Specific covalent binding of platelet-derived growth factor to human plasma α_2-macroglobulin

(mitogen/inflammation/atherosclerosis/transforming protein/plasma binding protein)

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ABSTRACT   Attempts to measure the platelet-derived growth factor (PDGF) in human plasma resulted in the discovery of a specific plasma binding protein. The ^{125}I-labeled PDGF (^{125}I-PDGF)-plasma binding protein complex retained mitogenic activity but lost reactivity against rabbit anti-PDGF antiserum. Copurification of the plasma binding protein and α_2-macroglobulin (α_2M) in human plasma, the formation of a complex between ^{125}I-PDGF and purified α_2M, and the comigration of the ^{125}I-PDGF-plasma binding protein complex and the ^{125}I-PDGF-α_2M complex in NaDodSO$_4$/polyacrylamide gel electrophoresis and in pore-limiting polyacrylamide gel electrophoresis strongly suggested that α_2M is the plasma binding protein for ^{125}I-PDGF. Immunoprecipitation of ^{125}I-PDGF-α_2M and ^{125}I-PDGF-plasma binding protein complexes by anti-human α_2M antiserum further established that α_2M and the plasma binding protein are the same molecule. Approximately 20% of ^{125}I-PDGF is complexed by α_2M; further ^{125}I-PDGF is complexed if the remaining ^{125}I-PDGF is incubated with additional α_2M. Complex formation of ^{125}I-PDGF with plasma or with α_2M was completely inhibited by 0.2 mM *p*-chloromercuric benzoate or 0.2 mM *N*-ethylmaleimide. The ^{125}I-PDGF-α_2M complex or ^{125}I-PDGF-plasma binding protein complex was not dissociated by 8 M urea, 1 M acetic acid, 0.1 M NaOH, or 1% NaDodSO$_4$ but was dissociated by 2-mercaptoethanol, suggesting that the covalent binding of ^{125}I-PDGF to α_2M occurs through a disulfide/sulfhydryl exchange reaction. The ^{125}I-PDGF-α_2M complex (780,000 daltons) appears to contain two molecules of ^{125}I-PDGF and two dimers of α_2M. The precise physiological role of the ^{125}I-PDGF-α_2M interaction is unknown. α_2M may serve to limit PDGF released locally at sites of blood vessel injury. Alternatively, because of the nearly complete homology between the partial amino acid sequence of PDGF and the predicted amino acid sequence of the transforming protein of the simian sarcoma virus, p28^{sis}, α_2M may play an important role in limiting the activity of a PDGF-like activity expressed by virus-transformed cells.

The platelet-derived growth factor (PDGF) is the principle mitogen in serum for cells of mesenchymal origin (1–3). PDGF also has a second major biological activity in being a powerful chemoattractant protein for inflammatory cells and for cells involved in wound repair (4–7). These properties of mitogenesis and chemotaxis suggest PDGF may be important in normal inflammation and repair and may be uniquely suited as a mediator in the abnormal process of atherosclerosis.

Recently, partial amino acid sequence analysis has demonstrated a striking sequence homology of human PDGF with the amino acid sequence predicted for the transforming protein of the simian sarcoma virus, p28^{sis}, suggesting that a PDGF-like protein may play a singularly important role in

cellular transformation by simian sarcoma virus or other transforming agents (8, 9).

PDGF is believed to be stored in α-granules of circulating platelets (10–13). Circulating platelets adhere to and are activated by exposed subendothelium when blood vessels are injured. Thus, PDGF likely binds and is active locally when endothelial integrity is compromised by injury (14, 15).

We attempted to assay PDGF in biological fluids by radioimmunoassay. A binding protein for ^{125}I-labeled PDGF (^{125}I-PDGF) was observed in human plasma that interfered with the radioimmunoassay (16), raising the important possibility that such a protein might bind and clear PDGF released into the systemic circulation. Thus, a plasma PDGF binding protein would limit the activity of PDGF to the immediate site of vessel injury or perhaps interfere with a PDGF-like activity expressed by virally transformed cells. This report identifies the PDGF plasma binding protein as α_2-macroglobulin (α_2-M).

