Correction. In the article “Specific covalent binding of platelet-derived growth factor to human plasma α2-macroglobulin” by Jung San Huang, Shuan Shian Huang, and Thomas F. Deuel, which appeared in number 2, January 1984, of Proc. Natl. Acad. Sci. USA (81, 342-346), the printer omitted the arrow in Fig. 3B indicating the position of α2-macroglobulin. A correct Fig. 3 is reproduced here.

![Graph](image_url)

**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 x 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 α2M by trypsin assay and by immunodiffusion. The purity of α2M obtained from the main fractions of the second protein peak is >95%. The α2M obtained only showed the slow form after electrophoresis in pore-limiting polyacrylamide gels. (B and C) NaDodSO4/polyacrylamide 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 125I-PDGF. Each fraction (100 µl from fraction 163 to fraction 193) was incubated with 100 ng of 125I-PDGF. After incubation at room temperature for 30 min, 6 µl of the reaction mixture was analyzed with NaDodSO4/PAGE (5% gel) followed by autoradiography. The arrows shown in (B) and (C) indicate the locations of α2M and the 125I-PDGF-α2M complex, respectively. A smaller percentage of 125I-PDGF complexes with α2M formed at pH 6.0; the optimal pH for complexation of 125I-PDGF to α2M is 7.4.

Correction. In the article “Increasing the intracellular Na+ concentration induces differentiation in a pre-B lymphocyte cell line” by Philip M. Rosoff and Lewis C. Cantley, which appeared in number 24, December 1983, of Proc. Natl. Acad. Sci. USA (80, 7547-7550), the following corrections should be noted. On page 7547, the first line of the right-hand column should read “fetal calf serum (GIBCO)/10 µM Na pyruvate/10 mM Hepes.” On page 7548, the second line of the legend to Table 1 should read “... was used at a final concentration of 400 µM...."
Specific covalent binding of platelet-derived growth factor to human plasma \( \alpha_2 \)-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 \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)M) in human plasma, the formation of a complex between \( ^{125} \)I-PDGF and purified \( \alpha_2 \)M, and the comigration of the \( ^{125} \)I-PDGF-plasma binding protein complex and the \( ^{125} \)I-PDGF-\( \alpha_2 \)M complex in NaDodSO\(_4\)/polyacrylamide gel electrophoresis and pore-limiting polyacrylamide gel electrophoresis strongly suggested that \( \alpha_2 \)M is the plasma binding protein for \( ^{125} \)I-PDGF. Immunoprecipitation of \( ^{125} \)I-PDGF-\( \alpha_2 \)M and \( ^{125} \)I-PDGF-plasma binding protein complexes by anti-human \( \alpha_2 \)M antiserum further established that \( \alpha_2 \)M and the plasma binding protein are the same molecule. Approximately 20\% of \( ^{125} \)I-PDGF is complexed by \( \alpha_2 \)M; further \( ^{125} \)I-PDGF is complexed if the remaining \( ^{125} \)I-PDGF is incubated with additional \( \alpha_2 \)M. Complex formation of \( ^{125} \)I-PDGF with plasma or with \( \alpha_2 \)M was completely inhibited by 0.2 mM \( \beta \)-chloromercuric benzoate or 0.2 mM \( N \)-ethylmaleimide. The \( ^{125} \)I-PDG-F-\( \alpha_2 \)M 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 \( \alpha_2 \)M occurs through a disulfide/sulfhydryl exchange reaction. The \( ^{125} \)I-PDGF-\( \alpha_2 \)M complex (780,000 daltons) appears to contain two molecules of \( ^{125} \)I-PDGF and two dimers of \( \alpha_2 \)M. The precise physiological role of the \( ^{125} \)I-PDGF-\( \alpha_2 \)M interaction is unknown. \( \alpha_2 \)M 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\(_{st}\), \( \alpha_2 \)M 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\(_{st}\), suggesting 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 \( \alpha \)-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 \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)M).

