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Platelet-derived growth factor stimulates tyrosine-specific protein kinase activity in Swiss mouse 3T3 cell membranes

(phosphotyrosine/cell growth/DNA synthesis)

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ABSTRACT **Platelet-derived growth factor (PDGF) stimulates the incorporation of ^{32}P from [γ-^{32}P]ATP into a M_r ≈170,000 protein by an endogenous tyrosine-specific protein kinase in membrane preparations of Swiss mouse 3T3 cells. Epidermal growth factor (EGF), but not fibroblast growth factor (FGF) or insulin, stimulates limited incorporation of ^{32}P into a protein of similar molecular weight. The ligand concentration required for half-maximal activity ($S_{0.5}$) for PDGF stimulation of phosphorylation is 50 ng/ml; saturation is achieved at 300 ng/ml. The $S_{0.5}$ for ATP is 15 μM. Mg^{2+} or Mn^{2+} is required for protein kinase activity. Stimulation by PDGF results in the preferential phosphorylation of tyrosine residues in this M_r ≈170,000 membrane protein. The M_r ≈170,000 protein can be resolved into M_r ≈180,000 and 160,000 components in 4% NaDodSO$_4$ gels. PDGF stimulates ^{32}P incorporation preferentially into the M_r ≈180,000 and less extensively into the M_r ≈160,000 protein. EGF stimulates ^{32}P incorporation predominantly into a protein of M_r ≈160,000. The similarity of PDGF and EGF in stimulating phosphotyrosine-specific protein kinase activity and the stimulation of a similar activity by viral transformation (*src*) genes suggest that a common mechanism may exist for the phenotypic expression of increased DNA synthesis and cell growth stimulated by these separate factors.**

The mechanisms whereby polypeptide growth factors stimulate target cells to synthesize DNA and to enter cell division remain unresolved. Recently, epidermal growth factor (EGF) was shown to stimulate the phosphorylation of M_r ≈150,000 and M_r ≈170,000 membrane proteins in A431 epidermal carcinoma cells (1, 2). Phosphotyrosine has been identified in hydrolysates of the protein phosphorylated by the membrane protein kinase activity expressed in target cells after exposure to EGF; this protein has been isolated and shown to contain protein kinase activity itself and to be associated with EGF binding activity (3–6). This EGF stimulation of protein kinase activity is of additional interest because of recent findings that the product of the Rous sarcoma transformation gene (*src*) is a protein kinase that also specifically phosphorylates tyrosine residues in target proteins (7–10).

We recently purified the platelet-derived growth factor (PDGF) to apparent homogeneity. Two separate, equally active proteins—PDGF I and PDGF II—were separated and found to be glycoproteins of different carbohydrate composition but with essentially identical amino acid compositions (11, 12). PDGF I and PDGF II bind to Swiss mouse 3T3 cells equally well with an apparent K_d of ≈10^{-9} M, a figure nearly identical with the apparent K_m measured for the PDGF-dependent stimulation of DNA synthesis (ref. 13; unpublished data).

We also recently observed the stimulation of phosphorylation of a cytoplasmic M_r ≈33,000 protein within 3 min after the addition of PDGF to confluent Swiss mouse 3T3 cells (14). Phosphoserine but not phosphotyrosine was observed in hydrolysates of this protein. We now report a PDGF-stimulable, tyrosine-specific protein kinase activity in isolated membranes from Swiss mouse 3T3 cells.

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MATERIALS AND METHODS

Materials. PDGF was purified to apparent homogeneity by established methods (12). EGF and fibroblast growth factor (FGF; pituitary) were obtained from Kor Biochemicals (Cambridge, MA). Bovine insulin was purchased from Sigma. [γ-^{32}P]ATP at 5–10 Ci/mmol (1 Ci = 3.7 × 10^{10} becquerels) was purchased from New England Nuclear. Phosphoserine and phosphothreonine were purchased from Sigma. Phosphotyrosine was synthesized by the method of Mitchell and Lunan (15).