MATERIALS AND METHODS

Materials. IgGsorb was obtained from the Enzyme Center (Boston); Na^{125}I (17 Ci/mg; 1 Ci = 37 GBq), Bolton–Hunter ^{125}I-labeled reagent (^{125}I-reagent; 2 Ci/mol), and [*methyl-^{3}H*]thymidine (79.4 Ci/mmol), from New England Nuclear; α_2M from human plasma (lot 102F-9360), *N*-ethylmaleimide, and CH$_3$NH$_2$, from Sigma; and rabbit anti-human α_2M antisera (lot 010403), from Calbiochem–Behring. Rabbit anti-human PDGF antiserum and human plasma (prepared from blood with EDTA as anticoagulant) were prepared as described (16).

Methods. *Polyacrylamide gel electrophoresis (PAGE).* NaDodSO$_4$/PAGE (5% and 15% gels) was carried out as described by Laemmli (17). Pore-limiting PAGE (5%, pH 8.6) was used as described by Van Leuven *et al.* (18).

Mitogenic activity assay and purification of PDGF. The mitogenic activity assay of PDGF was measured as described (19). PDGF was purified by described methods (19). In the experiments to follow, only PDGF II was utilized.

Iodination of PDGF. Iodination of PDGF II with IODO-GEN was carried out as described (20). ^{125}I-PDGF ($\approx$16 μCi/μg) was stored in 0.1 M acetic acid/0.1% human serum albumin at −20°C. PDGF also was iodinated with the Bolton–Hunter ^{125}I-reagent described by New England Nuclear (instructions for use) as modified from Bolton and Hunter (21). The specific activity of ^{125}I-PDGF was 65 μCi/μg.

Complex formation of ^{125}I-PDGF with human plasma and α_2M. ^{125}I-PDGF (100 ng) was incubated with 10 μl of human plasma or 10 μl of human α_2M (1 mg/ml) in 100 μl of 5 mM Hepes, pH 7.4/0.15 M NaCl. After incubation at room temperature for 30 min, 5–10 μl of the reaction mixture was immediately mixed with 5 μl of a NaDodSO$_4$ sample solution

Abbreviations: α_2M, α_2-macroglobulin; PDGF, platelet-derived growth factor; PAGE, polyacrylamide gel electrophoresis.
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(0.25 M Tris·HCl, pH 6.8/8% NaDodSO$_4$/20% glycerol) and subjected to NaDodSO$_4$/PAGE (5% gel). Complex formation of ^{125}I-PDGF and α_2M was effectively complete after 10 min at 22°C; 20% ^{125}I-PDGF is bound with saturating quantities of α_2M, and an additional 10–20% is bound if the remaining ^{125}I-PDGF is exposed subsequently to α_2M after removal of the ^{125}I-PDGF-α_2M complex with anti-human α_2M antisera.

RESULTS

Previous experiments had shown that ^{125}I-PDGF bound to a plasma protein of ≈280,000 daltons and that the ^{125}I-PDGF-plasma binding protein complex was not dissociated by NaDodSO$_4$ with or without other denaturing reagents (16). The specificity of this interaction for PDGF was tested by incubation of plasma with ^{125}I-PDGF in the presence of unlabeled PDGF and of other proteins (Fig. 1). Unlabeled PDGF (4 μg/0.1 ml) decreased the formation of the complex between plasma and ^{125}I-PDGF (100 ng/0.1 ml); at 50 μg/0.1 ml, unlabeled PDGF almost completely blocked the formation of the ^{125}I-PDGF-plasma binding protein complex. Both anionic and cationic proteins were tested for an effect on complex formation. Epidermal and fibroblast growth factors, insulin, platelet factor 4, and protamine sulfate, each at 1 mg/ml, had no effect on complex formation (data not shown), suggesting that complex formation between ^{125}I-PDGF and the plasma binding protein is specific to PDGF. Protamine sulfate, a competitive inhibitor of PDGF binding to its specific cell surface receptor (20), did not block the interaction of ^{125}I-PDGF with the plasma binding protein, suggesting that the binding of ^{125}I-PDGF to the plasma binding protein is different from the binding of ^{125}I-PDGF to its cell surface receptor and that the highly cationic property of PDGF alone is not responsible for binding.

