Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6756–6760, September 1986
Biochemistry

Purified human platelet-derived growth factor receptor has ligand-stimulated tyrosine kinase activity

(polypeptide growth factor/cell membrane)

SUBAL BISHAYEE*, ALONZO H. ROSS†, RICHARD WOMER*, AND CHARLES D. SCHER*

*Children's Hospital of Philadelphia and Department of Pediatrics, University of Pennsylvania, Philadelphia, PA 19104; and †Wistar Institute, Philadelphia, PA 19104

Communicated by Werner Henle, June 2, 1986

ABSTRACT **The platelet-derived growth factor receptor (PDGF-R), a 180-kDa single-chain polypeptide, was purified from membranes of the human osteogenic sarcoma cell line MG-63. Purification was achieved by treatment of membranes with PDGF and ATP, followed by solubilization with nonionic detergent and successive chromatography on solid-phase anti-phosphotyrosine monoclonal antibody and DEAE-cellulose. The PDGF-R, which was estimated to be 50–80% pure by $NaDodSO_4$/polyacrylamide gel electrophoresis of ^{32}P-labeled preparations, was free of contaminating epidermal growth factor receptor and had no detectable phosphatase activity. It specifically bound ^{125}I-labeled PDGF, a reaction quantified by binding of the ligand–PDGF-R complex to the anti-phosphotyrosine antibody. The purified receptor displayed PDGF-stimulatable tyrosine kinase activity, assayed by autophosphorylation of PDGF-R at tyrosine residues and by phosphorylation of angiotensin II. The K_m for ATP in the autophosphorylation reaction was 7.5 μM. Addition of PDGF did not change the K_m but increased the V_{max} 1.7-fold.**

Platelet-derived growth factor (PDGF) and its analogues initiate cellular replication by binding to a specific high-affinity membrane receptor (PDGF-R) (1–4). The molecular mechanism by which this interaction stimulates cellular growth is not entirely clear. However, PDGF-R appears to be a tyrosine kinase (5–8), and in this respect is similar to certain other growth factor receptors (e.g., the epidermal growth factor receptor, EGF-R) and certain oncogene products (reviewed in ref. 9). It is tempting to hypothesize that PDGF-induced tyrosine kinase activity is essential for mitogenesis. However, it has not been shown that tyrosine kinase activity is intrinsic to the receptor.

Daniel *et al.* (10) have recently purified PDGF-R from mouse 3T3 cells. Purification utilized a solid-phase antibody directed against phosphotyrosine and was based on the finding that PDGF stimulates tyrosine-specific phosphorylation of PDGF-R in intact cells. However, the purified preparation did not have PDGF-stimulatable tyrosine kinase activity. We have modified this technique to purify PDGF-R from membranes of a human osteogenic sarcoma cell line and now show that the purified soluble receptor has tyrosine kinase activity.

MATERIALS AND METHODS

Membrane Phosphorylation. Plasma membranes were prepared from confluent MG-63 human osteogenic sarcoma cells (11) as described (12), using Ca^{2+}- and Mg^{2+}-free buffers supplemented with 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride. The isolated membranes (protein concentration 0.3–0.9 mg/ml) were incubated at 4°C in 20 mM Hepes, pH 7.4/100 μM sodium vanadate/2 mM $MgCl_2$/0.5 mM $MnCl_2$ with PDGF for 20 min. The reaction was initiated by addition of γ-^{32}P-labeled or unlabeled ATP; it was terminated 20–30 min later by addition of EDTA to 5 mM followed by centrifugation. In certain cases, suramin, an anionic heterocyclic compound, was added to dissociate PDGF from the receptor (4). The pellet was solubilized in Nonidet P-40 (NP-40)-containing buffer A [10 mM Hepes, pH 7.4/50 mM NaCl/30 mM sodium pyrophosphate/50 mM NaF/5 mM EDTA/100 μM sodium vanadate/0.5% NP-40/1 mM phenylmethylsulfonyl fluoride/leupeptin (10 μg/ml)] for immunoprecipitation. For large-scale purification, glycerol was added to 10% (vol/vol) and the solution was clarified by centrifugation for 20 min at 4°C at 40,000 rpm in a 50 Ti rotor (Beckman).

