Characterization of the Receptor for Platelet-derived Growth Factor on Human Fibroblasts

DEMONSTRATION OF AN INTIMATE RELATIONSHIP WITH A 185,000-DALTON SUBSTRATE FOR THE PLATELET-DERIVED GROWTH FACTOR-STIMULATED KINASE*

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The receptor for platelet-derived growth factor (PDGF) on human foreskin fibroblasts has been characterized. The molecular weight of the PDGF-receptor complex was estimated by affinity labeling techniques to about 200,000, as determined by sodium dodecyl sulfate-gel electrophoresis performed under reducing conditions. Subtraction of the M, of reduced PDGF (18,000 to 15,000) gives a M, for the receptor proper of 185,000 (±10,000). The mobility in sodium dodecyl sulfate-gel electrophoresis was similar whether or not reducing agents were present, suggesting that the receptor may be a single chain protein. The hydrodynamic size of the 125I-PDGF-receptor complex after solubilization with Triton X-100, corresponded to a M, of ~320,000, as determined by gel chromatography. Subtraction of the M, contributions from Triton X-100 and PDGF, respectively, gives a M, of ~200,000 for the receptor itself, an estimate in good agreement with the value obtained from the affinity-labeling experiments. Several lectins were analyzed for their ability to inhibit binding of 125I-PDGF to its receptor. It was found that wheat germ agglutinin and a lectin from Crotalaria juncea were effective inhibitors and that their inhibitory effects could be neutralized by N-acetylglucosamine and galactose, respectively, suggesting that the receptor contains these sugars.

The properties of the receptor were compared with those of a 185,000-Da component, being the major substrate for the membrane-bound PDGF-stimulated kinase. It was found that the 185,000-Da component behaved similar to the PDGF receptor in sodium dodecyl sulfate-gel electrophoresis, performed with or without reducing agents present. Further, the 185,000-Da component co-eluted with the PDGF receptor on a Sepharose 6B column, and had affinity for the same lectins that inhibited the binding of 125I-PDGF to its receptor. Finally, the 185,000-Da component had affinity for PDGF immobilized on Sepharose beads, suggesting that it has PDGF-binding activity.

We conclude that the PDGF receptor and the 185,000-Da substrate for the PDGF-dependent kinase are intimately related and probably identical molecules.

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1 The abbreviations used are: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; phosphate-buffered saline, 137 mM NaCl, 2.7 mM KCl, 8.4 mM NaHPO₄, 1.47 mM KH₂PO₄, pH 7.3; HSAB, N-hydroxysuccinimidyl-4-azido benzate; DSS, disuccinimidyl suberate; SDS, sodium dodecyl sulphate; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; WGA, wheat germ agglutinin; ConA, concanavalin A.

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Affinity Labeling Experiments with DSS—Human foreskin fibroblasts were grown to confluency on 50-mm Petri dishes. After washing once in binding medium, cells were incubated in binding medium at 4 °C for 90 min together with 10 ng/ml of 125I-PDGF (radioabeled as described (12) to a specific activity of 20,000 cpm/ng). Cells were then washed 3 times in binding medium and then incubated at 20 °C for 10 min. Supernatants were then removed, cells were scraped from the dishes as described above, and analyzed by SDS-gel electrophoresis.

Results and Discussion

The affinity of fibroblasts for PDGF was demonstrated in two ways: (i) by co-localization of PDGF with chick embryo fibroblast nuclear membrane markers (26), and (ii) by co-localization of PDGF with A-23187. Furthermore,pdGF receptor characterization

In the course of experiments, we noticed that the PDGF receptor in its autophosphorylated form seemed to be bound to a protein with a molecular weight of 185,000, which was not detected in the unphosphorylated receptor. This protein seems to be closely related to the PDGF receptor, and its presence in the binding medium suggests that the PDGF receptor is associated with other proteins.