MATERIALS AND METHODS Materials. IgG sorb 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/mmol), and [\( ^{3} \)H]thymidine (79.4 Ci/mmol), from New England Nuclear; \( \alpha_2 \)M from human plasma (lot 102F-9360), N-ethylmaleimide, and CH\(_2\)NH\(_2\) from Sigma; and rabbit anti-human \( \alpha_2 \)M antiserum (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 Leerun 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.

Indication of PDGF. Indication of PDGF II with IODOG-GEN was carried out as described (20). \( ^{125} \)I-PDGF (16 \( \mu \)Ci/\( \mu \)g) was stored in 0.1 M acetic acid/0.1% human serum albumin at \(-20^\circ\)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 \( \mu \)Ci/\( \mu \)g.

Complex formation of \( ^{125} \)I-PDGF with human plasma and \( \alpha_2 \)M. \( ^{125} \)I-PDGF (100 ng) was incubated with 10 \( \mu \)l of human plasma or 10 \( \mu \)l of human \( \alpha_2 \)M (1 mg/ml) in 100 \( \mu \)l of 5 mM Heps, pH 7.4/0.15 M NaCl. After incubation at room temperature for 30 min, 5–10 \( \mu \)l of the reaction mixture was immediately mixed with 5 \( \mu \)l of a NaDodSO\(_4\) sample solution.

Abbreviations: \( \alpha_2 \)M, \( \alpha_2 \)-macroglobulin; PDGF, platelet-derived growth factor; PAGE, polyacrylamide gel electrophoresis.

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RESULTS

Previous experiments had shown that 125I-PDGF bound to a plasma protein of ~280,000 daltons and that the 125I-PDGF-plasma binding protein complex was not dissociated by NaDodSO4 with or without other denaturing reagents (16). The specificity of this interaction for PDGF was tested by incubation of plasma with 125I-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 125I-PDGF (100 ng/0.1 ml); at 50 µg/0.1 ml, unlabeled PDGF almost completely blocked the formation of the 125I-PDGF-plasma binding protein complex. Both anionic and cationic proteins were tested for an effect on complex formation. Epidermal and fibroblast growth factors, insulin, transferrin, albumin, and protamine sulfate, each at 1 mg/ml, had no effect on complex formation (data not shown), suggesting that complex formation between 125I-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 125I-PDGF with the plasma binding protein, suggesting that the binding of 125I-PDGF to the plasma binding protein is different from the binding of 125I-PDGF to its cell surface receptor and that the highly cationic property of PDGF alone is not responsible for binding.

The 310,000-dalton 125I-PDGF-plasma binding protein complex was first detected when 125I-PDGF was incubated with human plasma and analyzed by NaDodSO4/PAGE. When the complex was analyzed in reduced NaDodSO4 gels, complex formation was not observed, suggesting that complex formation might result from sulfhydryl/disulfide or di-sulfide exchange of either of the PDGF polypeptide subunit A or B chains and the plasma binding protein. We then tested whether the 125I bound to the 280,000-dalton plasma binding protein was 125I-labeled single chain or intact 125I-PDGF. The 125I-PDGF complex was isolated with Bio-Gel A-1.5m, reduced with 5% 2-mercaptoethanol, and analyzed with NaDodSO4/PAGE. 125I-PDGF was dissociated from the 125I-PDGF-plasma binding protein complex by reduction. Both PDGF A and B chains were identified (Fig. 2; lane 3). In Fig. 2, free 125I-PDGF (lane 1) and reduced 125I-PDGF (lane 2) are shown for comparison. Thus, 125I-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 125I-reagent were compared; each formed an identical complex with the plasma binding protein, suggesting that the inherent binding properties of PDGF to the plasma binding protein was not an artifact of iodination (22). Pretreatment of the 125I-PDGF-plasma binding protein complex with 8 M urea, 0.1 M acetic acid, 0.1 M NaOH, or 1% NaDodSO4 at 100°C for 10 min did not affect the mobility of the complex in NaDodSO4/PAGE, suggesting that a covalent bond linked the 125I-PDGF-plasma binding protein complex.

