiveness of Swiss mouse 3T3 cells to each protein (16).

A quantitative assessment of the potency of the antisera was next made using sequential dilution (Fig. 2). With ^{125}I-PDGF at 5 ng/ml, 50% precipitation of ^{125}I-PDGF was observed at a dilution of antisera of 1:5,000; 8% of the ^{125}I-PDGF was precipitated at a 1:40,000 dilution.

The antiserum was then tested to see whether it would block the binding of ^{125}I-PDGF to Swiss mouse 3T3 cells and the PDGF-dependent mitogenic response of 3T3 cells (Fig. 3). Increasing concentrations of antiserum effectively blocked the binding of ^{125}I-PDGF to Swiss mouse 3T3 cells. Complete inhibition of ^{125}I-PDGF binding to 3T3 cells was observed when 500 μg of antiserum was added to the binding assay. As the concentration of antiserum is reduced, the inhibition of

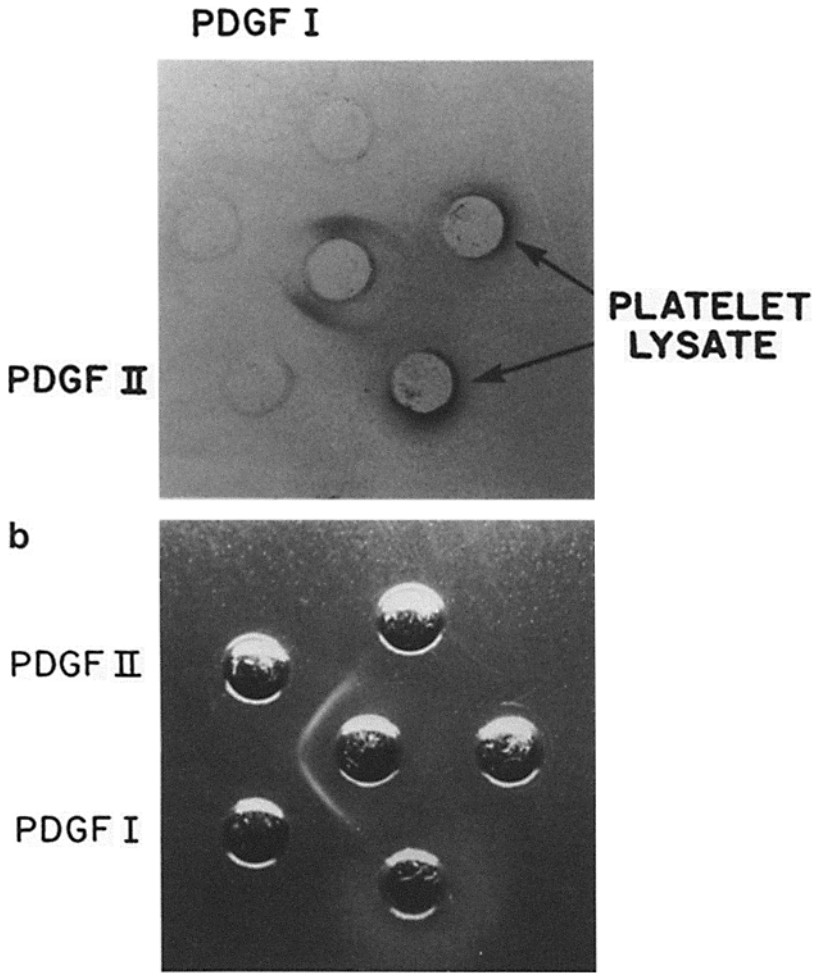

FIGURE 1 (*a*) Immunodiffusion of human PDGF II, human PDGF I and human platelet lysates against rabbit anti-PDGF antiserum. The concentrations of PDGF II and PDGF I were 0.5 mg/ml. The platelet lysates were added at a concentration of 1 U platelets/0.1 ml PBS. The center well contains antiserum. The immunodiffusion plates were stained with Coomassie Brilliant Blue. (*b*) Immunodiffusion of human PDGF II and human PDGF I against rabbit anti-PDGF II antiserum. The concentrations of PDGF I and PDGF II were 0.5 mg/ml in H_2O.