Immunopurification. The 2G8 anti-phosphotyrosine monoclonal antibody (13, 14) was purified from ascitic fluid by protein A-Sepharose affinity chromatography or ammonium sulfate precipitation and affinity chromatography using a phosphotyramine-derivatized Sepharose column. The antibody was coupled to CNBr-activated Sepharose (15 mg of antibody per ml of Sepharose) in 10 mM sodium phosphate, pH 7.4/0.15 M NaCl (14). The solubilized membranes in buffer A were stirred with solid-phase antibody for 2 hr at 4°C; the gel was extensively washed with buffer A, followed by buffer B (10 mM Hepes, pH 7.0/30 mM NaCl/1 mM phenylmethylsulfonyl fluoride/0.2% NP-40), and bound material was eluted with buffer B containing 40 mM phenyl phosphate.

DEAE-Cellulose and Wheat Germ Agglutinin (WGA)-Agarose Chromatography. For DEAE-cellulose purification the anti-phosphotyrosine eluate was incubated for 1 hr at 4°C with DE-52 (Whatman) equilibrated with buffer B; PDGF-R was eluted with 10 mM Hepes, pH 7.0/0.2% NP-40/0.25 M NaCl. For purification with WGA, the anti-phosphotyrosine eluate was incubated for 1 hr at 4°C with 50 μl of WGA-agarose (7 mg of protein per ml; Vector Laboratories, Burlingame, CA) equilibrated with buffer C (10 mM Hepes, pH 7.4/50 mM NaCl/0.2% NP-40/10% glycerol/1 mM phenylmethylsulfonyl fluoride). The PDGF-R was eluted with 3 mM chitotriose in buffer C without phenymethylsulfonyl fluoride.

EGF-R Detection. To quantify contamination with EGF-R, purified preparations were incubated (as above) with a solid-phase anti-EGF-R monoclonal antibody (no. 425) directed against the external domain of EGF-R (a gift from M. Das, University of Pennsylvania, Philadelphia and M. Herlyn, Wistar Institute, Philadelphia) and washed. The EGF-R was eluted by heating to 100°C with Laemmli (15) sample buffer containing 1% $NaDodSO_4$ and 5% 2-mercaptoethanol.

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Abbreviations: PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; EGF, epidermal growth factor; EGF-R, EGF receptor; NP-40, Nonidet P-40; WGA, wheat germ agglutinin.

Tyrosine Kinase Activity. To measure autophosphorylation, PDGF-R in 20 μl of 10 mM Hepes, pH 7.4/2 mM $MgCl_2$/0.5 mM $MnCl_2$/0.1% NP-40/5% glycerol was incubated in the presence or absence of PDGF at 20°C for 10 min; [γ-^{32}P]ATP was added and the mixture was incubated for another 10 min. The reaction was terminated by addition of 20 μl of double-strength Laemmli sample buffer and heating at 100°C for 3 min.

To measure phosphorylation of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), PDGF-R was preincubated at 20°C for 10 min with or without PDGF as above; angiotensin II (Sigma) was added to 2 mM, and [γ-^{32}P]ATP, to 100 μM. The reaction was terminated 20 min later by addition of 2 μl of bovine serum albumin (1 mg/ml) and 40 μl of 5% (wt/vol) trichloroacetic acid. After 20 min at 4°C, the samples were centrifuged and 20-μl aliquots of supernatants were spotted onto phosphocellulose squares that were then washed for 15 min in 30% acetic acid, followed by 10% acetic acid and acetone (16, 17). The amount of ^{32}P incorporated into the peptide was determined by scintillation counting; background counts obtained in the absence of the peptide were subtracted.

Phospho Amino Acid Analysis. Membrane-associated ^{32}P-labeled PDGF-R was obtained by treating membranes with PDGF in the presence of [γ-^{32}P]ATP. The ^{32}P-labeled PDGF-R was purified by successive chromatography on solid-phase anti-phosphotyrosine antibody and DEAE-cellulose. The sample was further purified by $NaDodSO_4$/polyacrylamide gel electrophoresis, eluted in water, and lyophilized. The purified unlabeled receptor, prepared as described above, was labeled by treatment with PDGF in the presence of [γ-^{32}P]ATP. The reaction was terminated by addition of bovine serum albumin to 125 μg/ml and perchloric acid to 5% (wt/vol). The precipitate was isolated by centrifugation and washed with acetone.