The 310,000-dalton ^{125}I-PDGF-plasma binding protein complex was first detected when ^{125}I-PDGF was incubated with human plasma and analyzed by NaDodSO$_4$/PAGE. When the complex was analyzed in reduced NaDodSO$_4$ gels, complex formation was not observed, suggesting that complex formation might result from sulfhydryl/disulfide or disulfide/disulfide exchange of either of the PDGF polypeptide-subunit A or B chains and the plasma binding protein. We then tested whether the ^{125}I bound to the 280,000-dalton plasma binding protein was ^{125}I-labeled single chain or intact ^{125}I-PDGF. The ^{125}I-PDGF complex was isolated with Bio-Gel A-1.5m, reduced with 5% 2-mercaptoethanol, and analyzed with NaDodSO$_4$/PAGE. ^{125}I-PDGF was dissociated from the ^{125}I-PDGF-plasma binding protein complex by reduction. Both PDGF A and B chains were identified (Fig. 2, lane 3). In Fig. 2, free ^{125}I-PDGF (lane 1) and reduced ^{125}I-PDGF (lane 2) are shown for comparison. Thus, ^{125}I-PDGF binds to the plasma binding protein as the intact protein; a disulfide linkage may be the linkage forming the complex itself, although present data is insufficient to fully establish this point. PDGF iodinated by the IODO-GEN method or with the Bolton–Hunter ^{125}I-reagent were compared; each formed an identical complex with the plasma binding protein, suggesting that the inherent binding properties of ^{125}I-PDGF to the plasma binding protein was not an artifact of iodination (22). Pretreatment of the ^{125}I-PDGF-plasma binding protein complex with 8 M urea, 1 M acetic acid, 0.1 M NaOH, or 1% NaDodSO$_4$ at 100°C for 10 min did not affect the mobility of the complex in NaDodSO$_4$/PAGE, suggesting a covalent bond linked the ^{125}I-PDGF-plasma binding protein complex.

The ^{125}I-PDGF-plasma binding protein complex was tested for mitogenic activity with 3T3 cells. The ^{125}I-PDGF-plasma binding protein complex retained ≈50% of the mitogenic activity of free PDGF (Table 1). The complex lost most of its
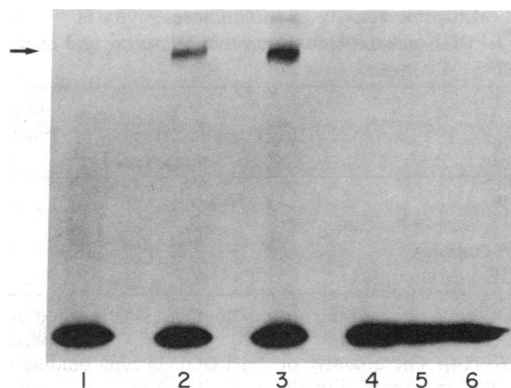

FIG. 1. Effect of unlabeled PDGF on the formation of ^{125}I-PDGF-plasma binding protein complex. Human plasma (100 μl) was treated with 100 ng of ^{125}I-PDGF in the presence of different concentrations of unlabeled PDGF: 0 (lanes 3 and 6), 40 (lanes 2 and 5), and 500 (lanes 1 and 4) μg/ml. After reaction at room temperature, 5 μl of the reaction mixture was then subjected to NaDodSO$_4$/PAGE (5% gel) and autoradiography without reduction (lanes 1–3) or with reduction (lanes 4–6). The arrow indicates the ^{125}I-PDGF-plasma binding protein complex. The radioactive material at the bottom of each gel is ^{125}I-PDGF, which runs with the tracking dye in 5% gels.

antigenic activity when tested against specific rabbit anti-human PDGF.

Attempts were made to identify the ^{125}I-PDGF binding activity in plasma. Purification of the plasma binding protein with ammonium sulfate (30–60% of saturation) and with DEAE-Sephacel column chromatography [0.025 M Tris·HCl (pH 8.5) with a linear salt gradient from 0 to 0.5 M NaCl] was attempted. The ^{125}I-PDGF plasma binding activity copurified with α_2M in both systems (data not shown). Copurification of the ^{125}I-PDGF-plasma binding protein complex and α_2M was then demonstrated by gel permeation chromatography (Ultrogel AcA34/Ultrogel AcA22, 2:1, vol/vol) of a 5.5–12.5% polyethylene glycol precipitate of human plasma (23). In this system, α_2M appears in a symmetrical protein peak with a purity ≥ 95% (Fig. 3A). The descending limb of the α_2M-containing peak shows α_2M as the predominant protein (Fig. 3B, Coomassie blue stain), coinciding directly with the
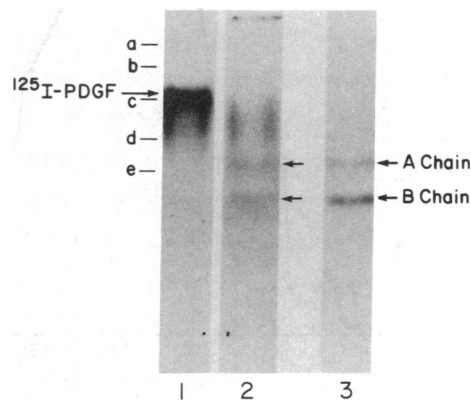