Cell Culture and Membrane Preparation. A plasma membrane-enriched fraction from Swiss mouse 3T3 cells was prepared by a modification of the procedure of Whittenberger and Glaser (16). Cells were grown to confluency in Dulbecco's modified Eagle's medium (GIBCO) containing 10% fetal calf serum, washed with Ca^{2+}- and Mg^{2+}-free Hanks' salt solution buffered with 20 mM Hepes at pH 7.4, incubated for 10 min at 37°C with Ca^{2+}- and Mg^{2+}-free Hanks' solution containing 1 mM EDTA, scraped in cold Ca^{2+}- and Mg^{2+}-free Hanks' solution containing bovine serum albumin at 5 mg/ml, and collected by centrifugation at 150 × *g* for 5 min at 4°C. The cells were suspended in 0.25 M sucrose/10 mM Tris·HCl, pH 7.4/0.2 mM $MgCl_2$/bovine serum albumin at 5 mg/ml (sucrose, Tris·HCl buffer), and disrupted by nitrogen bomb cavitation (800 pounds per square inch; 1 pound per square inch = 6,895 pascals). The disrupted cells were centrifuged at 31,000 × *g* for 20 min, resuspended in sucrose, Tris·HCl buffer, and layered on 25% Ficoll (wt/vol). After centrifugation at 100,000 × *g* for 8 hr, the membrane fraction at the interface was collected, suspended in 10 mM Hepes at pH 7.4, and pelleted by centrifugation at 39,000 × *g* for 20 min. Protein was determined as described by Lowry *et al.* (17). Membranes were stored at −70°C until use.

Membrane Phosphorylation Reaction and NaDodSO$_4$ Gel Electrophoresis. The reaction mixture contained the following materials, unless otherwise indicated: membranes (40 μg of protein), 20 mM Hepes buffer (pH 7.4), 20 mM $MgCl_2$, PDGF (15 ng), and bovine serum albumin (10 μg) in a final volume of 50 μl. The reaction mixtures were preincubated for 30 min at 4°C with PDGF; labeled [γ-^{32}P]ATP (15–30 μM; 5–10 Ci/mmol) was then added (4°C), followed by 10% trichloroacetic

Abbreviations: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; $S_{0.5}$, ligand concentration required for half-maximal activity.

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acid at an appropriate time. The trichloroacetic acid precipitates were washed twice, solubilized in 2% $NaDodSO_4$/2% 2-mercaptoethanol/10% glycerol/0.004% bromophenol blue/60 mM Tris·HCl, pH 6.8, and heated at 100°C for 2 min. Electrophoresis was performed in 7.5% polyacrylamide gels with 0.1% $NaDodSO_4$ (18). NaOH treatment of gels was performed by the method of Cheng and Chen (19) after fixation and Coomassie blue staining. Destained gels were dried and subjected to autoradiography for 1–3 days by using Kodak X-Omat AR film. In other experiments, gels were cut, solubilized in 30% H_2O_2 at 80°C for 5 hr, and assayed directly for radioactivity.

Analysis of Phosphorylated Amino Acids. The M_r ≈170,000 protein was extracted from gels in 0.05 M NH_4HCO_3 containing 0.1% $NaDodSO_4$, as described by Beemon and Hunter (20). Fifty micrograms of carrier bovine serum albumin was added, and the protein was precipitated by 20% trichloroacetic acid at 4°C, washed with ethanol, and successively washed with ethanol/ether (1:1) and suspended in 6 M HCl for partial acid hydrolysis at 100°C for 2 hr in tubes sealed under vacuum. The HCl was removed by evaporation and the hydrolysates were dissolved in a marker mixture containing phosphoserine, phosphothreonine, and phosphotyrosine (1 mg/ml each) and analyzed on cellulose MN 300 (Brinkmann, Westbury, NY) (0.1 mm) thin-layer plates by electrophoresis at pH 1.9 (acetic acid/formic acid/H_2O, 78:25:897) for 60 min at 1.5 kV, followed by electrophoresis at pH 3.5 (acetic acid/pyridine/H_2O, 1:20:379) for 40 min at 1 kV. The markers were detected by staining with ninhydrin.

RESULTS

To establish preliminarily whether PDGF activated a membrane protein kinase, 3T3 cell membranes were preincubated with or without PDGF (300 ng/ml) for 30 min at 4°C and were incubated at 4°C for 10 min with [γ-^{32}P]ATP (25 μM). Fig. 1 shows an autoradiograph (*Left*) with a marked increase in ^{32}P-labeled protein(s) from PDGF-stimulated membranes migrating at M_r ≈170,000. Phosphotyrosine and phosphothreonine are substantially more stable to hydrolysis by strong base than is phosphoserine. Therefore, the gels were incubated with 1 M NaOH for 1 hr at 37°C and analyzed by autoradiography (*Right*).