The labeled PDGF-R samples were hydrolyzed with 6 M HCl for 2 hr at 100°C. The hydrolysate was dried *in vacuo* and redissolved in a marker mixture containing phosphoserine, phosphothreonine, and phosphotyrosine. The phospho amino acids were analyzed on cellulose thin-layer plates by electrophoresis at pH 3.5 in acetic acid/pyridine/H_2O (1:20:379) at 1 kV for 40–60 min (14, 18). For two-dimensional electrophoresis, the plates were dried, rotated 90°, and run at pH 1.9 in acetic acid/88% formic acid/H_2O (78:25:897) at 1 kV for 60 min. The markers were detected by ninhydrin staining, and ^{32}P-labeled amino acids were visualized by autoradiography.

Purification and Iodination of PDGF. PDGF was purified from human platelets (American Red Cross) as described (19). Most experiments were performed with Blue Sepharose (Pharmacia)-purified PDGF (specific activity 100,000 units/mg). Occasional experiments were done with PDGF further purified on Bio-Gel P-60 (in 5 mM glycine, pH 10/0.1 mM EDTA), which had a specific activity of 300,000 units/mg and was 25–50% pure. The concentration of PDGF is presented in terms of nanomoles of active growth factor for all experiments (20). Bio-Gel-purified PDGF was iodinated by the chloramine-T method. An electrophoretically homogenous iodinated preparation was obtained by adsorption to and elution from MG-63 cells (to be described elsewhere). The ^{125}I-labeled PDGF had a specific radioactivity of 50,000–80,000 cpm/ng.

Binding of ^{125}I-Labeled PDGF to the Purified Receptor. Tyrosine-phosphorylated soluble PDGF-R was incubated with ^{125}I-labeled PDGF in 50 μl of 20 mM Hepes, pH 7.4/0.15 M NaCl/100 μM sodium vanadate/bovine serum albumin (2.5 mg/ml)/0.1% NP-40 for 1 hr at 22°C. Then 50 μl of 1:1 (vol/vol) slurry of anti-phosphotyrosine antibody-Sepharose was added and incubation continued for 1 hr. The gel was removed by centrifugation and washed twice, and bound material was eluted with 500 μl of buffer containing 40 mM sodium phenyl phosphate.

RESULTS

PDGF-R Purification. We have found, in agreement with Graves *et al.* (11), that MG-63 cells have 23,000 PDGF binding sites per cell, which have a dissociation constant of 5×10^{-11} M (data not shown). Treatment of MG-63 membranes with PDGF in the presence of [γ-^{32}P]ATP stimulated phosphorylation of a 180-kDa phosphoprotein (the PDGF-R) and other proteins (data not shown). Bound PDGF was removed by treatment with suramin (4), and the PDGF-R was purified by use of a solid-phase anti-phosphotyrosine monoclonal antibody (13, 14). $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography of the affinity-purified preparation revealed prominent phosphorylated proteins of 180, 116, 90, and 29 kDa; phosphorylation of the 180-kDa species, but not the other proteins, increased (approximately 10-fold) when the membranes were treated with PDGF prior to solubilization and affinity chromatography (Fig. 1). Thus the 180-kDa protein has the characteristics previously ascribed to PDGF-R.

The ^{32}P-labeled PDGF-R was further purified by chromatography on either WGA-Sepharose (10) or DEAE-cellulose. Both procedures initially appeared to give a similar degree of purification, with the 180-kDa PDGF-R comprising 70–80% of the labeled protein (Fig. 2). An unlabeled PDGF-R preparation purified by anti-phosphotyrosine and WGA chromatography was iodinated and shown to be 70–80% homogeneous (Fig. 2, lane 4).