FIG. 2. NaDodSO$_4$/polyacrylamide (15%) gel autoradiographs of ^{125}I-PDGF, reduced ^{125}I-PDGF, and reduced ^{125}I-PDGF-plasma binding protein complex. ^{125}I-PDGF-plasma binding protein complex was obtained from Bio-Gel A-1.5m (0.9 × 52 cm column) after ^{125}I-PDGF was incubated with human plasma. The ^{125}I-PDGF-plasma binding protein complex did not migrate into the separating gel (15%). About 3,000 cpm were applied per lane. Lanes: 1, ^{125}I-PDGF; 2, ^{125}I-PDGF reduced with 5% 2-mercaptoethanol; and 3, ^{125}I-PDGF-plasma binding protein complex reduced with 2-mercaptoethanol. Protein markers: a, bovine serum albumin; b, ovalbumin; c, carbonic anhydrase; d, soybean trypsin inhibitor; and e, lysozyme.

Table 1.   Mitogenic activity and immunoreactivity of ^{125}I-PDGF, of the ^{125}I-PDGF-plasma binding protein complex, and of the ^{125}I-PDGF-α_2M complex

	Mitogenic activity $\times 10^{-3}$, units/nmol	Immuno-reactivity,* %
^{125}I-PDGF	17.4	100
^{125}I-PDGF-plasma binding protein complex	7.2	3
^{125}I-PDGF-α_2M complex	6.4	2

^{125}I-PDGF-plasma binding protein complex was isolated on a column of Bio-Gel A-1.5m (0.9 × 52 cm) from a mixture of plasma and ^{125}I-PDGF (14). The quantity of ^{125}I-PDGF-plasma binding protein complex was measured directly from the content of ^{125}I-PDGF in the complex. It was assumed that the complex contained equal moles of ^{125}I-PDGF and α_2M (dimer). ^{125}I-PDGF-α_2M was isolated on a column of Bio-Gel A-1.5m (0.9 × 52 cm) from a mixture of ^{125}I-PDGF and α_2M (1 mg/ml).
*About 85% of 5 ng of ^{125}I-PDGF was immunoprecipitated with 10 μl of anti-PDGF antisera; this figure was taken as 100% immunoreactivity.

^{125}I-PDGF-plasma binding protein complex (Fig. 3C, autoradiography).

The ^{125}I-PDGF complexes, after incubation of ^{125}I-PDGF with human plasma [Fig. 4A (Coomassie blue stain), lanes 1 and 2, and Fig. 4B (autoradiography), lanes 1 and 2] and with purified α_2M (Fig. 4A and B, lanes 5 and 6), were then compared by electrophoresis in 5% NaDodSO$_4$/polyacrylamide gels and in 5% pore-limiting gels (data not shown). ^{125}I-PDGF forms a complex with α_2M; the ^{125}I-PDGF-plasma binding protein complex and the ^{125}I-PDGF-α_2M complex

comigrated in both electrophoretic systems. The ^{125}I-PDGF-α_2M complex was not dissociated by 1 M acetic acid, 8 M urea, 0.1 M NaOH, or 1% NaDodSO$_4$ (10 min at 100°C).

CH$_3$NH$_2$ inhibits the binding of proteases to α_2M (24). CH$_3$NH$_2$ was tested to see if it would inhibit complex formation of ^{125}I-PDGF and α_2M; CH$_3$NH$_2$ did not inhibit the formation of the ^{125}I-PDGF-α_2M complex (Fig. 4B, lanes 7 and 8) or of the ^{125}I-PDGF-plasma binding protein complex (data not shown). However, the sulfhydryl-blocking reagents, N-ethylmaleimide and p-chloromercuric benzoate, completely blocked complex formation between ^{125}I-PDGF and human plasma (Fig. 4B, lanes 3 and 4). Similar results were found with purified α_2M (data not shown). These results provide additional support that the plasma binding protein/α_2M may bind ^{125}I-PDGF through a disulfide/sulfhydryl exchange reaction.