The 125I-PDGF-plasma binding protein complex was tested for mitogenic activity with 3T3 cells. The 125I-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 125I-PDGF-plasma binding protein complex. Human plasma (100 µl) was treated with 100 ng of 125I-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 NaDodSO4/PAGE (5% gel) and autoradiography without reduction (lanes 1–3) or with reduction (lanes 4–6). The arrow indicates the 125I-PDGF-plasma binding protein complex. The radioactive material at the bottom of each gel is 125I-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 125I-PDGF binding activity in plasma. Purification of the plasma binding protein with ammonium sulfate (30–60% saturation) and with DEAE-Sepharcel 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 125I-PDGF plasma binding activity copurified with α2M in both systems (data not shown). Copurification of the 125I-PDGF-plasma binding protein complex and α2M was then demonstrated by gel permeation chromatography (Ultrigel AcA34/Ultrigel AcA22, 2:1, vol/vol) of a 5–12.5% polyethylene glycol precipitate of human plasma (23). In this system, α2M appears in a symmetrical protein peak with a purity ≥95% (Fig. 3A). The descending limb of the α2M-containing peak shows α2M as the predominant protein (Fig. 3B, Coomassie blue stain), coinciding directly with the

FIG. 2. NaDodSO4/polyacrylamide (15%) gel autoradiographs of 125I-PDGF, reduced 125I-PDGF, and reduced 125I-PDGF-plasma binding protein complex. 125I-PDGF-plasma binding protein complex was obtained from Bio-Gel A-1.5m (0.9 × 52 cm column) after 125I-PDGF was incubated with human plasma. The 125I-PDGF-plasma binding protein complex did not migrate into the separating gel (15%). About 3,000 cpm were applied per lane. Lanes: 1, 125I-PDGF; 2, 125I-PDGF reduced with 5% 2-mercaptoethanol; and 3, 125I-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-$\alpha_2$M complex

<table>
<thead>
<tr>
<th></th>
<th>Mitogenic activity</th>
<th>Immunoreactivity, %</th>
</tr>
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<tbody>
<tr>
<td>$^{125}$I-PDGF</td>
<td>17.4</td>
<td>100</td>
</tr>
<tr>
<td>$^{125}$I-PDGF-plasma binding protein complex</td>
<td>7.2</td>
<td>3</td>
</tr>
<tr>
<td>$^{125}$I-PDGF-$\alpha_2$M complex</td>
<td>6.4</td>
<td>2</td>
</tr>
</tbody>
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$^{125}$I-PDGF-plasma binding protein complex was isolated on a column of Bio-Gel A-1.5m (0.9 x 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 $\alpha_2$M (dimer). $^{125}$I-PDGF-$\alpha_2$M was isolated on a column of Bio-Gel A-1.5m (0.9 x 52 cm) from a mixture of $^{125}$I-PDGF and $\alpha_2$M (1 mg/ml).

*About 85% of $^{125}$I-PDGF was immunoprecipitated with 10 $\mu$L 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 $\alpha_2$M (Fig. 4A and B, lanes 5 and 6), were then compared by electrophoresis in 5% NaDodSO4/polyacrylamide gels and in 5% pore-limiting gels (data not shown). $^{125}$I-PDGF forms a complex with $\alpha_2$M; the $^{125}$I-PDGF-plasma binding protein complex and the $^{125}$I-PDGF-$\alpha_2$M complex comigrated in both electrophoretic systems. The $^{125}$I-PDGF-$\alpha_2$M complex was not dissociated by 1 M acetic acid, 8 M urea, 0.1 M NaOH, or 1% NaDodSO4 (10 min at 100°C). CH3NH2 inhibits the binding of proteases to $\alpha_2$M (24). CH3NH2 was tested to see if it would inhibit complex formation of $^{125}$I-PDGF and $\alpha_2$M; CH3NH2 did not inhibit the formation of the $^{125}$I-PDGF-$\alpha_2$M 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 $\alpha_2$M (data not shown). These results provide additional support that the plasma binding protein/$\alpha_2$M may bind $^{125}$I-PDGF through a disulfide/sulfhydryl exchange reaction.