Scatchard analysis demonstrated that intact MG-63 cells have 200,000 EGF-R per cell (data not shown). To learn whether the purified PDGF-R preparations were contaminated with EGF-R, a 170-kDa glycosylated tyrosine kinase (21–23), both purified preparations were chromatographed on a solid-phase mouse monoclonal antibody directed against the external domain of human EGF-R (antibody to be described elsewhere). Gel electrophoresis revealed that about 25% of the WGA-purified 180-kDa protein bound to the anti-EGF-R antibody (Fig. 2, lanes 1–3). In contrast, after DEAE-cellulose chromatography the EGF-R was undetectable (lanes 5 and 6). Thus, PDGF-R used in subsequent

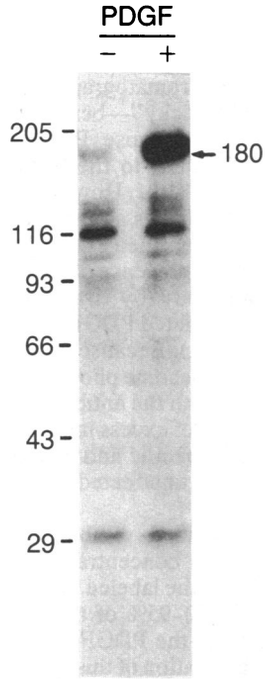

FIG. 1. PDGF stimulates the phosphorylation of the 180-kDa plasma membrane PDGF-R *in vitro*. MG-63 cell membranes (30 μg of protein) were incubated for 15 min at 4°C with or without 10 nM PDGF. [γ-^{32}P]ATP (80 Ci/mmol; 1 Ci = 37 GBq) was then added to a final concentration of 12.5 μM; the reaction was terminated 15 min later, and the tyrosine-phosphorylated proteins were isolated using a solid-phase anti-phosphotyrosine antibody and were analyzed by autoradiography after $NaDodSO_4$/polyacrylamide gel electrophoresis. Markers at left indicate molecular mass (kDa) of standard proteins run in parallel.

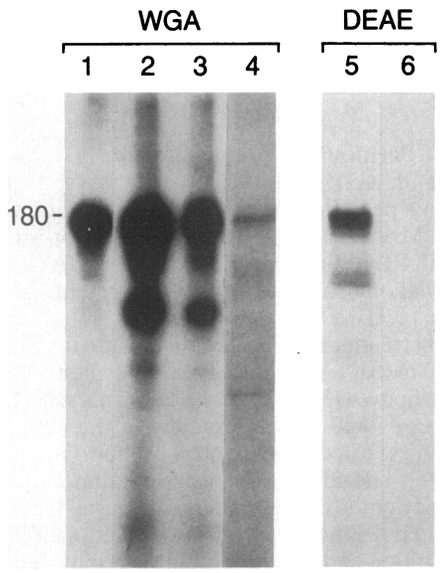

FIG. 2. Purity of PDGF-R. After purification of PDGF-R from PDGF-treated membranes by chromatography on anti-phosphotyrosine-Sepharose, the PDGF-R was further purified by chromatography on WGA-agarose or DEAE-cellulose. (*Left*) WGA-purified ^{32}P-labeled PDGF-R was chromatographed on anti-EGF-R-Sepharose and analyzed by gel electrophoresis. Lane 1: ^{32}P-labeled PDGF-R preparation (10,000 cpm) was incubated with the Sepharose-conjugated antibody, and the gel-bound radioactivity, after dissociation with Laemmli sample buffer, was analyzed. Lane 2: unadsorbed proteins (5000 cpm). Lane 3: input (2500 cpm). Lane 4: ^{125}I-labeled PDGF-R preparation. (*Right*) In a separate experiment, DEAE-cellulose-purified ^{32}P-labeled PDGF-R was chromatographed on the anti-EGF-R-Sepharose. Lane 5: input (2500 cpm). Lane 6: proteins eluted from the immobilized antibody with Laemmli sample buffer. Proteins were visualized by autoradiography.

experiments was highly purified and free of EGF-R. Its isoelectric point of 4.6–4.9 (data not shown) is similar to that found for the mouse PDGF-R (7, 8).

A dose–response analysis revealed that treatment of MG-63 membranes with 6 nM PDGF for 30 min in the presence of 25 μM [γ-^{32}P]ATP stimulated maximal receptor phosphorylation (data not shown). To prepare soluble receptor, membranes were treated with PDGF and unlabeled ATP in a similar fashion to assure maximal PDGF-R phosphorylation, and phosphorylated receptor was purified by anti-phosphotyrosine and DEAE-cellulose chromatography.