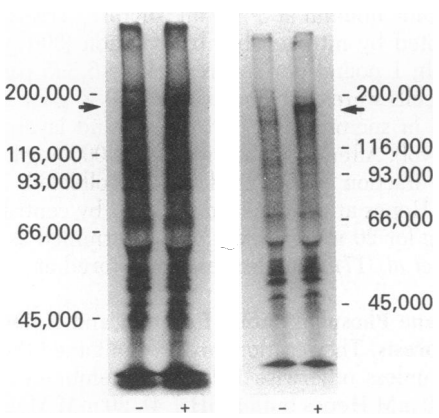

FIG. 1. PDGF-stimulable phosphorylation of Swiss mouse 3T3 cell membrane protein. The phosphorylation assays were performed in the absence (−) and presence (+) of PDGF at 300 ng/ml as described. The phosphorylated samples were subjected to 7.5% $NaDodSO_4$ gel electrophoresis and submitted to autoradiography (*Left*). Other gels were treated by 1 M NaOH for 60 min at 37°C and submitted to autoradiography (*Right*). Molecular weight standards used for calibration of the gels are shown for comparison. The arrows indicate the M_r ≈170,000 protein.

The radiolabeled protein(s) at M_r ≈170,000 are more prominent in comparison with other phosphorylated protein bands after alkaline treatment, suggesting that the M_r ≈170,000 protein may contain [^{32}P]phosphotyrosine or [^{32}P]phosphothreonine. Experiments at temperatures higher than 4°C consistently resulted in diminished apparent ^{32}P incorporation into protein—a result ascribed to hydrolysis of ^{32}P-labeled proteins by endogenous phosphatases.

Fig. 2 shows the time course of the PDGF-stimulable ^{32}P incorporation into the M_r ≈170,000 protein. Membranes preincubated for 30 min at 4°C in the presence or absence of PDGF at 300 ng/ml were incubated with [γ-^{32}P]ATP for the times indicated. Autoradiographs of membrane proteins after $NaDodSO_4$ gel electrophoresis were prepared and the band migrating at M_r ≈170,000 was extracted and radioactivity was measured. An approximately 2.7-fold increase in ^{32}P incorporation at 30 min was found in membranes exposed to PDGF in comparison to that in non-PDGF-exposed membranes. Increased activity over baseline was seen as early as 2 min. At 10 min, a 2.5-fold increase was demonstrated. The relatively reduced ^{32}P incorporation into the M_r ≈170,000 protein(s) at 60 min is likely due to release of incorporated ^{32}P by endogenous membrane phosphatases. The baseline stimulatory activity may derive from carry-over stimulation by serum PDGF in the growth medium or may arise from non-PDGF-stimulable protein kinase activity.

Membranes were exposed to PDGF for different times and incorporation of ^{32}P into the M_r ≈170,000 protein was measured. As shown in Fig. 3, PDGF-stimulable phosphorylation became maximal 20 min after exposure to PDGF and persisted at this level over the next 40 min of incubation.

Fig. 4 shows the dose response of ^{32}P incorporation into the M_r ≈170,000 protein with increasing concentrations of PDGF. Maximal stimulation of ^{32}P incorporation occurs at ≈10^{-8} M PDGF. The ligand concentration required for half-maximal activity ($S_{0.5}$) for PDGF-stimulated ^{32}P incorporation into the M_r ≈170,000 protein is ≈50 ng/ml. These results contrast with other PDGF-dependent responses previously observed. The maximal mitogenic response of intact Swiss mouse 3T3 cells to PDGF is observed at ≈1.3×10^{-9} M; the apparent K_m is ≈0.7

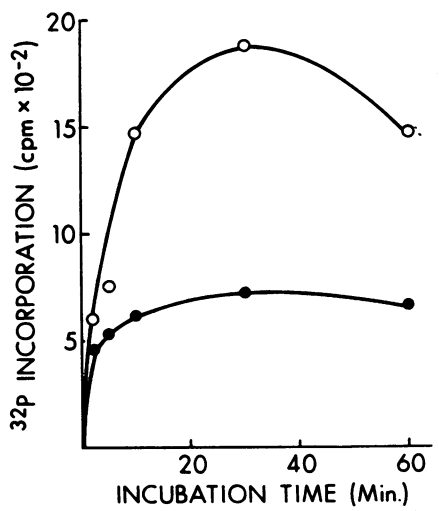

FIG. 2. Time course of ^{32}P incorporation into the M_r ≈170,000 protein. Membranes were incubated in the absence (●) or presence (○) of PDGF at 300 ng/ml. Reactions were terminated at the indicated times after addition of [γ-^{32}P]ATP. The phosphorylated samples were subjected to $NaDodSO_4$ gel electrophoresis and the M_r ≈170,000 protein was cut from gels and radioactivities were measured as described.