Rabbit anti-human α_2M antisera was used to provide additional evidence that α_2M is the ^{125}I-PDGF plasma binding protein. Plasma samples were incubated with ^{125}I-PDGF and specific anti-α_2M antisera or with nonimmune serum. The immunocomplexes were precipitated with IgGsorb and analyzed by NaDodSO$_4$/PAGE. A protein migrating as α_2M in the immunoprecipitate from plasma incubated with specific antisera was seen (Fig. 5A, lane b; Coomassie blue staining); this protein was not found in the immunoprecipitate from plasma incubated with nonspecific antisera (Fig. 5A, lane a). Autoradiograms of these NaDodSO$_4$ gels showed the ^{125}I-PDGF-plasma binding protein complex in the immunoprecipitate from specific anti α_2M antisera (Fig. 5B, lane b) but not in the immunoprecipitate when nonimmune serum was used (Fig. 5B, lane a). Control experiments established that the antisera was specific for α_2M (immunodiffusion analysis)
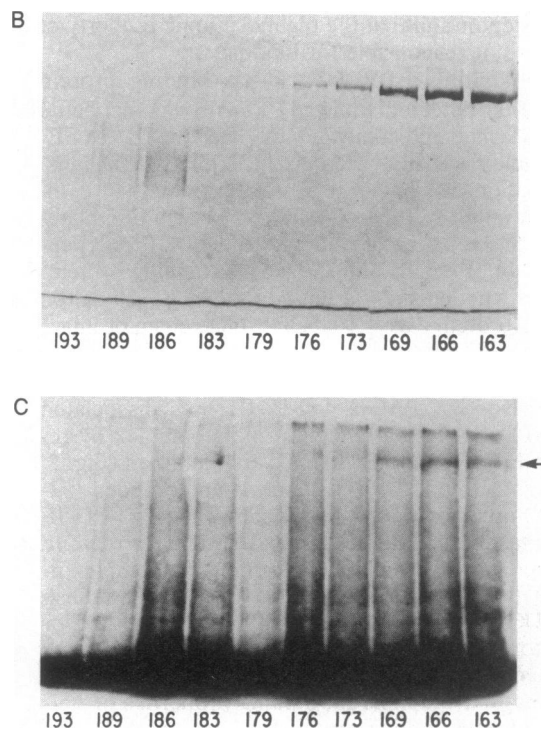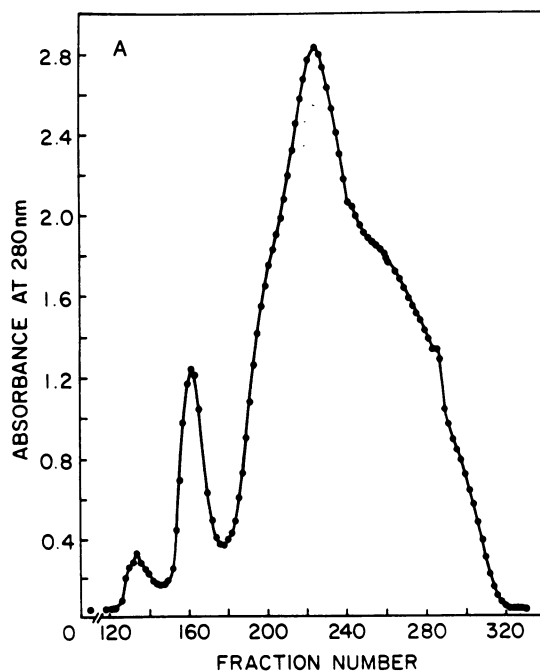