PDGF Binding. Because PDGF is "sticky"—being both highly charged and hydrophobic—and because PDGF is relatively large (32 kDa), binding of PDGF to the soluble receptor cannot be quantified readily (10). However, a PDGF-binding assay was developed that takes advantage of the binding of autophosphorylated highly purified PDGF-R to the anti-phosphotyrosine antibody; in the presence of PDGF-R, ^{125}I-labeled PDGF bound to the antibody (by virtue of its tight association with tyrosine-phosphorylated PDGF-R) in a concentration-dependent manner (Table 1). Because no ATP was present, PDGF itself could not have become phosphorylated. Association of ^{125}I-labeled PDGF with the antibody–receptor complex was inhibited by addition of excess unlabeled PDGF, allowing determination of both specific and nonspecific binding. Nonspecific binding was unaffected by the presence of PDGF-R.

Autophosphorylation. The highly purified soluble unlabeled PDGF-R was treated with various concentrations of PDGF in the presence of [γ-^{32}P]ATP and the labeled proteins were analyzed by gel electrophoresis; 70–95% of the label was found at 180 kDa, corresponding to the PDGF-R (data not shown). PDGF stimulated phosphorylation of this species

Table 1. PDGF binds to the highly purified PDGF-R

Membrane treatment	^{125}I-labeled PDGF, nM	Binding, cpm		
		Total	Nonspecific	Specific
PDGF	0.2	619	125	494
	0.5	1414	300	1114
No PDGF	0.2	142	110	32
	0.5	400	350	50

MG-63 membranes were treated with 100 μM ATP in the presence or absence of PDGF (10 nM), and the phosphorylated PDGF-R was purified by chromatography on solid-phase anti-phosphotyrosine antibody and DEAE-cellulose. Each receptor preparation (from 100 μg of membrane protein) was incubated with ^{125}I-labeled PDGF (53,000 cpm/ng); PDGF binding to PDGF-R was determined by the ability of the complex to bind to the anti-phosphotyrosine antibody. Nonspecific binding was determined by addition of 35 nM unlabeled PDGF.

in a dose-dependent fashion that plateaued at 2.5 nM (Fig. 3*A*). There was a >2-fold increase in PDGF-R autophosphorylation over baseline. This value is a minimal estimate of tyrosine phosphorylation, because purification of PDGF-R with the anti-phosphotyrosine antibody was dependent upon phosphorylation of the receptor (see Fig. 1) and, hence, some sites were already phosphorylated. Furthermore, such low ligand-induced phosphorylation has also been noted for the purified EGF-R (17). Phosphorylation of the tyrosine-phosphorylated purified receptor was not a coupled dephosphorylation–phosphorylation reaction, since no phosphatase