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]ATP (3000 Ci/mmol) was purchased from New England Nuclear and carrier-free Na125I from Amersham. DSS was from Pierce Chemical Co., and HSAB was a kind gift from Dr. L. Ragnarsson, Institute of Medical Genetics, Uppsala University. Lectin from Crotalaria juncea (2 mg) was coupled to 1 ml of activated CH-Sepharose (Pharmacia) according to the instructions provided by the manufacturer. PDGF was purified as described by Johnson et al. (11). The estimated purity was >98%, as determined by silver staining of proteins after SDS-gel electrophoresis, except for PDGF used for preparation of the PDGF-Sepharose gel. In the latter case, a PDGF preparation of 90% purity was used. 250 μg of this preparation was coupled to 1 ml of activated CH-Sepharose.

Cells—Human foreskin fibroblasts (cell line AG 1523, obtained from the Human Genetic Cell Repository, Institute for Medical Research, Camden, NJ) were grown as described (12).

Preparation of 125I-HSAB-PDGF—A fresh stock solution of HSAB (100 mM) in dimethyl sulfoxide was diluted in NaCl/Pi, and added at different concentrations (100, 50, 25, or 0 μg/ml) to test tubes containing 2.5 μg each of lyophilized PDGF. The cross-linker was added from light in all steps of the procedure. After incubation for 30 min at 20 °C, 0.25 μCi [125I]T and 10 μl of a solution of chloramine-T (2 mg/ml) were added. The samples were then mixed by pipetting for 1 min at room temperature. The reaction was then stopped by the addition of 25 μl of sodium metabisulphite (2 mg/ml in phosphate-buffered saline). Unreacted 125I was removed by chromatography on Sephadex G-25 in 1 M acetic acid containing 1 mg/ml bovine serum albumin. The resulting specific activities of the different 125I-HSAB-PDGF derivatives were ~20,000 cpm/ng of protein; 125I radioactivity was determined in a gamma counter at 70% efficiency.

Affinity Labeling Experiments with 125I-HSAB-PDGF—Confluent cultures of human fibroblasts in 50-mm Petri dishes (approximately 105 cells) were washed once in binding medium containing buffered saline containing 0.9 mM CaCl2, 0.8 mM MgSO4, and 1 mg/ml bovine serum albumin). Separate cultures were then incubated in 2 ml of binding medium containing 20 ng/ml of radiolabeled PDGF derivatized with HSAB at the different cross-linker concentrations indicated above. As a control for specificity, 125I-HSAB-PDGF was also incubated with cells in the presence of unlabeled PDGF (0.5 μg/ml). Washed cells then were washed 3 times in binding medium and given 2 ml of phosphate-buffered saline and subject to UV irradiation for 10 min. During this time, cell cultures were kept on ice at a 10-cm distance from the lamp (250 watts). In order to avoid unnecessary damage to proteins, the UV light was filtered through a 1 cm-layer of 10% acetone in a glass vessel. Cells were then scraped from the culture dishes with a rubber policeman and collected by centrifugation at 10,000 × g for 30 s. The cell pellets were then solubilized in 80 μl of 1% Triton X-100, 10% glycerol, 20 mM HEPES, pH 7.4, for 15 min at 0 °C, centrifuged at 10,000 × g for 5 min; the supernatants were then analyzed SDS-gel electrophoresis.

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After washing 3 times in binding medium, incubation was continued with [125I]PDGF (10 ng/ml) in this medium for 1 h. Cells were then washed another 3 times with the same medium. Cell-associated radioactivity was solubilized by incubation of the cell cultures with 0.5 ml of 1% Triton X-100, 10% glycerol, 20 mM HEPES, pH 7.4, 1 mg/ml bovine serum albumin for 30 min at 20°C (12), and determined in a γcounter. Using this protocol, about 95% of the binding is specific in the sense that it can be inhibited by an excess of unlabelled PDGF (12).