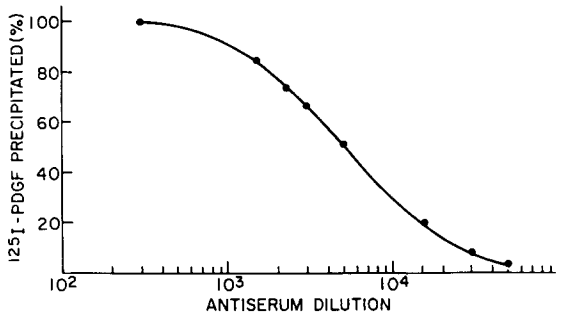

FIGURE 2 Anti-PDGF serum dilution curve. The assay medium contained 5 ng of ^{125}I-PDGF (10,000 cpm/ng) and 10 μl of various dilutions of anti-PDGF antiserum in 0.3 ml of RIA assay buffer. The immunoprecipitation with IgGsorb was carried out as described in Materials and Methods. The radioactivity precipitated with nondiluted antiserum was taken as 100%.

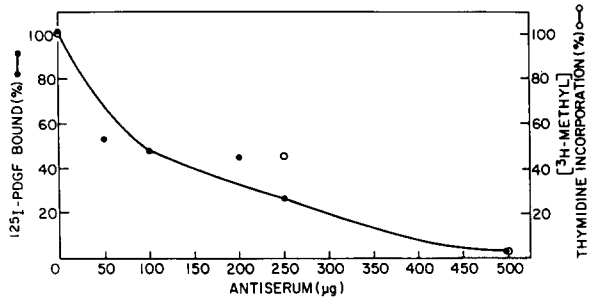

FIGURE 3 Effect of anti-PDGF antiserum on the binding of ^{125}I-PDGF to 3T3 fibroblasts and on the PDGF-dependent [^{3}H-methyl]thymidine incorporation into DNA of 3T3 fibroblasts. Assays were performed at 30 ng/ml of ^{125}I-PDGF and PDGF, respectively. PDGF or ^{125}I-PDGF was preincubated with antiserum at room temperature for 2 h before assay. The binding and mitogenic activities in the absence of antisera were taken as 100%.

^{125}I-PDGF binding is progressively reduced. The antiserum also effectively inhibits in parallel the PDGF-dependent stimulation of [^{3}H]thymidine incorporation into DNA of Swiss mouse 3T3 cells.

Samples of PDGF were then measured using the radioimmunoassay developed (Fig. 4). Direct measurement of PDGF over concentrations from 0.2 to 2 ng/ml was readily obtained. Repetitive experiments provided identical results; when known samples were tested as unknowns, accurate estimates of PDGF concentrations were reproducibly obtained. The radioimmunoassay was then used to measure PDGF levels in 12 samples of human plasma; 55.0 ± 11 ng/ml PDGF was found (Table I). Eight samples of human serum were measured; PDGF levels were found to be 103 ± 16 ng/ml. The high levels of plasma PDGF found (≈55 ng/ml) raised the possibility that PDGF was released from platelets during preparation of plasma. Both PF4 and PDGF activities have been localized to α-granules in platelets (6–9, 21, 22); radioimmunoassay of PF4 in plasma samples provides a sensitive index of platelet release during plasma preparation (19). PF4 levels were measured and found to be from 14 ng/ml to 52 ng/ml in the plasma samples (courtesy of Dr. Shirley Levine,

University of Texas Health Science Center, San Antonio). PF4 is present at ≃5 μg/ml in human serum (19, 21, 22). The plasma samples used to estimate PDGF levels thus contained <1% the PF4 released in serum, providing direct evidence that significant release of platelet α-granule constituents did not occur during the collection/preparation of plasma.

