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Dimerization of B-type Platelet-derived Growth Factor Receptors Occurs after Ligand Binding and Is Closely Associated with Receptor Kinase Activation*

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Platelet-derived growth factor (PDGF) was found to induce dimerization of purified B-type PDGF receptors, as analyzed by sodium dodecyl sulfate gel electrophoresis after covalent cross-linking using disuccinimidyl suberate. PDGF-BB was 20-fold more effective than PDGF-AB; PDGF-AA was without effect. The dimerization was dose-dependent and was maximal at 0.5–2 μg/ml PDGF-BB; at higher concentrations dimerization was less abundant. This indicates that dimerization occurred when one PDGF-BB molecule bound two receptor molecules. The dimerization correlated to activation of the tyrosine kinase of the receptor, determined as autophosphorylation, but was not dependent on phosphorylation reactions because it occurred also in the absence of ATP. Furthermore, dimerization of the receptor correlated with the ability to phosphorylate phosphofructokinase, an exogenous substrate. The complex of ligand and receptor dimer was stable; it resisted electrophoresis under nondenaturing conditions, as well as gel chromatography.

The present data indicate that intermolecular mechanisms are involved in signal transduction from the external ligand binding domain to the internal effector domains of the B-type PDGF receptor.

Platelet-derived growth factor (PDGF)[1] is a major mitogen in serum for connective tissue-derived cells in culture (for a review see Ref. 1). It is a 30-kDa dimeric molecule composed of disulfide-bonded polypeptide chains (denoted A and B) (2). All three possible isoforms of the two chains, PDGF-AA, PDGF-AB, and PDGF-BB, have been identified and purified from natural sources (3–6). In addition, the transforming activity of simian sarcoma virus has been found to be exerted by a factor structurally similar to PDGF-BB (reviewed in Ref. 7). The different isoforms have been found to differ in functional activities (8), most likely due to different binding specificities to two separate receptor classes (9, 10); the A-type PDGF receptor binds all three isoforms of PDGF, whereas the B-type receptor binds PDGF-BB with high affinity and PDGF-AB with lower affinity but does not bind PDGF-AA with any appreciable affinity.

The B-type PDGF receptor, which transduces a potent mitogenic signal, has been purified and characterized; it is a 170–185-kDa[2] transmembrane glycoprotein with a ligand-activated protein tyrosine kinase activity associated with its cytoplasmic domain (reviewed in Ref. 11). The receptor has been purified from cultured cells (12, 13) and tissues (14), and monoclonal (15, 16) as well as polyclonal (14, 17) antibodies are available. The receptor is synthesized as a 160-kDa precursor which is processed to a mature size of about 180 kDa (15, 17, 18). In addition, cDNA has been cloned for the murine (19) and human (20–22) B-type PDGF receptor. The primary sequence predicts that the external part of the receptor is organized as five immunoglobulin-like domains. The A-type receptor is smaller than the B-type receptor; it is synthesized as a 140-kDa precursor that maturates to a size of 170 kDa (23). The nature of the signals it transduces remains to be elucidated.

The fact that many growth factor receptors and oncogene products are protein tyrosine kinases suggests that tyrosine phosphorylation of specific substrates is important in growth stimulation (reviewed in Ref. 24). It is not clear, however, how binding of a ligand to the external domain of a growth factor receptor induces activation of the kinase associated with the intracellular part of the receptor.

In this paper we show that ligand binding induces dimerization of the B-type PDGF receptor and that dimerization is intimately associated with activation of the receptor kinase.

MATERIALS AND METHODS

PDGF—PDGF was purified from human platelets as described (25). The preparation used in the present work consisted of 70% PDGF-AB and 30% PDGF-BB. PDGF-AB was purified from the PDGF preparation using immobilized metal ion affinity chromatography (4). PDGF-AA and PDGF-BB were purified from supernatants of yeast cells transfected with DNA constructs encoding the short variant of the A chain and the B chain of PDGF, respectively.[3]

PDGF B-type Receptor—PDGF B-type receptor was purified from Triton X-100-solubilized membranes prepared from porcine uterus as described (14). Experiments were performed with receptor preparations that were purified to homogeneity (Figs. 1–3) or with receptor preparations partially purified by chromatographies on wheat germ agglutinin (WGA)-Sepharose and a fast protein liquid chromatography Mono Q column (Figs. 4–8) (14).