The ability of certain monosaccharides to neutralize the lectin-induced inhibition of [125I]PDGF binding was investigated in the following way. Cells were first incubated as described above with lectins at 100 μg/ml (WGA, ConA, the lectin from Crotalaria juncea, or no addition). The cultures were then washed 3 times in binding medium and reincubated in 250 μl of this medium at 4°C for 60 min, together with different monosaccharides at 100 mM (α-methylmannoside, α-methylglucoside, N-acetylglucosamine, galactose, or no addition). After washing 3 times in binding medium, the [125I]PDGF-binding capacity of the cells was determined as described above.

To investigate the lectin-binding specificity of the substrates for the PDGF-dependent kinase, the following experiment was performed. Aliquots of human fibroblast membranes (10 μg) were incubated for 10 min at 0°C in 0.5% Triton X-100, 20 mM HEPES, pH 7.4, 5 mM MnCl₂, 1 mg/ml bovine serum albumin in the absence or presence of 75 nM PDGF in a total volume of 40 μl; 15 μM [32P]ATP (containing 2.3 μCi of radioactivity) was then added, and incubation prolonged for another 10 min at 0°C. After the addition of a 1000-fold excess of unlabelled ATP to inhibit further incorporation of [32P]radioactivity, samples were incubated for 30 min at 0°C with immobilized lectins (40 μl of packed Sepharose beads containing WGA, ConA or the lectin from Crotalaria juncea, respectively). At this time, the beads were washed in the centrifuge, 3 times with 0.2% Triton X-100, 10% glycerol, 0.15 M NaCl, 20 mM Tris, pH 7.4, one time with the same buffer containing a higher ionic strength (0.5 M NaCl), and finally one additional time with 20 mM Tris, pH 7.4. The material adsorbed to the beads was then desorbed by incubation for 3 min at 95°C in SDS-sample buffer and analyzed by SDS-gel electrophoresis.

Experiments with PDGF-Sepharose—The interaction between PDGF-Sepharose and the substrates for the PDGF-dependent kinase was investigated in the following manner. Human fibroblast membranes (20 μg) were incubated for 30 min at 0°C in 0.5% Triton X-100, 15% glycerol, 20 mM HEPES, pH 7.4, 75 mM NaCl, 1 mg/ml bovine serum albumin and PDGF-Sepharose (20 μl of packed beads) in a total volume of 75 μl. A parallel sample was treated similarly and incubated with an equal amount of a control gel consisting of an identical gel coupled with ethanolamine. After incubation, the samples were centrifuged at 10,000 × g for 1 min; the supernatants and the pellets were then used separately in the phosphorylation reaction. Aliquots (25 μl) of the supernatants were incubated for 10 min at 0°C with 3 mM MnCl₂ in the presence or absence of 75 nM PDGF; at this time [32P]ATP was added to a final concentration of 15 μM (4.5 μCi of radioactivity), and incubation prolonged for another 10 min. Samples were then analyzed by SDS-gel electrophoresis. The pellet beads were first washed 3 times with 0.2% Triton X-100, 20% glycerol, 50 mM NaCl, 20 mM Tris, pH 7.4, in the centrifuge and then subjected to phosphorylation conditions as described above. The material adsorbed to the beads was then desorbed by incubation for 3 min at 95°C in SDS-sample buffer and analyzed by SDS-gel electrophoresis. The amount of [32P]radioactivity associated with the 185-kDa component was quantified by densitometric scanning of the autoradiogram.

RESULTS

Affinity Labeling of the PDGF Receptor with [125I]HSAB-PDGF—In order to obtain information on the molecular structure of the PDGF receptor on human foreskin fibroblasts, affinity labeling of the receptor was performed. In the first set of experiments, the photocatalyzable cross-linker HSAB was used. PDGF was derivatized with this cross-linker at three different molar ratios and then iodinated. After binding to cells at 4°C for 90 min in the dark, the cell cultures were washed and exposed to UV light for 10 min. Cell-associated radioactivity was then analyzed by SDS-gel electrophoresis. As can be seen in Fig. 1, A–C, under reducing conditions, bands with M, values 190,000 to 210,000 appeared. These were the only high M, components seen, and are likely to represent [125I]PDGF-receptor complexes. The bands were most marked in lane A, corresponding to the highest degree of derivatization of PDGF. The total amount of radioactivity in this molecular weight region was, however, low (<1%); the major part of radioactivity appeared at the position of reduced PDGF, indicating a low efficiency of the cross-linking reaction. In the control, where underderivatized PDGF was used, faint bands with M, values 190,000 to 210,000 were occasionally also observed (Fig. 1, lane D). This suggests that a limited spontaneous covalent cross-linking of [125I]PDGF to its receptor may occur in conjunction with exposure to UV light.