A receptor-competition assay in which PDGF is measured by its capacity to compete with ^{125}I-PDGF binding to the PDGF receptor on the surface of Swiss mouse 3T3 cells was then used to assess the high plasma levels of PDGF found by radioimmunoassay (Fig. 5). PDGF levels measured by radioimmunoassay were compared with levels measured by receptor-competition (Table I). PDGF levels were substantially <10 ng/ml in human plasma; ≃50 ng/ml PDGF was found in human serum when PDGF is measured by receptor competition. Due to the limitations in the sensitivity of the receptor competition assay at PDGF levels <10 ng/ml, the levels of plasma PDGF present could not be accurately determined even when plasma concentrations were raised to 20%. Plasma and serum samples were assayed by the standard mitogenic assay. Human serum contained ≃64 ng/ml of PDGF; no mitogenic activity was found in human plasma

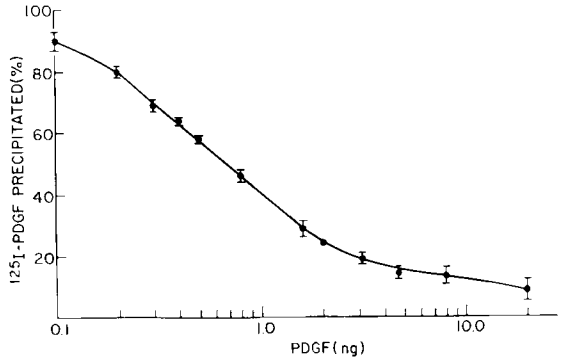

FIGURE 4 Standard curve of the radioimmunoassay with rabbit anti-PDGF serum. The procedure for radioimmunoassay is described in Materials and Methods. The samples were assayed in quadruplicates. Each point represents the mean ± SE of measurements obtained from five different experiments.

TABLE I

Measurement of PDGF in Human Plasma and Serum by Using Radioimmunoassay, Receptor-Competition Assay, and Mitogenic Assay

	RIA	RCA	Mitogenic assay
	ng/ml	*ng/ml*	*ng/ml*
Human serum*	103 ± 16	50 ± 9.0	64 ± 4
Human plasma*	55 ± 1	<10‡	0§

RIA, radioimmunoassay. *RCA*, receptor-competition assay.

* Each value represents the mean ± SE of results obtained from eight human serum samples or 12 human plasma samples. Three levels of human serum and plasma (2.5 times dilution, 5, 10, and 20 μl) were assayed ±0.2 ng PDGF as internal standard in radioimmunoassays, and two levels of human serum and plasma (10 and 20 μl) were assayed ±2 ng purified PDGF (as internal standard) in receptor-competition assays.

‡ Levels of PDGF in plasma samples <10 ng/ml were difficult to quantitate because high amounts of plasma decrease the nonspecific binding and the quantitation of specific ^{125}I-PDGF binding is less accurate. At concentrations of 10–20% plasma, because of decreased nonspecific binding in the assay an apparent value of PDGF in plasma is seen. The dose response to PDGF, however, is not linear.

§ A small mitogenic activity was found in human plasma. However, no dose response of mitogenic activity of PDGF in increasing concentrations was observed.

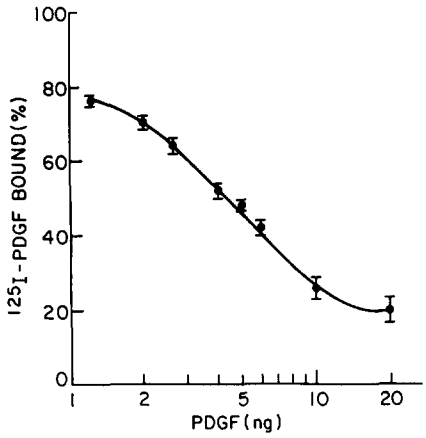

FIGURE 5 Standard curve of ^{125}I-PDGF receptor-competition assay with mouse Swiss 3T3 fibroblasts. The assay medium (1 ml) consisted of 5 ng of ^{125}I-PDGF (1 × 10^4 cpm/ng) and various amounts of authentic PDGF (0–20 ng) in 5 mM HEPES buffer, pH 7.4, containing 5% plasma-derived sera. The assay samples were tested in duplicate. Each point represents the mean ± SE of measurements obtained from four different experiments.