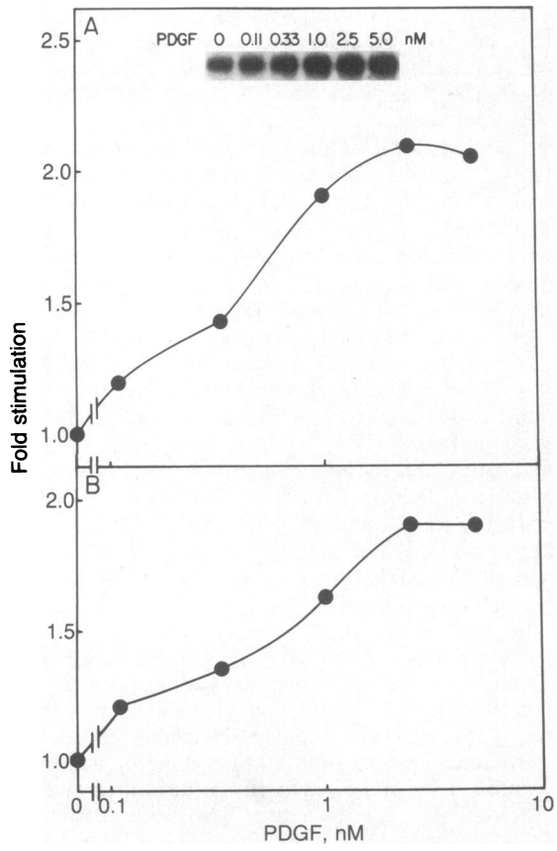

FIG. 3. Autophosphorylation of PDGF-R and phosphorylation of angiotensin II are dependent on PDGF concentration. Soluble PDGF-R was incubated with various concentrations of PDGF and [γ-^{32}P]ATP in absence (*A*) or presence (*B*) of 2 mM angiotensin II. (*A*) Autophosphorylation of PDGF-R was quantified by scanning autoradiograms of the electrophoresed samples (see *Inset*) with a densitometer. (*B*) Phosphorylation of angiotensin II. In the absence of PDGF, 900 fmol of phosphate were incorporated into the peptide.

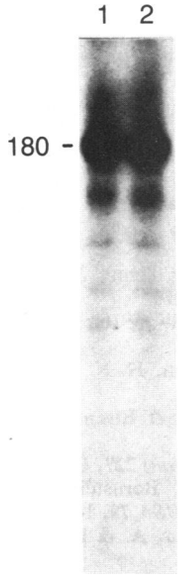

FIG. 4. Lack of phosphatase activity of the purified PDGF-R. Soluble PDGF-R (from 100 μg of membrane protein) was treated with PDGF and [γ-^{32}P]ATP (25 μM; 80 Ci/mmol) for 10 min to phosphorylate the receptor. Unlabeled ATP was added to 1 mM, and the reaction continued for 0 (lane 1) or 60 (lane 2) min. The reaction mixture was diluted into an equal volume of 2× Laemmli sample buffer (15) and heated at 100°C for 3 min, and the products were analyzed by gel electrophoresis.

activity was detectable in these soluble PDGF-R preparations (Fig. 4).

Phosphorylation of Angiotensin II. Incubation of various concentrations of PDGF with the soluble PDGF-R and angiotensin II, which contains tyrosine but not serine or threonine, stimulated phosphorylation of the peptide in a dose-dependent fashion (Fig. 3*B*). This increase plateaued at a level 2-fold greater than baseline, a value similar to that found for EGF-R-stimulated phosphorylation of synthetic peptides (16). PDGF alone had no effect (data not shown). Thus PDGF-treated PDGF-R can phosphorylate other peptides; since angiotensin II contains tyrosine but not serine or threonine, this finding confirms that purified soluble PDGF-R is a tyrosine kinase.

Effect of ATP Concentration. Incubation of the purified PDGF-R with, or without, PDGF and various concentrations of [γ-^{32}P]ATP resulted in the ATP-dependent phosphorylation of the receptor. A double-reciprocal plot of the rate of phosphorylation vs. the ATP concentration was linear, yielding a K_m for ATP of 7.5 μM whether or not PDGF was present (Fig. 5). PDGF activated the kinase by increasing the V_{max} approximately 1.7-fold without affecting the affinity for ATP.

Phospho Amino Acid Analysis of PDGF-R. Cell-membrane-associated PDGF-R was purified after treatment with PDGF and [γ-^{32}P]ATP. Phospho amino acid analysis showed that 40% of the ^{32}P was found in phosphotyrosine, the rest being in phosphoserine and phosphothreonine (data not shown). In contrast, two-dimensional electrophoresis of a hydrolysate of highly purified PDGF-R that had been labeled in solution by treatment with [γ-^{32}P]ATP and PDGF revealed that virtually all of the ^{32}P was in phosphotyrosine (Fig. 6); less than 5% of the ^{32}P was in phosphoserine.

DISCUSSION

The highly purified, soluble PDGF-R has been shown to have PDGF-stimulatable tyrosine kinase activity. PDGF-R phosphorylates itself, angiotensin II (Fig. 3*B*), and a *src*-related peptide (data not shown) in a dose-dependent manner. This tyrosine kinase activity is not due to EGF-R, since the latter can be resolved from PDGF-R by ion-exchange chromatography. An important question is how PDGF-R, a polypeptide isolated from cell membranes by virtue of PDGF-stimulated tyrosine autophosphorylation (a reaction that has been run to near completion), can undergo additional PDGF-stimulated tyrosine autophosphorylation. One possibility is that