The specificity of the reaction was investigated by adding unlabeled PDGF (500 ng/ml) together with the [125I]-labeled derivatives of PDGF during binding to cells. Under these conditions, the formation of the 190,000 to 210,000 bands was inhibited (Fig. 1). Addition of EGF, insulin, or fibroblast growth factor together with [125I]HSAB-PDGF did not inhibit the formation of the high M, bands (not shown). This supports the notion that the 190,000 to 210,000 bands represent covalent complexes between the PDGF receptor and [125I]HSAB-PDGF. Subtraction of the molecular weight of reduced PDGF

A higher amount of radioactivity appeared in the 200-kDa region in the unreduced gels compared to the reduced gels. The reason for this is not known, but it may be related to the fact that PDGF consists of two disulfide-linked chains; if only one of the chains is cross-linked to the receptor, radioactivity associated with the other is lost upon reduction.
PDGF Receptor Characterization

Fig. 2. Affinity labeling of the PDGF receptor using DSS. $^{125}\text{I}-\text{PDGF}$ was bound to human fibroblasts and the binding stabilized by various concentrations of DSS as described under "Experimental Procedures." Samples were analyzed by SDS-gel electrophoresis and autoradiography.

(15,000 to 18,000), gives a $M_r$ for the PDGF receptor of 175,000–195,000 (see Discussion).

Analysis of the mobility of the $^{125}\text{I}-\text{PDGF}$-receptor complex by SDS-gel electrophoresis in the absence of reducing agents, gave essentially the same result as when dithiothreitol was present (Fig. 1). This suggests that the receptor is a single chain protein.

Affinity Labeling of the PDGF Receptor by use of a Bifunctional Cross-linker—In another set of experiments, a homobifunctional cross-linker was used to covalently stabilize the binding of $^{125}\text{I}-\text{PDGF}$ to its receptor. Cells were first given $^{125}\text{I}-\text{PDGF}$ for 90 min at 4°C, washed, and incubated together with various amounts of DSS for 15 min at room temperature. Cell-associated radioactivity was analyzed by SDS-gel electrophoresis. As can be seen in Fig. 2, at low concentrations of DSS, $^{125}\text{I}-\text{PDGF}$ was cross-linked to form a complex of $M_r$ 200,000 to 220,000. When the DSS concentration was raised, additional complexes of $M_r$ ~400,000 and higher were formed. In analogy with the results of the photoaffinity labeling experiments, the electrophoretical pattern was similar whether or not reducing agents were present during analysis (Fig. 2). Control experiments showed that the formation of high $M_r$, $^{125}\text{I}$-labeled complexes was inhibited if unlabeled PDGF was included during the binding to the cells (not shown).

In conclusion, the experiments with the homobifunctional cross-linker, DSS support the results obtained with the photocatalyzable cross-linker HSAB, i.e. the PDGF-receptor complex has a $M_r$ of ~200,000 and probably consists of one single polypeptide chain.