(Table I); these levels are consistent with those found using the receptor-competition assay.

The levels of PDGF in plasma/serum found by radioimmunoassay were thus higher than levels found in the mitogenic and receptor competition assays. Subtraction of plasma value from the serum values obtained by radioimmunoassay resulted in a corrected serum level of ≃50 ng PDGF/ml, a value now consistent with serum PDGF levels obtained by both the mitogenic and receptor-competition assays.

To account for the high levels of PDGF in plasma, an immunologically cross-reactive protein or a protein interfering with the radioimmunoassay was then sought. 2 ml of plasma was pretreated with IgGSorb to remove endogenous human IgG. The plasma was then incubated with 20 μl of specific anti-PDGF antiserum (or 20 μl of nonimmune rabbit serum, as control). After incubation overnight at 4°C, the plasma solution was mixed with 50 μl of 35% (wt/vol) protein A-agarose gel suspension, stirred for 3 h and washed sequentially with 1 ml of phosphate-buffered saline (PBS), with 1 ml of 0.1% Tween 80 in PBS and 0.5 M NaCl, and with 1 ml of PBS solution. The protein A-sepharose gel was then suspended in 200 μl of 0.3 M sodium phosphate buffer (pH 7.5) and iodinated with 0.5 mCi/5 nmol Na^{125}I in a test tube coated with 40 μg of iodogen at room temperature for 1 h (17). The iodinated protein A-sepharose gel was washed with PBS solution, suspended in 1% SDS, and subjected to SDS PAGE (7.5% and 15% acrylamide gels). No difference in the ^{125}I patterns in the autoradiograms were found between plasma samples treated with immune serum and nonimmune serum.

The failure to demonstrate immunocross-reactive material in human plasma in this and in other experiments (immunodiffusion analysis) prompted us to investigate possible plasma proteins which might interact with ^{125}I-PDGF, thereby interfering with the RIA assay. Incubation of ^{125}I-PDGF with plasma followed by gel permeation on Bio-Gel A 1.5 m showed a distinct ^{125}I-labeled fraction (peak C) migrating sustantially faster than peak A or peak B; peak C is not found when ^{125}I-PDGF is incubated without plasma (Fig. 6*a*). The two forms (peak A and peak B) found in gel permeation