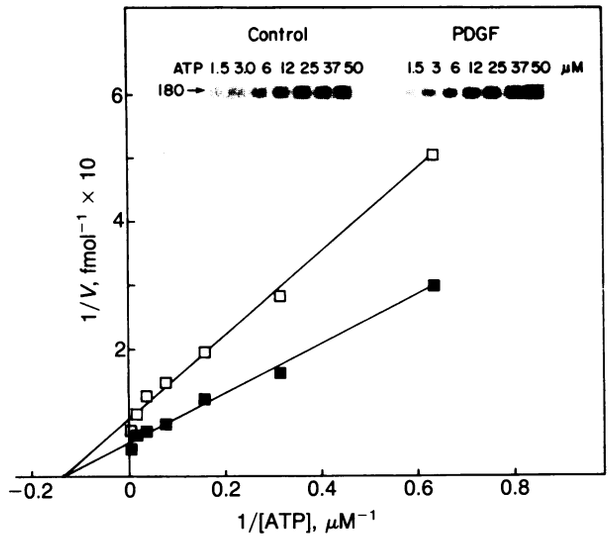

FIG. 5. ATP dependence of autophosphorylation. Purified PDGF-R (from 50 μg of membrane protein) was incubated in the presence (■) or absence (□) of 1.3 nM PDGF for 10 min. Then [γ-^{32}P]ATP (27 μCi/mmol) was added to the final concentration shown and the reaction continued for 5 min. The phosphorylated proteins were analyzed by autoradiography after gel electrophoresis (*Insets*); the extent of receptor phosphorylation was determined by measuring the radioactivity of the 180-kDa protein band in a scintillation counter.

solubilization exposes a cryptic phosphate acceptor site(s) within the PDGF-R.

The soluble PDGF-R has been shown to bind PDGF, a function of its external domain. Such binding has hitherto been difficult to quantify for the soluble receptor. Daniel *et al.* (10) incorporated the soluble PDGF-R within liposomes to show that the purified receptor could bind the ligand. The present study exploited the phosphotyrosines within the soluble PDGF-R to quantify ^{125}I-labeled PDGF binding. The ligand–receptor complex was immobilized by a solid-phase anti-phosphotyrosine monoclonal antibody, thereby allowing quantification of PDGF binding.

Soluble PDGF-R has also been shown to have tyrosine kinase activity, which appears to be a function of its cytoplasmic domain. This activity phosphorylates both exogenous substrates and the receptor itself in an ATP-dependent fashion. The K_m for ATP (7.5 μM) in the autophosphorylation reaction is similar to that of the purified EGF-R (24). However, in contrast to EGF-stimulated EGF-R autophos-

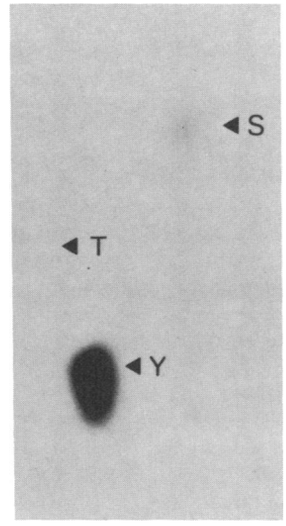

FIG. 6. Highly purified PDGF-R is phosphorylated on tyrosine. After soluble PDGF-R was treated with PDGF and [γ-^{32}P]ATP, it was hydrolyzed and the labeled phospho amino acids were identified after two-dimensional electrophoresis. The positions of marker phosphoserine (S), phosphothreonine (T), and phosphotyrosine (Y) are shown.

phorylation, addition of PDGF to PDGF-R does not affect the K_m for ATP.

The mechanism of autophosphorylation is not clear. It is not known whether autophosphorylation is an intra- or intermolecular event. The soluble EGF-R has recently been shown to have intrapeptide autophosphorylating activity (17). The development of the soluble PDGF-R kinase assay should allow resolution of this problem.