Analysis of $M_r$ of Kinase Substrates in the Nonreduced State—PDGF has been reported to stimulate phosphorylation in human fibroblast membranes (17). The molecular mass of the major substrates for the PDGF-stimulated kinase has been estimated to 185 and 130 kDa by SDS-gel electrophoresis in the presence of reducing agents (18). The 130-kDa component has been shown to be a proteolytic degradation product of the 185-kDa component. In order to allow a closer molecular comparison of the 185-kDa component and the PDGF receptor, we analyzed the mobility of the 185-kDa component also in SDS-gel electrophoresis without reducing agents present. Fig. 3 shows that the major substrate for the PDGF-stimulated kinase migrated as a 185,000 protein under nonreducing as well as reducing conditions. Thus, the behavior of the 185-kDa component in SDS-gel electrophoresis, with or without reducing agents present, was similar to that expected for the PDGF receptor, as judged from the affinity-labeling experiments (Figs. 1 and 2).

Comparative Analysis of Hydrodynamic Size—Gel chromatography was used to investigate whether the binding of $^{125}\text{I}$-PDGF to its receptor was stable after solubilization of the membranes with Triton X-100. $^{125}\text{I}$-PDGF was first bound to its receptor on intact human fibroblasts at 4°C. Cells were then washed and cell-associated radioactivity solubilized with a buffer containing Triton X-100, and applied to a column of Sepharose 6B eluted with the same buffer. As can be seen in Fig. 4A, after this procedure, the major part of the radioactivity eluted early in the chromatogram in a broad peak at a $K_w$ of about 0.3. In the control, where $^{125}\text{I}$-PDGF was added together with unlabeled PDGF to Triton-solubilized material from the same cells, the major part of the radioactivity eluted later on the column, in two peaks co-eluting with $^{125}\text{I}$-PDGF.
PDGF Receptor Characterization

A

eluted volume (ml)

FIG. 4. Chromatography on Sepharose 6B of \(^{125}\)I-PDGF-receptor complex and membrane kinase substrates after solubilization with Triton X-100. For experimental details, see “Experimental Procedures.” A, human fibroblasts incubated with \(^{125}\)I-PDGF; B, human fibroblast membranes were subjected to standard phosphorylation conditions in the presence of PDGF and run on the column; individual fractions from the chromatogram were analyzed by SDS-gel electrophoresis and the radioactivity in the 185-kDa component quantified.

B

FIG. 5. Inhibition by lectins of binding of \(^{125}\)I-PDGF to human fibroblasts. For experimental details, see “Experimental Procedures.” 100% corresponds to 4200 cpm bound/1.5 \(\times\) \(10^5\) cells. •, WGA; O—O, Crotalaria juncea lectin; ▲—▲, ConA.

\((K_v = 0.7)\) and \(\text{free } ^{125}\text{I} (K_v = 1.0), \) respectively. This indicates that \(^{125}\)I-PDGF forms a high \(M\) complex with its receptor which is stable in the presence of Triton X-100. Calibration of the column using water-soluble standard proteins of known \(M\), revealed that the \(^{125}\)I-PDGF-receptor complex eluted between ferritin \((M_r = 440,000)\) and catalase \((M_r = 232,000), \) at a position corresponding to a \(M,\) of about 320,000. This figure includes, however, the weight of the detergent attached to the receptor. Triton X-100 form micelles of \(M,\) \(~ 90,000,\) Thus, to obtain a \(M,\) for the receptor proper, 90,000 and in addition 30,000 for the PDGF molecule, has to be subtracted from 320,000. This gives a \(M,\) around 200,000 for the receptor, which is in good agreement with the estimate derived from affinity labeling experiments.

Fig. 4B shows an experiment where membranes from human fibroblasts were first solubilized with Triton X-100, and subsequently phosphorylated in the presence of PDGF. The sample was run on the same Sepharose 6B column and aliquots of the effluent fractions analyzed by SDS-gel electrophoresis and autoradiography. The amount of radioactivity associated with the 185-kDa component was then quantified. As can be seen, the elution position of this component was similar to the elution position of the \(^{125}\)I-PDGF-receptor complex.