Rabbit anti-human $\alpha_2$M antisera was used to provide additional evidence that $\alpha_2$M is the $^{125}$I-PDGF plasma binding protein. Plasma samples were incubated with $^{125}$I-PDGF and specific anti-$\alpha_2$M antisera or with nonimmune serum. The immunocomplexes were precipitated with IgG sorb and analyzed by NaDodSO4/PAGE. A protein migrating as $\alpha_2$M 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 NaDodSO4 gels showed the $^{125}$I-PDGF-plasma binding protein complex in the immunoprecipitate from specific anti-$\alpha_2$M 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 $\alpha_2$M (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 x 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 $\alpha_2$M by trypsin assay and by immunodiffusion. The purity of $\alpha_2$M obtained from the main fractions of the second protein peak is >95%. The $\alpha_2$M obtained only showed the slow form after electrophoresis in pore-limiting polyacrylamide gels. (B and C) NaDodSO4/polyacrylamide gel Coomassie brilliant blue staining patterns (B) and autoradiograms (C) of the fractions from 2.1 Ultrogel AcA34/Ultrogel AcA22 column chromatography after reaction with $^{125}$I-PDGF. Each fraction (100 $\mu$L from fraction 163 to fraction 193) was incubated with 100 ng of $^{125}$I-PDGF. After incubation at room temperature for 30 min, 6 $\mu$L of the reaction mixture was analyzed with NaDodSO4/PAGE (5% gel) followed by autoradiography. The arrows shown in (B) and (C) indicate the locations of $\alpha_2$M and the $^{125}$I-PDGF-$\alpha_2$M complex, respectively. A smaller percentage of $^{125}$I-PDGF complexes with $\alpha_2$M formed at pH 6.0; the optimal pH for complexation of $^{125}$I-PDGF to $\alpha_2$M is 7.4.
The immunoprecipitates of plasma by precipitation diograph (B) of $^{125}$I-PDGF incubated with human plasma and $\alpha_2$M on NaDodSO4/polyacrylamide gel (5%). Human plasma (100 μl; lanes 1–4) or $\alpha_2$M (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 by NaDodSO4/PAGE followed by autoradiography. Lanes: and precipitated $^{125}$I-PDGF-$\alpha_2$M complex from solution under identical conditions to those used with plasma above (Table 2). The protein precipitated by the anti-$\alpha_2$M antisera had an identical migration to the plasma $^{125}$I-PDGF binding protein in NaDodSO4/PAGE. Although the $\alpha_2$M content in plasma samples (≈20 μg/10 μl) is about double that of the sample of purified $\alpha_2$M (10 μg/10 μl), about equal amounts of $^{125}$I-PDGF complexes were immunoprecipitated by the rabbit anti-human $\alpha_2$M (Table 2); thus, 10 μg of $\alpha_2$M is fully saturated with respect to the $^{125}$I-PDGF added (100 ng of $^{125}$I-PDGF per 0.1 ml).

Table 2. Immunoprecipitation of human plasma and $\alpha_2$M with rabbit anti-human $\alpha_2$M antisera in the presence of $^{125}$I-PDGF

<table>
<thead>
<tr>
<th>Immunoprecipitate, cpm</th>
<th>Anti-human $\alpha_2$M antisera</th>
<th>Nonimmune serum</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td>5,157 ± 160</td>
<td>677 ± 94</td>
</tr>
<tr>
<td>$\alpha_2$M</td>
<td>4,982 ± 138</td>
<td>644 ± 122</td>
</tr>
</tbody>
</table>

Human plasma (10 μl) or $\alpha_2$M 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 $\alpha_2$M antibody 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, 30 μl of 10% IgGsorb was added and then incubated further at room temperature for 2 hr. The IgGsorb 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-$\alpha_2$M complex with antisera was observed; ≈10% of $^{125}$I-PDGF was complexed with $\alpha_2$M in this experiment, as estimated by measurement in NaDodSO4 gels.