Autophosphorylation Assay—The autophosphorylation assay was performed essentially as described (14). Briefly, PDGF B-type receptor, approximately 100 ng, was incubated at 0 °C with various con-

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[1] The abbreviations used are: PDGF, platelet-derived growth factor; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DSS, disuccinimidyl suberate; EGF, epidermal growth factor; WGA, wheat germ agglutinin.

[2] The size of the B-type PDGF receptor differs depending on the source, probably due to differences in glycosylation and to species differences. The receptor purified from porcine uterus that was used in this study is 170 kDa; after autophosphorylation it migrates as 175 kDa in SDS gel electrophoresis (14).

[3] Östman, A., Bäckström, G., Fong, N., Betsholtz, C., Wernstedt, C., Hellman, U., Westermark, B., Valenzuela, P., and Heldin, C.-H. (1989) *Growth Factors*, in press.

centrations of ligands. The incubation mixture had a total volume of 40 μl and contained (final concentrations): 0.1% Triton X-100, 5% glycerol, 0.5 mM EGTA, 0.5 mM dithiothreitol, 20 mM Hepes, pH 7.4, 180 mM NaCl, 3 mM $MnCl_2$, 1 mg/ml bovine serum albumin. After 10 min of incubation, 15 μM [^{32}P]ATP (containing 5×10^6 cpm of radioactivity) was added and incubation continued for 10 min at 0 °C. The incorporation of radioactivity was interrupted by addition of unlabeled ATP and phenylphosphate at 1.5 and 4.0 mM, respectively.

Phosphorylation of Exogenous Substrate—The exokinase activity of the receptor was determined using phosphofructokinase as a substrate. Partially purified receptor was incubated with different concentrations of PDGF-BB for 10 min at 0 °C, using the same conditions as described above for the autophosphorylation assay. Phosphofructokinase (200 μg/ml) was then added together with 15 μM [^{32}P]ATP (containing 10^7 cpm of radioactivity) and the incubation prolonged for another 5 min at 30 °C. The incubation was interrupted as described above.

Cross-linking—Samples were cross-linked by incubation with 0.2 mM DSS for 30 min at room temperature. DSS was dissolved in dimethyl sulfoxide and added at 4% (v/v) final concentration to the samples. The cross-linking reaction was blocked by addition of 50 mM methylammonium chloride, 20 mM Hepes, pH 7.4.

SDS-Gel Electrophoresis—Samples were analyzed by SDS-gel electrophoresis according to Blobel and Dobberstein (26), using 4–12% gradient polyacrylamide gels. The gels were subjected to autoradiography, and the incorporation of radioactivity in the receptor or in phosphokinase was determined as Cerenkov radiation of the corresponding gel pieces after excision from the gel.

Immunoblotting—Mono Q-purified PDGF B-type receptor was incubated with and without PDGF and then cross-linked, or not, with DSS. The samples were then subjected to SDS-gel electrophoresis. The proteins of the gel were electrophoretically blotted to nitrocellulose paper and subjected to immunoblotting using a PDGF B-type receptor antiserum (PDGFR-1), as described (27).

Nondenaturating Electrophoresis—Mono Q-purified receptor was subjected to autophosphorylation as described above, with and without PDGF (2.5 μg/ml) and then cross-linked, or not, with DSS. Samples were then subjected to nondenaturating electrophoresis in a 90 × 60 × 0.6-mm, 4–12% gradient polyacrylamide gel run at 4 °C in 0.1% Triton X-100, 25 mM Tris-HCl, 0.192 M glycine, pH 8.3 (28). The gel was first preelectrophoresed for 30 min at 200 V. Samples were then applied and run for 2.5 h at 200 V; the gel was then dried and subjected to autoradiography.