FIG. 3.   (A) Chromatographic profile of polyethylene glycol precipitates of human plasma on Ultrogel AcA34/Ultrogel AcA22. Human plasma (88 ml) was precipitated at 5.5–12.5% (wt/vol) polyethylene glycol as described by Barrett (23). The precipitates were dissolved in 20 ml of 0.1 M sodium citrate (pH 6.0) and then applied onto a column (5.0 × 72 cm) of Ultrogel AcA34/Ultrogel AcA22, 2:1 (vol/vol), and eluted with the same buffer. The flow rate and fractional volume were 20 ml/hr and 3 ml, respectively. The second protein peak from fractions 155–170 was identified as α_2M by trypsin assay and by immunodiffusion. The purity of α_2M obtained from the main fractions of the second protein peak is >95%. The α_2M obtained only showed the slow form after electrophoresis in pore-limiting polyacrylamide gels. (B and C) NaDodSO$_4$/poly-acrylamide gel Coomassie brilliant blue staining patterns (B) and autoradiographs (C) of the fractions from 2:1 Ultrogel AcA34/Ultrogel AcA22 column chromatography after reaction with ^{125}I-PDGF. Each fraction (100 μl from fraction 163 to fraction 193) was incubated with 100 ng of ^{125}I-PDGF. After incubation at room temperature for 30 min, 6 μl of the reaction mixture was analyzed with NaDodSO$_4$/PAGE (5% gel) followed by autoradiography. The arrows shown in (B) and (C) indicate the locations of α_2M and the ^{125}I-PDGF-α_2M complex, respectively. A smaller percentage of ^{125}I-PDGF complexes with α_2M formed at pH 6.0; the optimal pH for complexation of ^{125}I-PDGF to α_2M is 7.4.
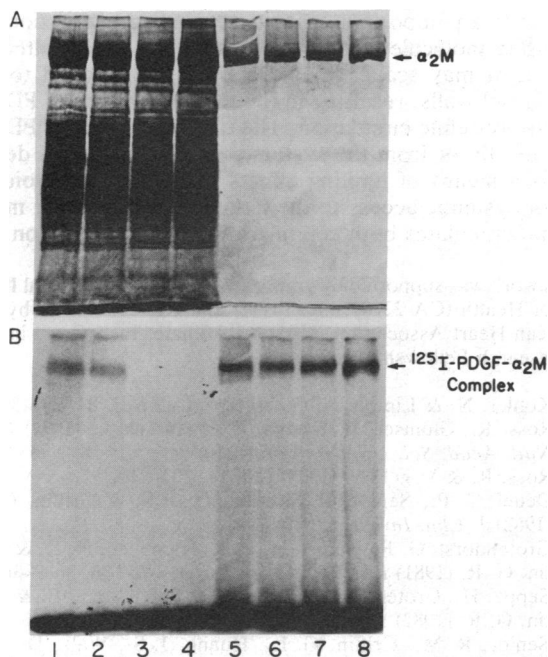
FIG. 4. Coomassie brilliant blue staining pattern (*A*) and autoradiograph (*B*) of ^{125}I-PDGF incubated with human plasma and α_2M on NaDodSO$_4$/polyacrylamide gel (5%). Human plasma (100 μl; lanes 1–4) or α_2M (1 mg/ml; lanes 5–8) in 0.025 M Tris·HCl, pH 8.0/0.1 M NaCl was incubated with 100 ng of ^{125}I-PDGF in the presence or absence of 2 mM *N*-ethylmaleimide or 20 mM CH$_3$NH$_2$. After reaction at room temperature for 30 min, 6 μl of the reaction mixture was analyzed with NaDodSO$_4$/PAGE followed by autoradiography. Lanes: 1 and 2, ^{125}I-PDGF and human plasma; 3 and 4, ^{125}I-PDGF, 2 mM *N*-ethylmaleimide, and human plasma; 5 and 6, ^{125}I-PDGF and α_2M; and 7 and 8, ^{125}I-PDGF, α_2M, and 20 mM CH$_3$NH$_2$.

and precipitated ^{125}I-PDGF-α_2M complex from solution under identical conditions to those used with plasma above (Table 2). The protein precipitated by the anti-α_2M antisera had an identical migration to the plasma ^{125}I-PDGF binding protein in NaDodSO$_4$/PAGE. Although the α_2M content in plasma samples ($\approx$20 μg/10 μl) is about double that of the
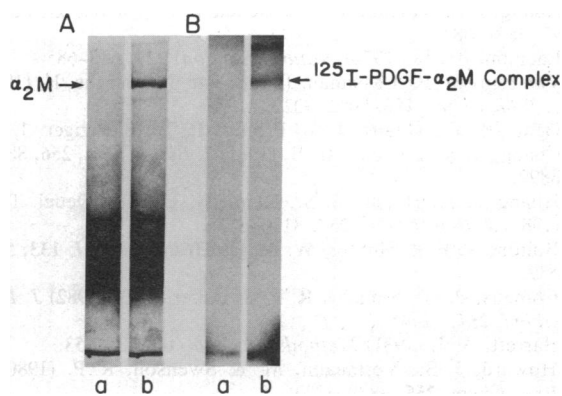

FIG. 5. Coomassie brilliant blue staining pattern (*A*) and autoradiograph (*B*) of the immunoprecipitates of ^{125}I-PDGF-plasma binding protein complex by rabbit anti-α_2M antiserum. The immunoprecipitation of ^{125}I-PDGF-plasma binding protein complex in human plasma by rabbit anti-α_2M antisera was described in Table 2. The immunoprecipitates with nonimmune rabbit serum/IgGsorb (lanes a) and with rabbit specific anti-α_2M antiserum/IgGsorb (lanes b) were analyzed with NaDodSO$_4$/PAGE (5% gel) followed by autoradiography. In addition to the ^{125}I-PDGF-α_2M complex (arrow), some of the ^{125}I-PDGF complex consistently was found at the junction of the stacking gel with the running gel.