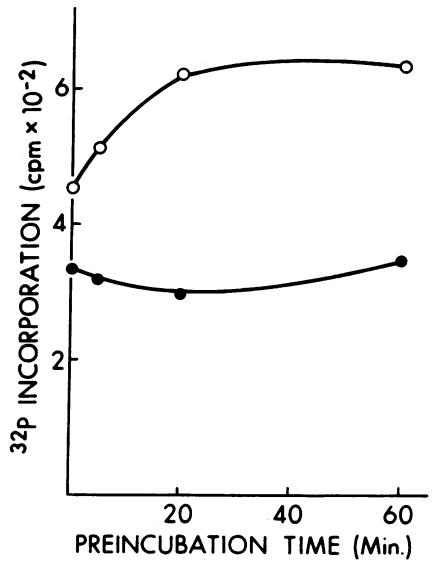

FIG. 3. Effect of preincubation with PDGF on M_r ≈170,000 protein phosphorylation. Membranes were incubated in the absence (●) or presence (○) of PDGF at 4°C for indicated times. Phosphorylation with [γ-^{32}P]ATP was allowed to proceed for 5 min at 4°C.

$\times 10^{-9}$ M (13). Very similar results have been found with direct binding experiments (unpublished data) and with the PDGF-dependent phosphorylation of a cytoplasmic protein found in 3T3 cells (14). Receptor affinity for PDGF may be modified during preparation of 3T3 cell membranes; this has not been directly tested.

Other growth stimulatory proteins were compared with PDGF. Fig. 5 compares the incorporation of ^{32}P into the M_r ≈170,000 protein stimulated by PDGF, EGF, FGF, and insulin—all at 100 nM. Neither FGF nor insulin was effective. EGF stimulated ^{32}P incorporation at ≈50% of the level stimulated by PDGF. Slight differences were consistently noted in the gel patterns of the phosphorylated proteins between PDGF- and EGF-stimulated 3T3 cell membranes.

Fig. 6 shows the dose–response of ATP on ^{32}P incorporation into the M_r ≈170,000 protein by using membranes previously incubated with PDGF. The $S_{0.5}$ of ATP for stimulation of ^{32}P

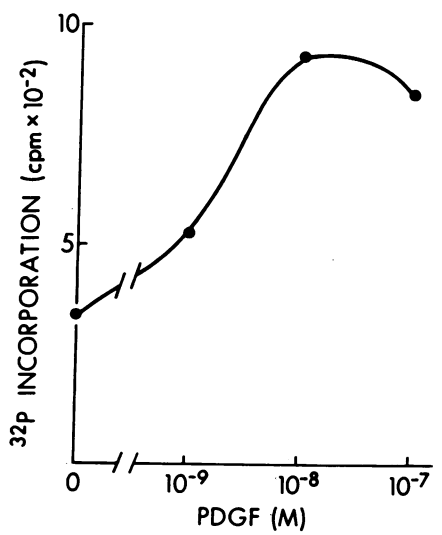

FIG. 4. Effect of PDGF concentration on M_r ≈170,000 protein phosphorylation. Membrane phosphorylation was performed at the PDGF concentrations indicated as described.

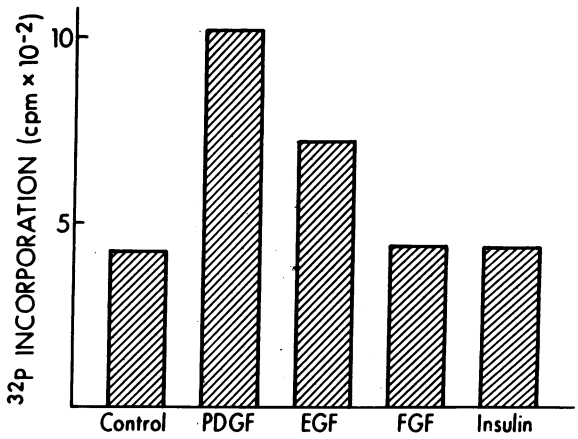

FIG. 5. Effect of other growth factors on M_r ≈170,000 protein phosphorylation. Assays were performed as described for PDGF and growth factors were added in a final concentration of 0.1 μM as indicated.

incorporation is ≈15 μM for PDGF-stimulated and unstimulated membranes.