Gel Chromatography—Mono Q-purified receptor was subjected to the autophosphorylation assay in the absence or presence of PDGF-BB (2.5 μg/ml). In order to remove a major part of [^{32}P]ATP, samples were applied to a 50-μl column of WGA-Sepharose and the column washed with 1 ml of 0.2% Triton X-100, 0.15 M NaCl, 20 mM Hepes, pH 7.4, 10% glycerol, 1 mM EGTA. The PDGF B-type receptor was eluted with 200 μl of 0.3 M *N*-acetylglucosamine in this buffer, applied to a Superose 6 column attached to a fast protein liquid chromatography apparatus, and eluted in 0.2% Triton X-100, 0.15 M NaCl, 10% glycerol, 1 mM dithiothreitol, 20 mM Hepes, pH 7.4. The column was operated at room temperature at a flow rate of 300 μl/min, and 0.3-ml fractions were collected. The ^{32}P radioactivity in each fraction was determined as Cerenkov radiation. The obtained peaks were subjected to cross-linking with DSS and analyzed by SDS-gel electrophoresis and autoradiography after concentration on disposable Amicon filters.

In another experiment, 300 μl of Mono Q-purified receptor was incubated for 30 min at 0 °C in the absence or presence of PDGF-BB (2.5 μg/ml). Samples were then subjected to gel chromatography on the Superose 6 column. Fractions (500 μl each) from the area of the chromatogram where receptor dimers and monomers elute were individually concentrated to about 50 μl and subjected to the autophosphorylation assay in the absence and presence of PDGF-BB (2.5 μg/ml). Samples were then cross-linked with DSS and subjected to SDS-gel electrophoresis and autoradiography.

RESULTS

Ligand Binding Induces Dimerization of the B-type PDGF Receptor—PDGF induces autophosphorylation of B-type PDGF receptor purified from porcine uterus (14). In order to investigate whether receptor activation involves dimerization of receptors, experiments with the homobifunctional cross-linker DSS were performed. Incubation of pure PDGF B-type receptor with [^{32}P]ATP and PDGF led to stimulation of autophosphorylation of the 170-kDa receptor (Fig. 1). Addition of DSS to the incubation mixture after the phosphorylation reaction led to a shift of almost all radioactivity from 170 to about 350 kDa; this shift was not seen for the low amount of background autophosphorylation obtained in the absence of PDGF (Fig. 1). The size of the 350-kDa complex is consistent with the possibility that it consists of two receptors. The lack of resolution in the high M_r region of the SDS gel makes it impossible to determine whether the complex also contains the ligand(s). No indication that ligand binding-induced receptor oligomerization of multimerization was obtained; no components larger than the 350-kDa complex were seen, even when DSS concentrations up to and including 2 mM were used. In particular, no radioactive material was observed on top of the separating gel or on top of the spacer gel (not shown). In conclusion, the data indicate that PDGF induces dimerization of the B-type PDGF receptor.

Next, we investigated the ability of the various isoforms of PDGF to induce dimerization of the B-type PDGF receptor. As shown in Fig. 2, PDGF-BB was 20-fold more efficient than PDGF-AB. The doses of PDGF-BB and PDGF-AB required to induce half-maximal receptor dimerization were similar to those that gave half-maximal receptor autophosphorylation. PDGF-AA at concentrations up to 7.5 μg/ml had no ability on its own to induce receptor dimerization (Fig. 2), nor did it inhibit PDGF-BB-induced receptor dimerization (not shown). These observations indicate that receptor kinase activation is closely associated with dimerization.

Theoretically, the dimerization of PDGF B-type receptors could occur via at least two different mechanisms. On one hand, PDGF binding could induce a conformational change in the receptor that increases its affinity for another receptor

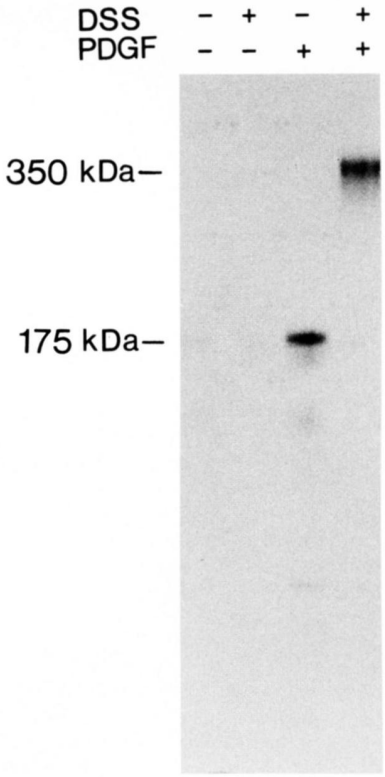

FIG. 1. **Induction of dimerization of the B-type PDGF receptor by PDGF.** Pure receptor was incubated with [^{32}P]ATP in the absence or presence of PDGF and then cross-linked, or not, with DSS. Samples were analyzed by SDS-gel electrophoresis and autoradiography.