Table 2. Immunoprecipitation of human plasma and α_2M with rabbit anti-human α_2M antiserum in the presence of ^{125}I-PDGF

| | Immunoprecipitate, cpm | |
	Anti-human α_2M antiserum	Nonimmune serum
Plasma	5,157 ± 160	677 ± 94
α_2 M	4,982 ± 138	644 ± 122

Human plasma (10 μl) or α_2M solution (100 μg/0.1 ml) in 100 μl of 5 mM Hepes/0.15 M NaCl, pH 7.4, reacted with 100 ng of ^{125}I-PDGF. After 30 min at room temperature, 10 μl of the reaction mixture was incubated with 10 μl of rabbit anti-human α_2M antiserum or rabbit nonimmune serum in 0.3 ml of 10 mM sodium phosphate buffer/0.5 M NaCl/0.1% Tween 80/0.02% sodium azide, pH 7.4, containing human serum albumin (1 mg/ml). After incubation at 4°C overnight, 50 μl of 10% IgGsorb was added and then incubated further at room temperature for 2 hr. The IgGsorb solution was centrifuged and washed three times with 1 ml of the same sodium phosphate buffer. The IgGsorb precipitate was then measured in a gamma counter. Complete precipitation of the ^{125}I-PDGF-α_2M complex with antisera was observed; $\approx$10% of ^{125}I-PDGF was complexed with α_2M in this experiment, as estimated by measurement in NaDodSO$_4$ gels.

sample of purified α_2M (10 μg/10 μl), about equal amounts of ^{125}I-PDGF complexes were immunoprecipitated by the rabbit anti-human α_2M (Table 2); thus, 10 μg of α_2M is fully saturated with respect to the ^{125}I-PDGF added (100 ng of ^{125}I-PDGF per 0.1 ml).

α_2M (dimer) obtained commercially or purified by the procedure of Barrett (23) had an estimated molecular mass of 280,000 daltons in nonreduced NaDodSO$_4$/polyacrylamide gels (5%) without mercaptoethanol. After reduction, the subunit mass of α_2M was $\approx$185,000 daltons. The α_2M dimer, which contains two disulfide-linked subunits ($\approx$185,000 daltons), behaved differently in nonreduced NaDodSO$_4$ gels, with an apparent molecular mass of 280,000 daltons. Based on our observations of ^{125}I-PDGF-α_2M (dimer) at $\approx$310,000 daltons, α_2M dimer at $\approx$280,000 daltons, and ^{125}I-PDGF at $\approx$30,000 daltons, we estimate that in 1 mol of the ^{125}I-PDGF-α_2M (tetramer) complex, 2 mol of ^{125}I-PDGF, and 2 mol of α_2M dimer ($\approx$360,000 daltons) are present; the molecular mass of ^{125}I-PDGF-α_2M (tetramer) is calculated to be 780,000 daltons, which is consistent with that observed with Bio-Gel A-1.5m gel permeation chromatography (16) of the ^{125}I-PDGF-plasma binding protein complex and of the ^{125}I-PDGF-α_2M complex (data not shown).

DISCUSSION

Our attempts to measure PDGF levels in human plasma by radioimmunoassay resulted in the discovery of a plasma binding protein for ^{125}I-PDGF (16). The ^{125}I-PDGF-plasma binding protein complex reacts poorly with specific rabbit anti-human PDGF antisera but retains $\approx$50% of the mitogenic activity. The binding of ^{125}I-PDGF to the plasma binding protein appears to be specific. Only a single species of ^{125}I-PDGF complex ($\approx$310,000 daltons) was found when ^{125}I-PDGF and human plasma were incubated together. Other proteins, including human serum albumin, protamine sulfate, and the epidermal, fibroblast, and nerve growth factors, did not inhibit the formation of ^{125}I-PDGF-plasma binding protein complex; unlabeled PDGF effectively competed for ^{125}I-PDGF binding to the plasma binding protein.