**FIG. 4.** Coomassie brilliant blue staining pattern (A) and autoradiograph (B) of the immunoprecipitates of $^{125}$I-PDGF-plasma binding protein complex by rabbit anti-$\alpha_2$M antisera. The immunoprecipitation of $^{125}$I-PDGF-plasma binding protein complex in human plasma by rabbit anti-$\alpha_2$M antisera was described in Table 2. The immunoprecipitates with nonimmune rabbit serum/IgGsorb (lanes a) and with rabbit specific anti-$\alpha_2$M antisera/IgGsorb (lanes b) were analyzed by NaDodSO4/PAGE (5% gel) followed by autoradiography. In addition to the $^{125}$I-PDGF-$\alpha_2$M complex, another complex was found at the junction of the stacking gel with the running gel.

**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 ≈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 (≈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 $\alpha_2$M (three methods), the formation of a similar complex of $^{125}$I-PDGF with purified human $\alpha_2$M, and comigration of $^{125}$I-PDGF-plasma binding protein complex and the $^{125}$I-PDGF-$\alpha_2$M complex in polyacrylamide gels strongly suggest that $\alpha_2$M is the $^{125}$I-PDGF binding protein in human plasma. Additional evidence was provided when specific anti-human $\alpha_2$M antisera precipitated both the $^{125}$I-PDGF-plasma bind-
ing protein complex and 125I-PDGF-α2M complex; these complexes migrated identically in NaDodSO₄ gels. 

α2M is one of the major protease inhibitors in human plasma. The reaction of α2M with proteases is initiated at the "bait region" of α2M, 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 α2M and the protease may be formed between an internal thio ester linkage of α2M and an ε amino group of the trapped protease. The apparent covalent binding of 125I-PDGF to α2M may be dependent upon a sulfhydryl/disulfide exchange reaction and be distinct from the interactions of proteases and α2M, based on the following observations: (i) sulfhydryl-blocking reagents (N-ethylmaleimide and iodoacetamide) completely prevent the binding of 125I-PDGF to α2M and have no effect on the binding of proteases to α2M (25); (ii) the 125I-PDGF-α2M complex can be dissociated by 2-mercaptoethanol and dithiothreitol, whereas the covalent complex of α2M-protease is resistant to these reducing agents; (iii) CH₂NH₂, an inhibitor of the protease-α2M covalent binding, does not inhibit 125I-PDGF binding to α2M; and (iv) no proteolytic activity has been found in preparations of homogeneous PDGF (unpublished results). Further support for the covalent nature of the 125I-PDGF-α2M 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% NaDodSO₄ (10 min at 100°C).

Recently, 125I-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 125I-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 125I-PDGF and its specific cell surface receptor during investigations of 125I-PDGF binding to Swiss mouse 3T3 cells (20). However, to exclude the possibility that the covalent binding property of 125I-PDGF to α2M is derived from the iodination procedure with IODO-GEN as oxidizing agent, 125I-PDGF prepared with the Bolton–Hunter 125I reagent was used to avoid possible side reactions of oxidation (21). 125I-PDGF prepared by either the IODO-GEN method or by the Bolton–Hunter 125I reagent formed an identical complex with α2M, thus establishing that the 125I-PDGF-α2M complex is not an artifact derived from the iodination with IODO-GEN.

α2M also forms complexes with several basic proteins (27); PDGF has the pI value of pI = 10.2. It is possible that α2M binds PDGF and these basic proteins through the same mechanism. However, a covalent bond has not been found in the complexes of α2M with these basic proteins (27) and, when these basic proteins were tested at 1 mg/ml, no influence on the covalent binding of 125I-PDGF to α2M was found (unpublished results). Thus the binding of 125I-PDGF to α2M appears to be unique, but additional experiments are required for the precise definition of the 125I-PDGF-α2M complex.

A physiological role of the PDGF-α2M complex formation has not been established. The striking similarity of the partial amino acid sequence of PDGF and that predicted for p28 (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, α2M may serve an important role in regulating expression of PDGF-like molecules into the extracellular space; alternatively, α2M 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.

This work was supported by grants awarded by the National Institutes of Health (CA 22409, HL 14147, and HL 22119) and by the American Heart Association, Missouri Affiliate, Inc. (Grant in Aid and Research Fellowship).