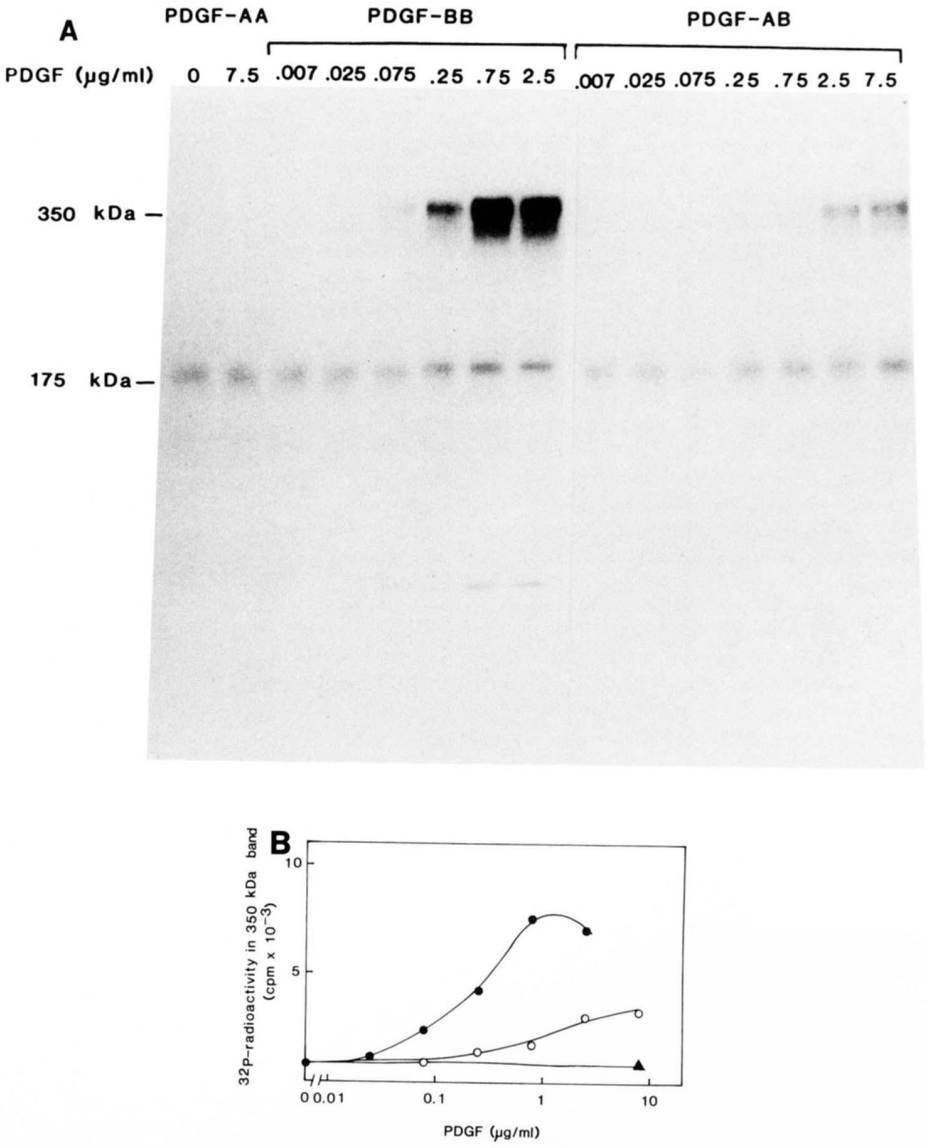

FIG. 2. **Induction of dimerization of the B-type PDGF receptor by different isoforms of PDGF.** Pure receptor was incubated with [^{32}P]ATP in the presence of different concentrations of PDGF-AA, PDGF-AB, or PDGF-BB and then cross-linked with DSS. Samples were then analyzed by SDS-gel electrophoresis and autoradiography (*A*). The 350-kDa bands were also excised from the gel and their ^{32}P radioactivity determined as Cerenkov radiation (*B*). ●, PDGF-BB; ○, PDGF-AB; ▲, PDGF-AA.