Inhibition of \(^{125}\)I-PDGF Binding to Fibroblasts by Lectins—To further characterize the PDGF receptor on human fibroblasts, several lectins with different carbohydrate-binding specificities were tested for their ability to compete with \(^{125}\)I-PDGF for binding. Fig. 5 shows that WGA (which binds N-acetylglucosamine residues) was an effective competitor; at 20 \(\mu g/ml\), WGA inhibited 50% of the binding of \(^{125}\)I-PDGF. The lectin from Crotalaria juncea (which binds galactose and N-acetylglactosamine residues) also had some inhibitory effect. Concanavalin A (which binds mannose and glucose residues) showed a limited inhibitory effect (Fig. 5). A small inhibitory effect was also observed with other lectins with similar specificity obtained from Lens culinaris, Pisum sativum, Vicia sativa, and Vicia ervilia (not shown). Lectins from the following sources, with the indicated specificities, were also tested and found not to interfere with binding of \(^{125}\)I-PDGF: Vicia villosa (N-acetylgalactosamine), peanut (galactose), Lotus tetragonolobus (fucose), and Helix pomatia (N-acetylgalactosamine). To minimize the possibility of interactions between \(^{125}\)I-PDGF and the lectins, the binding was performed in two steps; cells were first exposed to lectins, then washed and incubated with \(^{125}\)I-PDGF. Thus, the inhibition was likely to be due to an interaction between the lectin and the receptor.

Fig. 6 shows that the lectin-induced inhibition of \(^{125}\)I-PDGF binding could be neutralized by certain monosaccharides. Thus, N-acetylgalactosamine and galactose neutralized the inhibitions produced by WGA and the lectin from Crotalaria

FIG. 6. Neutralization by monosaccharides of the lectin-induced inhibition of \(^{125}\)I-PDGF binding to human fibroblasts. For experimental details see “Experimental Procedures.” 100% corresponds to 3900 cpm bound/1.5 \(\times\) \(10^5\) cells.
juncea, respectively. The small inhibition caused by ConA was neutralized by α-methylmannoside or α-methylglucoside. Thus, these findings were in concordance with the known specificities for the various lectins. The conclusion from these experiments is that the PDGF receptor contains carbohydrate, probably N-acetylglucosamine residues and galactose residues, in the vicinity of the PDGF-binding region.

Afinity of the Substrates for the PDGF-dependent Kinase for Various Lectins—To investigate whether the lectins which could inhibit 125I-PDGF-binding to its receptor, also bound the 185- and 130-kDa substrates for the PDGF-dependent membrane kinase, the following experiment was performed. Membranes from human fibroblasts were solubilized with Triton X-100 and subjected to the standard phosphorylation assay in the presence or absence of PDGF. Various lectins immobilized on Sepharose beads were then added to the incubations, and the adsorbed radioactivity analyzed by SDS-gel electrophoresis. Fig. 7 shows that the 185-kDa as well as the 130-kDa components were effectively adsorbed to WGA-Sepharose. Immobilized Crotalaria juncea lectin and ConA also bound a small amount of these components. Thus, the lectins which inhibited the binding of 125I-PDGF to its receptor, also recognized the 185- and 130-kDa substrates.

Afinity of the Substrates for PDGF-Sepharose—An important experiment in the evaluation of the hypothesis that the PDGF receptor and the 185-kDa component are the same protein, was to see whether the 185-kDa component had affinity for PDGF. For this purpose, fibroblast membranes were solubilized with Triton X-100 and exposed to PDGF-Sepharose and to a control gel consisting of ethanolamine-Sepharose, respectively. After incubation for 30 min at 0 °C, the unadsorbed fractions were incubated with [32P]ATP in the absence or presence of PDGF. Fig. 8 shows that the exposure of the solubilized membranes to PDGF-Sepharose resulted in a decrease in phosphorylation of the 185- and 130-kDa proteins in the unadsorbed fraction as compared to the solubilized membranes exposed to the control gel. When the corresponding PDGF-Sepharose beads were incubated with [32P]ATP after washing, a significant phosphorylation of the 185- and 130-kDa components was demonstrated (Fig. 8). The recovery of kinase activity on the beads, measured as stimulation of phosphorylation of the 185-kDa component, was estimated to be 10%. When the control beads were subjected to the same treatment, no phosphorylation was seen (Fig. 8). This indicates that the 185-kDa
component and also the PDGF-dependent kinase activity have affinity for PDGF.