The effects of divalent cations on the protein kinase activity were then studied. Membranes preincubated with PDGF were subsequently incubated with [γ-^{32}P]ATP and EDTA or divalent cations as shown in Table 1. Essentially no incorporation of ^{32}P was demonstrated when EDTA (1 mM) or Ca^{2+} (2 mM) was present during incubation. Mg^{2+} was strongly stimulatory; maximal stimulation occurred at 20 mM. Mn^{2+} also supported ^{32}P incorporation but to a lesser extent than found with Mg^{2+}. Cyclic AMP and cyclic GMP (0.1 mM) do not influence ^{32}P incorporation into the M_r ≈170,000 protein.

The results of partial base hydrolysis in gels suggested that the PDGF-stimulated incorporation of ^{32}P into the M_r ≈170,000 protein was tyrosine- or threonine-specific. Therefore, direct analysis of ^{32}P-phosphorylated amino acids was attempted in hydrolysates of the labeled protein. Fig. 7 presents results of autoradiographs of the phosphorylated amino acid content separated from these hydrolysates. Both phosphoserine and phosphotyrosine were identified by comparison with standard phosphorylated amino acids. PDGF appears to stimulate incorporation of ^{32}P into both serine and tyrosine residues. Be-

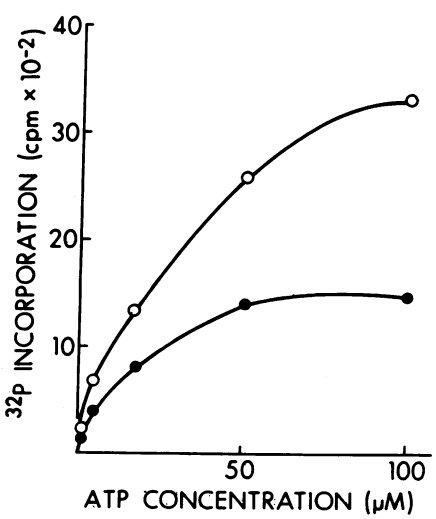

FIG. 6. Effect of [γ-^{32}P]ATP concentration on M_r ≈170,000 protein phosphorylation. The assays were performed in the absence (●) or presence (○) of PDGF as described, except [γ-^{32}P]ATP concentrations were modified as illustrated.

Table 1. Divalent cation requirement for the PDGF-stimulable phosphorylation of the M_r ≈170,000 3T3 cell membrane protein

Addition		^{32}P incorporation, cpm	
Cmpd.	Conc., mM	Without PDGF	With PDGF
EDTA	1	31	7
$MgCl_2$	4	347	676
	20	649	1,247
$MnCl_2$	1	203	723
	5	346	689
$CaCl_2$	2	55	56

cause of the low absolute radioactivity incorporated and because of losses in preparation, quantitation of ^{32}P incorporation into serine and tyrosine residues yielded variable results. Autoradiographs and quantitative tracings of single dimensional gels of hydrolysates yielded consistent results (Fig. 8). Free phosphate and phosphoserine were readily detected in hydrolysates from the M_r ≈170,000 protein from membranes with or without PDGF exposure. Non-PDGF-treated membranes showed essentially no phosphotyrosine, whereas phosphotyrosine was present in substantial quantities in hydrolysates of the M_r ≈170,000 protein from PDGF-treated membranes incubated with [γ-^{32}P]ATP. The absolute increase in tyrosine phosphorylation was greater than that of serine; PDGF preferentially stimulates tyrosine phosphorylation in the M_r ≈170,000 membrane protein. PDGF stimulated phosphorylation of tyrosine 10-fold, whereas PDGF stimulation of phosphorylation of serine was 1.5-fold. This relative increase in phosphotyrosine most likely is considerably greater than 10-fold; phosphotyrosine is substantially more acid labile than is phosphoserine (21), and thus preferentially hydrolyzed in the hydrolysis of the isolated protein.