or ligand-receptor complex. On the other hand, the dimeric PDGF molecule could simultaneously interact with two receptors, thereby binding them together. If the latter model is correct, one would expect that the dimerization would be maximal at a certain ligand concentration and that very high concentrations of ligand would not induce receptor dimerization. The result of an experiment using very high concentrations of PDGF-BB is consistent with this prediction; about 0.5 μg/ml[4] PDGF-BB gave maximal dimerization, and higher concentrations gave a significantly lower effect both on dimerization and autophosphorylation (Fig. 3).

Correlation between Receptor Dimerization and Stimulation of Receptor Exokinase Activity—Kinetic data indicate that the autophosphorylation of the B-type PDGF receptor occurs via an intramolecular rather than intermolecular mechanism.[5] It is not known, however, whether the receptors in a dimer phosphorylate themselves or each other. Thus, the correlation between receptor dimerization and autophosphorylation (Figs. 1–3) could be due either to an increase in receptor kinase activity or, if the receptor molecules in the dimer phosphorylate each other, to an increased availability of substrate for intermolecular phosphorylation. Therefore, the receptor kinase activity was also measured using an exogenous substrate, phosphofructokinase. As shown in Fig. 4, PDGF-BB induced the phosphorylation of phosphofructokinase in a concentration-dependent manner; the phosphorylation decreased at high PDGF-BB concentrations, and the phosphorylation of the exogenous substrate correlated perfectly to dimerization of the receptor.

Receptor Dimerization Is Not Dependent on Phosphorylation Reactions—The data described in Figs. 1–4 indicate that B-type PDGF receptor dimerization occurs in conjunction with PDGF receptor autophosphorylation. In order to investigate whether the dimerization is dependent on an active receptor kinase and/or receptor autophosphorylation, the following experiment was performed. A preparation of purified receptor was incubated with or without PDGF in the absence of ATP. Samples were then cross-linked with DSS, or not, and analyzed by SDS-gel electrophoresis and immunoblotting using an antiserum that reacts with the receptor (14). As shown in Fig. 5, PDGF induced the formation of a receptor complex of about 350 kDa which was seen after cross-linking with DSS; in the absence of covalent cross-linking, or in the

[4] The concentration of PDGF-BB that gave maximal dimerization varied between 0.5 and 2 μg/ml for different receptor preparations.

[5] L. Rönnstrand, unpublished observation.