PDGF treatment of membrane PDGF-R stimulates phosphorylation of serine, threonine, and tyrosine, whereas treatment of the highly purified receptor causes tyrosine-specific phosphorylation. Similar findings have been reported for two other growth factor receptors with tyrosine kinase activity, the insulin receptor (25) and the EGF-R (26). Thus, it appears that the phosphorylation on serine and threonine residues of PDGF-R in membranes is due to membrane-associated serine- and threonine-specific protein kinases. However, purified growth factor receptors, including PDGF-R, have intrinsic tyrosine kinase activity.

We thank W. Eberenz, O. M. Jackson, P. Y. Oh, and L. Engle for technical assistance. We thank Drs. M. Herlyn and M. Das for monoclonal antibody to EGF-R and M. Das for both EGF and helpful discussions. This work was supported by the Joseph Stokes, Jr., Research Institute, by American Cancer Society Institutional Research Grant IN-135F (to S.B.), by National Institutes of Health Grant CA25874 (to A.H.R.), by a National Cancer Institute Clinical Investigator Award (to R.W.), and by National Institutes of Health Grants CA34162 and CA38939 (to C.D.S.). In addition, C.D.S. and R.W. were supported by "The Eagles Fly for Leukemia" campaign.

1. Heldin, C. H., Westermark, B. & Wasteson, A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3664–3668.
2. Bowen-Pope, D. F. & Ross, R. (1982) *J. Biol. Chem.* **257,** 5161–5171.
3. Huang, J. S., Huang, S. S., Kennedy, B. & Deuel, T. F. (1982) *J. Biol. Chem.* **257,** 8130–8136.
4. Williams, L. T., Tremble, P. M., Lavin, M. F. & Sunday, M. E. (1984) *J. Biol. Chem.* **259,** 5287–5294.
5. Ek, B., Westermark, B., Wasteson, A. & Heldin, C. H. (1982) *Nature (London)* **295,** 419–420.
6. Nishimura, J., Huang, J. S. & Deuel, T. F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4303–4307.
7. Pike, L. J., Bowen-Pope, D. F., Ross, R. & Krebs, E. G. (1983) *J. Biol. Chem.* **258,** 9383–9390.
8. Frackelton, A. R., Jr., Tremble, P. M. & Williams, L. T. (1984) *J. Biol. Chem.* **259,** 7909–7915.
9. Hunter, T. & Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54,** 897–930.
10. Daniel, T. O., Tremble, P. M., Frackelton, A. R., Jr., & Williams, L. T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2684–2687.
11. Graves, D. T., Antoniades, H. N., Williams, S. R. & Owen, A. J. (1984) *Cancer Res.* **44,** 2966–2970.
12. Thom, D., Powell, A. J., Lloyd, C. W. & Rees, D. A. (1977) *Biochem. J.* **259,** 5287–5294.
13. Ross, A. H., Baltimore, D. & Eisen, H. N. (1981) *Nature (London)* **294,** 654–656.
14. Frackelton, A. R., Jr., Ross, A. H. & Eisen, H. N. (1983) *Mol. Cell. Biol.* **3,** 1343–1352.
15. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
16. Pike, L. J., Gallis, B., Casnellie, J. E., Bornstein, P. & Krebs, E. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1443–1447.
17. Biswas, R., Basu, M., Sen-Majumdar, A. & Das, M. (1985) *Biochemistry* **24,** 3795–3802.
18. Cooper, J. A., Sefton, B. M. & Hunter, T. (1983) *Methods Enzymol.* **99,** 387–402.
19. Scher, C. D., Engle, L. J., Eberenz, W. M., Ganguly, K. & Wharton, W. (1986) *J. Cell. Physiol.* **126,** 333–340.
20. Antoniades, H. N., Scher, C. D. & Stiles, C. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1809–1813.
21. Das, M., Miyakawa, T., Fox, C. F., Pruss, R. M., Aharonov, A. & Herschman, H. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2790–2794.
22. Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255,** 8363–8365.
23. Cohen, S., Fava, R. A. & Sawyer, S. T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6237–6241.
24. Das, M., Knowles, B., Biswas, R. & Bishayee, S. (1984) *Eur. J. Biochem.* **141,** 429–434.
25. White, M. F., Takayama, S. & Kahn, C. R. (1985) *J. Biol. Chem.* **260,** 9470–9478.
26. Rubin, P. A. & Earp, H. S. (1983) *J. Biol. Chem.* **258,** 5177–5182.