Because the M_r ≈170,000 phosphoprotein band was broad in autoradiographs of all gels (7.5%) analyzed, further analysis was attempted with PDGF- and EGF-stimulated membranes in 4% gels. The 4% gels separated ^{32}P-containing proteins of M_r ≈180,000 and of M_r ≈160,000 (Fig. 9). PDGF stimulated ^{32}P incorporation preferentially into the M_r ≈180,000 protein and

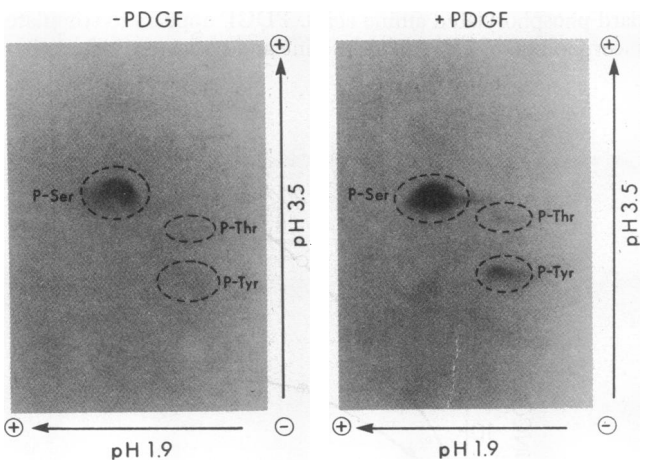

FIG. 7. Analysis of phosphorylated amino acids in the M_r ≈170,000 protein from control and PDGF-stimulated membranes. Four hundred micrograms of membrane protein was phosphorylated with or without PDGF as described and was subjected to 7.5% NaDodSO$_4$ gel electrophoresis. The M_r ≈170,000 protein was extracted and subjected to acid hydrolysis. Two-dimensional electrophoresis was then performed as described and autoradiographs were prepared. Phosphorylated amino acids were identified by comparison with standards stained with ninhydrin. P-Ser, phosphoserine; P-Thr, phosphothreonine; P-Tyr, phosphotyrosine.

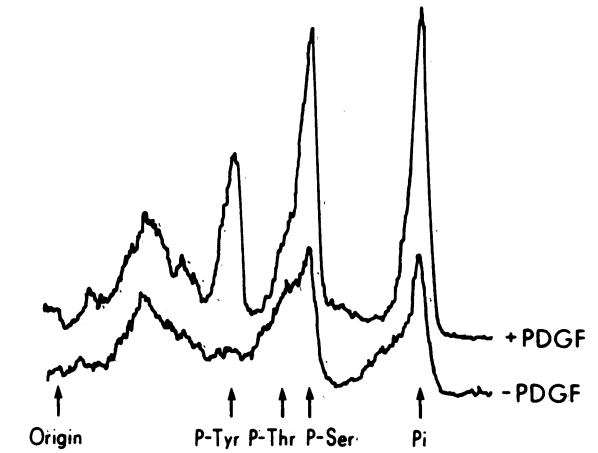

FIG. 8. Electrophoretic separation and densitometric scanning of phosphorylated amino acids. The M_r ≈170,000 protein was extracted and subjected to acid hydrolysis. Electrophoresis was performed at pH 3.5 for 40 min at 1 kV toward anode and the autoradiograph was scanned with an LKB soft laser scanning densitometer.

less extensively into the M_r ≈160,000 protein. EGF preferentially and consistently stimulated a protein of M_r ≈160,000. The relationship of the M_r ≈160,000 proteins stimulated to incorporate ^{32}P by PDGF and EGF is unknown. Initial one-dimensional peptide mapping by limited proteolytic cleavage of these M_r ≈160,000 proteins supports the finding that EGF and PDGF may stimulate different phosphoproteins. Preliminary one-dimensional peptide mapping of the PDGF-stimulated M_r ≈180,000 and M_r ≈160,000 proteins shows ^{32}P-labeled peptides migrating with an identical R_F, suggesting that the M_r ≈160,000 protein may be the proteolytic product of the M_r ≈180,000 protein.

DISCUSSION

Membranes isolated from Swiss mouse 3T3 cells are shown to contain a PDGF-stimulated protein kinase activity that incorporates ^{32}P from [γ-^{32}P]ATP into a M_r ≈170,000 protein. Maximal protein phosphorylation is obtained at PDGF at ≈300 ng/ml; the $S_{0.5}$ for PDGF is ≈50 ng/ml. The reaction is dependent