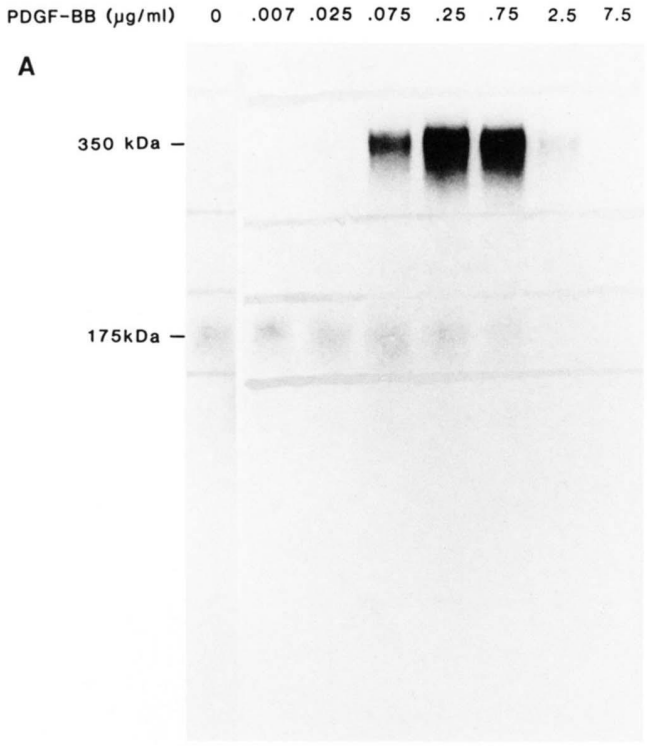

FIG. 3. **Concentration dependence of PDGF-BB-induced receptor dimerization.** Pure B-type PDGF receptor was incubated with [^{32}P]ATP and different concentrations of PDGF-BB. Samples were cross-linked with DSS and then analyzed by SDS-gel electrophoresis and autoradiography (*A*). The ^{32}P radioactivity in the 350-kDa bands were also determined as Cerenkov radiation after excision from the gel (*B*).

absence of PDGF, the receptor was detected, as expected, at 170 kDa.

Thus, the conclusion of this experiment is that receptor dimerization occurs directly after ligand binding and is not dependent on autophosphorylation of the receptor.

The Receptor Dimers Do Not Dissociate during Electrophoresis under Nondenaturating Conditions—The complex of ligand and receptor dimers dissociates in SDS-gel electrophoresis (Figs. 1–5). To investigate the stability of the complex, we subjected it to nondenaturating electrophoresis in the absence of SDS. As shown in Fig. 6, PDGF induced a slower migration of a portion of the autophosphorylated receptor that was not seen when the receptor was incubated in the absence of PDGF. An even larger proportion of slower migrating species was found when DSS was used to covalently stabilize the complex. Although this electrophoresis system

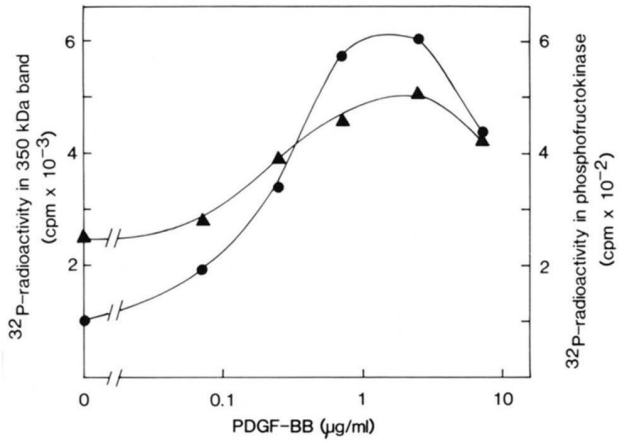

FIG. 4. **Concentration dependence of PDGF-BB-induced phosphorylation of an exogenous substrate.** Purified receptor was incubated with phosphofructokinase, [^{32}P]ATP, and different concentrations of PDGF-BB. Samples were cross-linked with DSS and then analyzed by SDS-gel electrophoresis and autoradiography. The ^{32}P radioactivity in the 350-kDa receptor dimer bands (●) and in the 80-kDa phosphofructokinase bands (▲) were determined as Cerenkov radiation after excision from the gel.