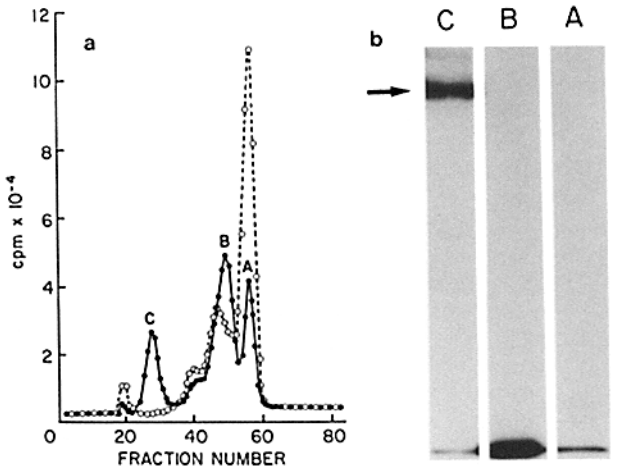

FIGURE 6 (*a*) Gel filtration profile of ^{125}I-PDGF incubated with or without human plasma on Bio-Gel A 1.5 m gel. 100 ng of ^{125}I-PDGF was incubated with 200 μl of human plasma or 200 μl of 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. After 20 min at room temperature, the reaction mixture was applied to a column of Bio-Gel A 1.5 m (0.9 × 52 cm) previously equilibrated with 10 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl and 0.01% sodium azide and human serum albumin (1 mg/ml). The frational volume was 0.7 ml and the flow rate was 20 ml/h. The radioactivity for each fraction was measured with a gamma counter. No significant differences in relative radioactive distribution in peak A, B, or C were found at concentrations of ^{125}I-PDGF from 10 ng/ml to 500 ng/ml. ●, + plasma. ○, − plasma. (*b*) SDS polyacrylamide gel autoradiographs of peak A, B, and C from Bio-Gel A 1.5 m gel permeation chromatography of ^{125}I-PDGF treated with human plasma. 20 μl of each fraction which contained the highest radioactivity in peak A, B, and C as shown in *a* was subjected to SDS 5% PAGE. After staining with Coomassie Brilliant Blue and destaining with 10% 2-propanol/10% acetic acid, the gels were then dried and exposed to Kodak XAR-5 films at −70°C with DuPont lightning-plus intensifying screens. Myosin, β-galactosidase, phosphorylase B, and bovine serum albumin were used as standard molecular weight markers. The arrow indicates the location of ^{125}I-PDGF-plasma binding protein complex. ^{125}I-PDGF appeared with the dye front in 5% SDS polyacrylamide gels. The molecular weight of the ^{125}I-PDGF-plasma-binding protein complex was measured as 310,000. The molecular weight of peaks A and B was 30,000 and co-migrated with unlabeled PDGF in 15% SDS polyacrylamide gels.

chromatography of ^{125}I-PDGF at neutral pH are consistently present; only a single peak (free PDGF) is seen on Bio-Gel P-100 in 1 M acetic acid (data not shown). Peak A is free PDGF and peak B is felt to be a noncovalent, self associated form of ^{125}I-PDGF. A sample from peak C was subjected to SDS gel electrophoresis and compared with samples from peaks A and B. Autoradiograms of these gels showed ^{125}I-PDGF associated with a high molecular weight protein in peak C (Fig. 6*b*). The molecular weight of this ^{125}I-PDGF-plasma-binding protein complex was estimated as 310,000. If the assumption is made that this complex contains equal molar ^{125}I-PDGF/plasma-binding protein, the molecular weight of this ^{125}I-PDGF plasma-binding protein is ≃280,000. The ^{125}I-PDGF-plasma-binding protein complex is not dissociated by boiling, by treatment with SDS, or by 1 M acetic acid. The ^{125}I-PDGF plasma-binding protein complex retained ≃3% apparent immunoreactivity. Experiments are in progress to characterize this plasma-binding protein and its interactions with PDGF. While the identity of this protein and the nature of its interactions with PDGF are not known, the finding of the ^{125}I-PDGF-plasma-binding protein complex provides direct evidence that the plasma PDGF levels measured in the RIA assay are due to complexing of the ^{125}I-PDGF added for assay with the plasma-binding protein, leaving less ^{125}I-PDGF associated with the antibody; the presence of an immunologically cross-reactive material could not be demonstrated. The ^{125}I-PDGF bound to this plasma protein is able to account fully for the apparent PDGF found in human plasma by radioimmunoassay.

DISCUSSION

PDGF has been difficult to purify. Small-scale purifications were initially achieved (23–25), and, more recently, the large-scale purification of PDGF has been reported (14–16, 26–28). Our laboratory (15) succeeded in separating PDGF into two homogeneous protein fractions (PDGF I and PDGF II) that were similar in amino acid composition but different in carbohydrate content. PDGF II was used to generate antisera; antiserum to PDGF II has been obtained from rabbits. Initial efforts to raise antisera with purified PDGF were not successful, perhaps because binding of the highly positively charged PDGF (isoelectric point [pI] ≃10.2, reference 15) at the injection site precluded delivery of adequate antigen to sites for processing. PDGF coupled to homocyanin subsequently proved to be highly immunogenic. The anti-PDGF antisera obtained precipitates ^{125}I-PDGF from solution; loss of PDGF mitogenic activity after reaction with antisera correlates directly with the decrease of ^{125}I-PDGF binding to 3T3 cells. Further evidence of specificity was found in immunodiffusion analysis; a single, smoothly fused immunoprecipitin line was obtained when the antiserum was tested against purified PDGF and against concentrated whole platelet lysates. No difference in immunological reactivity was found between PDGF I and II.