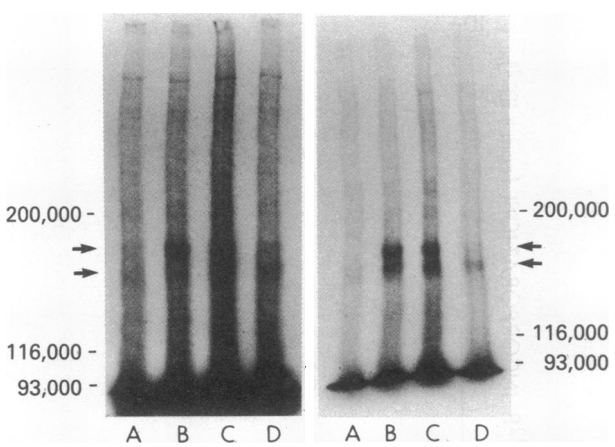

FIG. 9. M_r ≈170,000 protein separation by 4% NaDodSO$_4$ electrophoresis. Forty micrograms of membrane protein was phosphorylated as described and was subjected to 4% NaDodSO$_4$ gel electrophoresis. A, Control; B, PDGF at 300 ng/ml; C, co-electrophoresis of B and D; D, EGF at 600 ng/ml. (*Left*) Untreated gels; (*Right*) gels treated with 1 M NaOH for 1 hr at 37°C. The arrows indicate the M_r ≈180,000 and M_r ≈160,000 proteins.

upon divalent cations; Mg^{2+} is more effective than Mn^{2+}. The PDGF-stimulated ^{32}P incorporation is preferentially into tyrosine residues and to a lesser extent into serine residues. The broad band phosphorylated at $M_r \approx 170,000$ has been separated into a $M_r \approx 180,000$ and a $M_r \approx 160,000$ protein, although the $M_r \approx 160,000$ protein may be the proteolytic cleavage product of the $M_r \approx 180,000$ protein.

EGF and PDGF stimulate tyrosine-specific protein kinases in target cells which subsequently undergo DNA synthesis and cell division (1–4, 6, 22). Tyrosine-specific protein kinases also are found in cells transformed by the viral transformation gene (*src*), suggesting that a common mechanism may be shared for phenotypic expression of the cell growth characteristic of transformed and growth factor-stimulated cells (7, 23–30).

We have previously reported that PDGF stimulates phosphorylation of a $M_r \approx 33,000$ protein in Swiss mouse 3T3 cells (14). This protein phosphorylation was demonstrated in intact cells at 37°C. The PDGF concentration required for protein phosphorylation in intact cells ($S_{0.5}$ = 3 ng/ml) is much lower than is the concentration of PDGF required to stimulate phosphorylation in 3T3 cell membranes ($S_{0.5}$ = 50 ng/ml). The reason for the difference in sensitivity of response to PDGF is not known but reduced receptor affinity for PDGF in membrane preparations seems likely.

$M_r \approx 170,000$ and $\approx 150,000$ proteins are phosphorylated when A-431 human epidermal carcinoma cells are exposed to EGF. These proteins were not observed in two-dimensional gels of proteins obtained from whole cells but were observed when the EGF receptor was precipitated with specific antisera (6). ^{32}P phosphotyrosine was found in hydrolysates of that protein. The EGF-stimulated protein kinase activity is similar to the activity we have observed in 3T3 cell membranes exposed to PDGF. Both phosphorylation reactions require divalent cations, neither reaction requires cyclic nucleotides, both have a similar time course at 0°C, both have a similar apparent K_m for ATP, and both reactions are phosphotyrosine-specific (1, 4, 6). Important differences are found, however. PDGF receptors have no apparent affinity for EGF (unpublished data) and thus, PDGF and EGF appear to operate through different receptors. PDGF binding to A-431 cells is insignificant and membranes prepared from A-431 cells do not incorporate ^{32}P in response to PDGF. Cohen *et al.* (31) recently demonstrated that EGF stimulates phosphorylation of an affinity-purified EGF receptor. $M_r \approx 170,000$ and $M_r \approx 150,000$ components were identified. Our results with 3T3 cell membranes show a very similar pattern of protein phosphorylation stimulated by PDGF, suggesting that the protein phosphorylated by PDGF may be the PDGF receptor also. Preliminary results of crosslinking ^{125}I-labeled PDGF bound to 3T3 cells support the possibility that the $M_r \approx 170,000$ protein phosphorylated in our experiments is the PDGF receptor. However, EGF-stimulated protein phosphorylation is different from that stimulated by PDGF (Fig. 9), consistent with direct binding data showing that EGF does not compete with PDGF for binding to 3T3 cells.