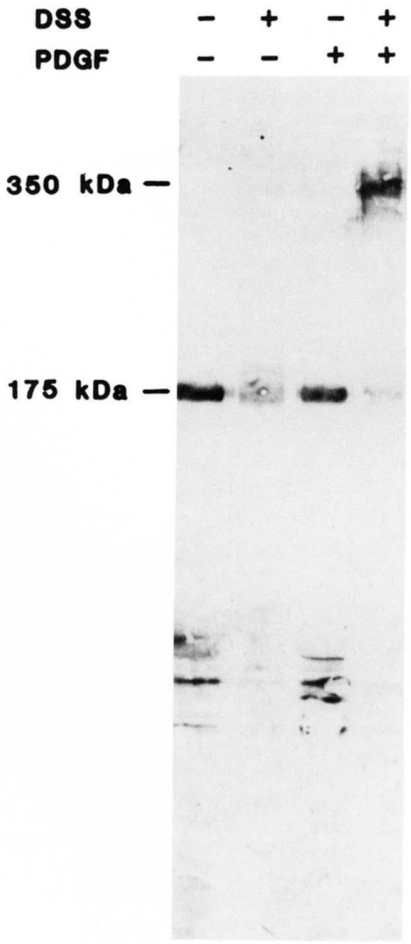

FIG. 5. **Analysis of PDGF B-type receptor dimerization by immunoblotting.** Purified PDGF B-type receptor was incubated in the absence or presence of PDGF and then cross-linked with DSS, or not. Samples were then run on SDS-gel electrophoresis blotted to a nitrocellulose filter. The blot was then incubated with a PDGF receptor antiserum. After washing, bound immunoglobulin was detected by incubation with ^{125}I-protein A. An autoradiogram of the blot is shown.

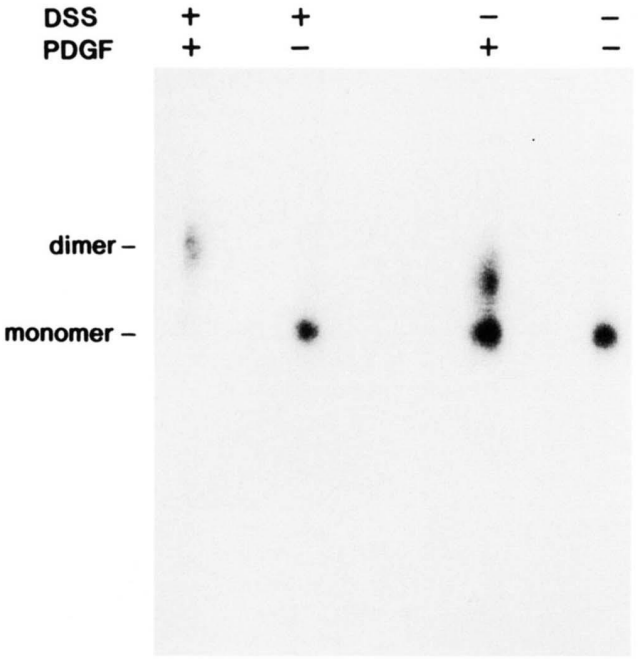

FIG. 6. **Analysis of B-type PDGF receptor dimerization by nondenaturating electrophoresis.** Purified receptor was incubated with [^{32}P]ATP in the absence or presence of PDGF and then cross-linked, or not, by DSS. Samples were analyzed by nondenaturating electrophoresis in the absence of SDS.

only gives a rough estimate of molecular size, the data are consistent with the notion that the slower and faster migration forms of the receptor correspond to receptor dimers and monomers, respectively. This is further verified by analysis by SDS-gel electrophoreses of parallel samples; the major part of the sample incubated in the presence of PDGF and cross-linked by DSS contained dimeric receptors (not shown). Thus the ligand-incubated dimeric receptor complex is partly stable during electrophoresis at pH 8.3 in the absence of SDS.

Preparation of Functionally Active Receptor Dimers and Monomers by Gel Chromatography—The fact that the complex between ligand and PDGF B-type receptor is sufficiently stable to resist nondenaturating electrophoresis (Fig. 6) prompted experiments aimed at separating native receptor dimers and monomers on a preparative scale by gel chromatography.

Mono Q-purified PDGF B-type receptor was incubated with [^{32}P]ATP in the absence or presence of PDGF-BB to allow receptor autophosphorylation. The receptors were then adsorbed to WGA-Sepharose and the major part of excess [^{32}P]ATP removed by washing. The samples were eluted by *N*-acetylglucosamine and then applied to gel chromatography on a Superose 6 column eluted with 0.2% Triton X-100, 0.15 M NaCl, 10% glycerol, 20 mM Hepes, pH 7.4.