Copurification of the ^{125}I-PDGF plasma binding protein and α_2M (three methods), the formation of a similar complex of ^{125}I-PDGF with purified human α_2M, and comigration of ^{125}I-PDGF-plasma binding protein complex and the ^{125}I-PDGF-α_2M complex in polyacrylamide gels strongly suggest that α_2M is the ^{125}I-PDGF binding protein in human plasma. Additional evidence was provided when specific anti-human α_2M antisera precipitated both the ^{125}I-PDGF-plasma bind-

ing protein complex and ^{125}I-PDGF-α_2M complex; these complexes migrated identically in NaDodSO$_4$ gels.

α_2M is one of the major protease inhibitors in human plasma. The reaction of α_2M with proteases is initiated at the "bait region" of α_2M, where a susceptible peptide bond is cleaved by the attacking enzyme and initiates a rapid conformational change, resulting in the trapping of the enzyme (23). A covalent bond between α_2M and the protease may be formed between an internal thio ester linkage of α_2M and an ε amino group of the trapped protease. The apparent covalent binding of ^{125}I-PDGF to α_2M may be dependent upon a sulfhydryl/disulfide exchange reaction and be distinct from the interactions of proteases and α_2M, based on the following observations: (i) sulfhydryl-blocking reagents (N-ethylmaleimide and iodoacetamide) completely prevent the binding of ^{125}I-PDGF to α_2M and have no effect on the binding of proteases to α_2M (25); (ii) the ^{125}I-PDGF-α_2M complex can be dissociated by 2-mercaptoethanol and dithiothreitol, whereas the covalent complex of α_2M–protease is resistant to these reducing agents; (iii) CH$_3$NH$_2$, an inhibitor of the protease-α_2M covalent binding, does not inhibit ^{125}I-PDGF binding to α_2M; and (iv) no proteolytic activity has been found in preparations of homogeneous PDGF (unpublished results). Further support for the covalent nature of the ^{125}I-PDGF-α_2M linkage was obtained by showing the stability of the complex to the denaturing conditions of 8 M urea, 1 M acetic acid, 0.1 M NaOH, and 1% NaDodSO$_4$ (10 min at 100°C).

Recently, ^{125}I-labeled epidermal growth factor was found to covalently link to its receptors, an observation subsequently explained by the fact that the covalent bond was derived from an artifact dependent upon the iodination of the factor with chloramine T (22). We have prepared ^{125}I-PDGF by the IODO-GEN method (20). IODO-GEN is a water-insoluble compound and chemically similar to chloramine T (26). We never found a covalent complex of ^{125}I-PDGF and its specific cell surface receptor during investigations of ^{125}I-PDGF binding to Swiss mouse 3T3 cells (20). However, to exclude the possibility that the covalent binding property of ^{125}I-PDGF to α_2M is derived from the iodination procedure with IODO-GEN as oxidizing agent, ^{125}I-PDGF prepared with the Bolton–Hunter ^{125}I reagent was used to avoid possible side reactions of oxidation (21). ^{125}I-PDGF prepared by either the IODO-GEN method or by the Bolton–Hunter ^{125}I-reagent formed an identical complex with α_2M, thus establishing that the ^{125}I-PDGF-α_2M complex is not an artifact derived from the iodination with IODO-GEN.

α_2M also forms complexes with several basic proteins (27); PDGF has the pI value of $\approx$10.2. It is possible that α_2M binds PDGF and these basic proteins through the same mechanism. However, a covalent bond has not been found in the complexes of α_2M with these basic proteins (27) and, when these basic proteins were tested at 1 mg/ml, no influence on the covalent binding of ^{125}I-PDGF to α_2M was found (unpublished results). Thus the binding of ^{125}I-PDGF to α_2M appears to be unique, but additional experiments are required for the precise definition of the ^{125}I-PDGF-α_2M complex.

A physiological role of the PDGF-α_2M complex formation has not been established. The striking similarity of the partial amino acid sequence of PDGF and that predicted for p28^{sis} (8, 9), the transforming protein of the simian sarcoma virus, suggests that viral and other cellular transformation events may be mediated by growth factor-like proteins. Thus, α_2M

may serve an important role in regulating expression of PDGF-like molecules into the extracellular space; alternatively, α_2M may scavenge PDGF not locally bound to injured vessel walls, resulting in the rapid clearance of PDGF from the systemic circulation. The rapid clearance of PDGF either locally or from the systemic circulation seems desirable as a means of limiting effects of PDGF and avoiding local or systemic access to the circulation of a potent mitogen that stimulates both cell migration and cell division.

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