The data presented indicate that PDGF stimulates the tyrosine-specific phosphorylation of a $M_r \approx 170,000$ membrane protein in Swiss mouse 3T3 cells. The function of this protein kinase/phosphoprotein activity is not known but may be related to the initiation of DNA synthesis and cell division characteristic of PDGF-stimulated cells or to receptor function *per se*. A phosphotyrosine-specific protein kinase is the specific product of the Rous sarcoma *src* (transformation) gene. A common important role for tyrosine phosphorylation in cell growth and in DNA synthesis may be shared in growth factor-stimulated and virus-transformed cells.

Note Added in Proof. Ek *et al.* (32) have reported the phosphorylation of tyrosine residues of membrane proteins with apparent M_r of 175,000 and 130,000.

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1. Carpenter, G., King, L., Jr., & Cohen, S. (1979) *J. Biol. Chem.* **254**, 4884–4891.
2. King, L., Jr., Carpenter, G. & Cohen, S. (1980) *Biochemistry* **19**, 1524–1528.
3. Cohen, S., Carpenter, G. & King, L., Jr. (1980) *J. Biol. Chem.* **255**, 4834–4842.
4. Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255**, 8363–8365.
5. Ehrhart, J.-C., Creuzet, C., Rollet, E. & Loeb, J. (1981) *Biochem. Biophys. Res. Commun.* **102**, 602–609.
6. Hunter, T. & Cooper, J. A. (1981) *Cell* **24**, 741–752.
7. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
8. Levinson, A. D., Courtneidge, S. A. & Bishop, J. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1624–1628.
9. Erikson, E. & Erikson, R. L. (1980) *Cell* **21**, 829–836.
10. Sefton, B. M., Hunter, T., Ball, E. B. & Singer, S. J. (1981) *Cell* **24**, 165–174.
11. Deuel, T. F., Huang, J. S., Proffitt, R. T., Chang, D. & Kennedy, B. (1981) *J. Supramol. Struct. Cell. Biochem. Suppl.* **5**, 128 (abstr.).
12. Deuel, T. F., Huang, J. S., Proffitt, R. T., Baenziger, J. U., Chang, D. & Kennedy, B. B. (1981) *J. Biol. Chem.* **256**, 8896–8899.
13. Huang, J. S., Proffitt, R. T., Baenziger, J. U., Chang, D., Kennedy, B. & Deuel, T. F. (1981) in *Differentiation and Function of Hematopoietic Cell Surfaces*, eds. Marchesi, V., Gallo, R. & Majerus, P. (Alan R. Lyss, New York), in press.
14. Nishimura, J. & Deuel, T. F. (1981) *Biochem. Biophys. Res. Commun.* **103**, 355–361.
15. Mitchell, H. K. & Lunan, K. D. (1964) *Arch. Biochem. Biophys.* **106**, 219–222.
16. Whittenberger, G. & Glaser, L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2251–2255.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
18. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
19. Cheng, Y. S. E. & Chen, L. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2388–2392.
20. Beemon, K. & Hunter, T. (1978) *J. Virol.* **28**, 551–566.
21. Plimmer, R. H. A. (1941) *Biochem. J.* **35**, 461–469.
22. Carpenter, G., King, L., Jr., & Cohen, S. (1978) *Nature (London)* **276**, 409–410.
23. Beemon, K. (1981) *Cell* **24**, 145–153.
24. Ghysdael, J., Neil, J. C. & Vogt, P. K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2611–2615.
25. Collett, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2021–2024.
26. Levinson, A. D., Oppermann, H., Levintow, L., Varmus, H. E. & Bishop, J. M. (1978) *Cell* **15**, 561–572.
27. Kawai, S., Yoshida, M., Segawa, K., Sugiyama, H., Ishizaki, R. & Tayoshima, K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6199–6203.
28. Feldman, R., Hanafusa, T. & Hanafusa, H. (1980) *Cell* **22**, 757–765.
29. Neil, J. C., Ghysdael, J. & Vogt, P. K. (1981) *Virology* **109**, 223–228.
30. Ghysdael, J., Neil, J. C., Wallbank, A. M. & Vogt, P. K. (1981) *Virology* **111**, 386–400.
31. Cohen, S., Ushiro, H., Stoscheck, C. & Chinkers, M. (1982) *J. Biol. Chem.* **257**, 1523–1531.
32. Ek, B., Westermark, B., Wasteson, A. & Heldin, C.-H. (1982) *Nature (London)* **295**, 419–420.