**DISCUSSION**

In this paper, we have shown that the PDGF receptor and the 185-kDa phosphoprotein, being the major substrates in the PDGF-stimulated phosphorylation reaction, have several characteristics in common. They have: 1) similar mobilities in SDS-gel electrophoresis with or without dithiothreitol present; 2) similar elution positions on Sepharose 6B after solubilization; and 3) similar lectin-binding specificities. Furthermore, the 185-kDa protein has affinity for PDGF-Sepharose. We conclude that they are closely related and probably identical.

The $M_r$ of the PDGF receptor was estimated by affinity-labeling using two different types of cross-linkers, i.e. photocatalyzable (HSAB) and homobifunctional (DSS). Use of the photocatalyzable cross-linker resulted in the formation of $^{125}$I-HSAB-PDGF-receptor complexes of $M_r$ of ~200,000, as determined by SDS-gel electrophoresis performed under reducing conditions. The homobifunctional cross-linker produced complexes of slightly higher $M_r$, 200,000 to 220,000. Since molecular weights in SDS-gel electrophoresis are most reliably estimated using reduced samples, we considered it most appropriate to estimate the $M_r$ for the PDGF receptor from the SDS-gel electrophoresis run performed in the presence of dithiothreitol. To obtain an $M_r$ for the PDGF receptor proper, the $M_r$ of the PDGF molecule has to be deducted from the $M_r$ of the complex. PDGF has a $M_r$ of 30,000 in its native form, and is composed of two disulphide-linked polypeptide chains of $M_r$ values 18,000 to 15,000. In the experiment where HSAB was used, the cross-linker did not to any appreciable extent covalently link the two chains to each other, since no radioactivity remained in the 30,000 molecular weight region after reduction (not shown). However, DSS caused such a cross-linking of the individual PDGF chains, resulting in the conservation of a 30-kDa form also in the presence of reducing agents (not shown). This may have accounted for the slightly higher $M_r$ obtained for the $^{125}$I-PDGF-receptor complex in the experiment where DSS was used, compared with the experiment where HSAB was used. Taken together, this suggested that one should subtract a $M_r$ of 18,000 to 15,000 for reduced PDGF from an $M_r$ of about 200,000 for the complex, which would give a $M_r$ of ~185,000 ($\pm$10,000) for the PDGF receptor in human fibroblasts. This value is slightly higher than that obtained by Glenn et al. (31) for the PDGF receptor on mouse 3T3 cells (164,000), as estimated from affinity labeling studies using a homobifunctional cross-linker. However, Glenn et al. (31) obtained a similar apparent $M_r$ for the unreduced $^{125}$I-PDGF-receptor complex on 3T3 cells (190,000) as we have obtained for the unreduced complex on human fibroblasts. The major reason for the difference in estimated $M_r$ for the receptor itself (164,000 versus 185,000) is due to the fact that in the studies by Glenn et al. (31) the $M_r$ for native PDGF was subtracted from the apparent $M_r$ of the unreduced complex, whereas we have subtracted the $M_r$ of reduced PDGF from the apparent $M_r$ of the reduced complex.

In the affinity labeling experiments using the photocatalyzable cross-linker, a small amount of $^{125}$I-PDGF was bound to the receptor even in the absence of cross-linker (Fig. 1). Similarly, a spontaneous covalent binding of $^{125}$I-EGF to its receptor has been observed (32, 33). This feature of $^{125}$I-EGF has been attributed to chemical modification of the EGF molecule in the course of iodination with the chloramine-T method (34). Since a similar procedure was used for radiolabeling of PDGF in the present studies, the spontaneous attachment of PDGF to its receptor, although less pronounced and also noticed only after UV irradiation, might be due to similar modifications of the PDGF molecule. The reason for the appearance of three distinct components in the $M_r$ region 190,000 to 210,000 after affinity labeling with the photocatalyzable cross-linker, is not known; possibly it might indicate proteolysis or damage to the receptor by UV irradiation.