Using partially purified antisera, a radioimmunoassay sensitive in measuring 0.2 ng PDGF/ml has been developed. 55 ng/ml PDGF was measured by radioimmunoassay in human plasma samples in which significant platelet release had not occurred during preparation as indicated by very low levels of PF4 in the samples; the PDGF activity in these plasma samples by [^{3}H]thymidine incorporation or by PDGF specific receptor competition assays was essentially nondetectable. Immunologically cross-reactive, biologically inactive proteins therefore were sought in human plasma. Using partially purified antisera, no immunocross-reactive material could be identified in immunodiffusion analysis or when immobilized antiserum was used to adsorb plasma. Binding of ^{125}I-PDGF to a plasma protein (≃280,000 mol wt) was then demonstrated after incubating plasma and ^{125}I-PDGF. The binding of ^{125}I-PDGF to the plasma-binding protein appears to account for the apparent PDGF levels in plasma by binding ^{125}I-PDGF that otherwise would bind to the antibody during radioimmunoassay. About 10–20% ^{125}I-PDGF added to samples of plasma or serum is bound to the PDGF-binding protein under conditions of assay. By use of internal standards, it can be established that the radioimmunoassay for PDGF in serum accurately reflects incremental increases of PDGF over and above PDGF bound to the binding protein. If the plasma radioimmunoassayable levels of "PDGFlike" material are subtracted from the serum RIA levels, ≃50 ng/ml PDGF is estimated in human serum. These levels of PDGF are consistent with levels measured in serum using the mitogenic

([^{3}H]thymidine incorporation) and receptor-competition assays (3T3 cells).

A radioimmunoassay for a human serum growth factor for BALB/c-3T3 cells has been previously reported by Antoniades and Scher (29); use of the radioimmunoassay demonstrated this serum growth factor in platelets. The molecular weight of this growth factor used as antigen for raising antisera was estimated to be 13,000 (SDS gel electrophoresis and Bio-Gel P-150 gel permeation chromatography) and its pI was 9.7. The relationship of the PDGF antigen used in our work (M_r ≃30,000) and the antigen reported previously (29) is not clear although it seems most likely that the proteins share a common origin. No plasma PDGF-binding activity was reported. Heldin et al. (30) also reported the development of antiserum against human PDGF. This antiserum inhibited the multiplication-stimulating activity of PDGF and partially but not completely cross-reacted with osteosarcoma-derived growth factor. A radioimmunoassay was developed with a sensitivity to 5 ng/ml; plasma and serum PDGF levels were not reported. Singh et al. (31) used a radioreceptor assay to measure PDGF in serum. They report ≃H15 ng/ml PDGF in normal serum and <1 ng/ml PDGF in serum derived from platelet-poor plasma. Radioimmunoassay for PDGF was not described.

Correlation between our three assay systems suggests that PDGF levels of ≃50 ng/ml are found in serum from normal humans. Serum PDGF levels from individuals may be difficult to measure, however, because of ^{125}I-PDGF binding to the 280,000 mol wt plasma protein observed in this work. Serum levels can be estimated, however, by doing simultaneous plasma and serum "levels" and correcting measured serum PDGF levels for the apparent PDGF levels found in plasma.

The presence of a 280,000 mol wt ^{125}I-PDGF-binding protein in human plasma is of major interest. Such a protein might serve to clear PDGF released locally by platelets at sites of injured endothelium, thereby limiting PDGF activity to that PDGF bound locally to cells and to subendothelium. The nature of the binding protein and its in vivo function are presently under investigation.

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