The appearance of components with $M_r$ 400,000 and higher after affinity-labeling with homobifunctional cross-linkers at high concentrations (Fig. 2), probably reflects the formation of PDGF receptor dimers and multimers, or the cross-linking of the PDGF receptor with the absorbed protein. Higher molecular weight components were not observed when the more specific photocatalyzable cross-linker, HSAB, was used (Fig. 1).

The finding that the 185-kDa substrate for the PDGF-dependent kinase has affinity for PDGF-Sepharose, suggests that the PDGF receptor and the 185-kDa component are the same protein. Other interpretations are, however, also possible; e.g. the 185-kDa component and the PDGF receptor may be distinct molecules, but linked to each other by noncovalent bonds, or, the 185-kDa component may have a nonspecific affinity for PDGF-Sepharose. However, in view of their similarities in $M_r$, single chain state and lectin-binding specificity, we consider it more likely that they are indeed identical proteins. The 130-kDa fragment of the 185-kDa component also bound to PDGF-Sepharose (Fig. 8). This suggests that the PDGF-binding activity, as well as the lectin-binding activity (Fig. 7), is associated with a common part of the molecules. The conclusions above were drawn from experiments where Triton-solubilized membranes were exposed to PDGF-Sepharose, then the adsorbed and unadsorbed fractions were subjected to phosphorylation separately. When human fibroblasts were first phosphorylated in the presence of PDGF and then subjected to PDGF-Sepharose, only a minor part of the radioactivity associated with the 185- and 130-kDa components was adsorbed to the beads (not shown). This is probably due to the fact that the soluble PDGF binds very tightly to these components, and therefore blocks the binding to the immobilized PDGF.

It follows from the experiment with PDGF-Sepharose that the kinase activity also has affinity for this gel. Analogous to the discussion above, this may be taken to indicate that the 185-kDa PDGF receptor molecule itself has kinase activity, or that the kinase activity resides in a different molecule which is associated with the receptor by noncovalent bonds, or, which has some nonspecific affinity for PDGF-Sepharose. Since autophosphorylation is a common feature among kinases, we consider it likely that the PDGF-stimulated phosphorylation of the 185-kDa component represents autophosphorylation of the PDGF-dependent kinase, although definitive proof of this is still lacking. The recovery of activity that we obtained, measured as stimulation of phosphorylation of the 185-kDa component, was 10%. This low recovery might have several explanations. Possibly only a fraction of the PDGF-dependent kinase activity has affinity for PDGF-Sepharose, or, it may be due to inactivation of the PDGF kinase during the experiment. It might also be due to a decreased accessibility of the immobilized kinase for its substrates. The latter explanation is possible also if the phosphorylation of the 185-kDa component represents an autophosphorylation, as suggested above, provided that the autophosphorylation is intramolecular.

Thus, the receptors for three different growth factors, PDGF, EGF, and insulin are associated with tyrosine-specific kinase activity. Interestingly, PDGF (35), as well as EGF and insulin (36), has been shown to also stimulate serine- or threonine-specific kinase activity in intact cells. In view of
the different amino acid specificity, this is likely to be a secondary event. It is possible that some of the substrates for the receptor-associated tyrosine kinases are serine- or threonine-specific kinases, which may be activated by phosphorylation on tyrosine. Studies have been initiated to identify substrates in intact cells for the different tyrosine kinases (20, 23, 37). It is important for the understanding of the mechanism of action of growth factors, to elucidate the function of substrate specificities of these receptor-associated kinases with those of the tyrosine kinases associated with the oncogenes of certain retroviruses. Substrates common to all, or many of these kinases would be particularly interesting in relation to a possible role in control of cell proliferation.

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