Fig. 7 shows that the ^{32}P radioactivity of the sample incubated in the presence of PDGF eluted at a position similar to that of thyroglobulin (M_r 669,000). As expected, the amount of high molecular weight ^{32}P radioactivity was lower in the sample incubated in the absence of PDGF. Furthermore, the radioactivity eluted later in the chromatogram at a position similar to that of ferritin (M_r 440,000) (Fig. 7*A*). Considering that each solubilized B-type PDGF receptor contains a Triton X-100 micelle of about 90 kDa (29), the elution positions of the receptors incubated in the presence and absence of PDGF-BB are reasonably close to those expected for the receptor dimer and monomer, respectively. Analysis by SDS-gel electrophoresis and autoradiography after cross-linking with DSS

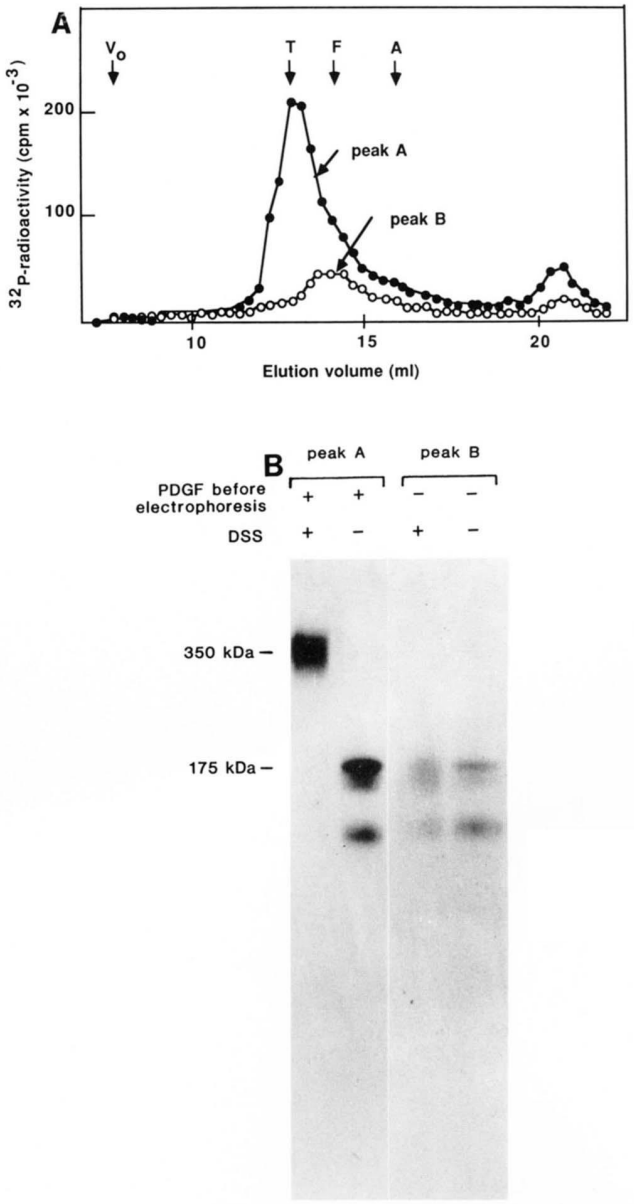

FIG. 7. **Analysis of B-type PDGF receptor dimerization by gel chromatography.** Purified receptor was incubated with [^{32}P]ATP in the absence (○) or presence (●) of PDGF-BB. After removal of the major part of unreacted [^{32}P]ATP by chromatography on WGA-Sepharose, samples were analyzed by gel chromatography on Superose 6. ^{32}P radioactivity in the fractions was determined as Cerenkov radiation (*A*), and the two peaks obtained (*peak A* and *peak B*) were analyzed, after cross-linking by DSS, by SDS-gel electrophoresis and autoradiography (*B*). The void volume (V_0) and the elution positions of thyroglobulin (*T*, M_r 669,000), ferritin (*F*, M_r 440,000), and aldolase (*A*, M_r 158,000) are indicated. The small peak eluting at 21 ml represents [^{32}P]ATP.

supported this notion; the peak eluting first represented a receptor dimer, whereas the peak eluting later represented a monomeric receptor (Fig. 7*B*).

Finally, we compared the kinetic properties of the ligand-induced PDGF B-type receptor dimers with those of receptor monomers after separation by gel chromatography. Mono Q-purified PDGF B-type receptor was incubated in the presence or absence of PDGF-BB and subjected to gel chromatography on Superose 6. Individual fractions were then concentrated and incubated with [^{32}P]ATP in the presence or absence of PDGF-BB. Essentially all receptor kinase activity of the

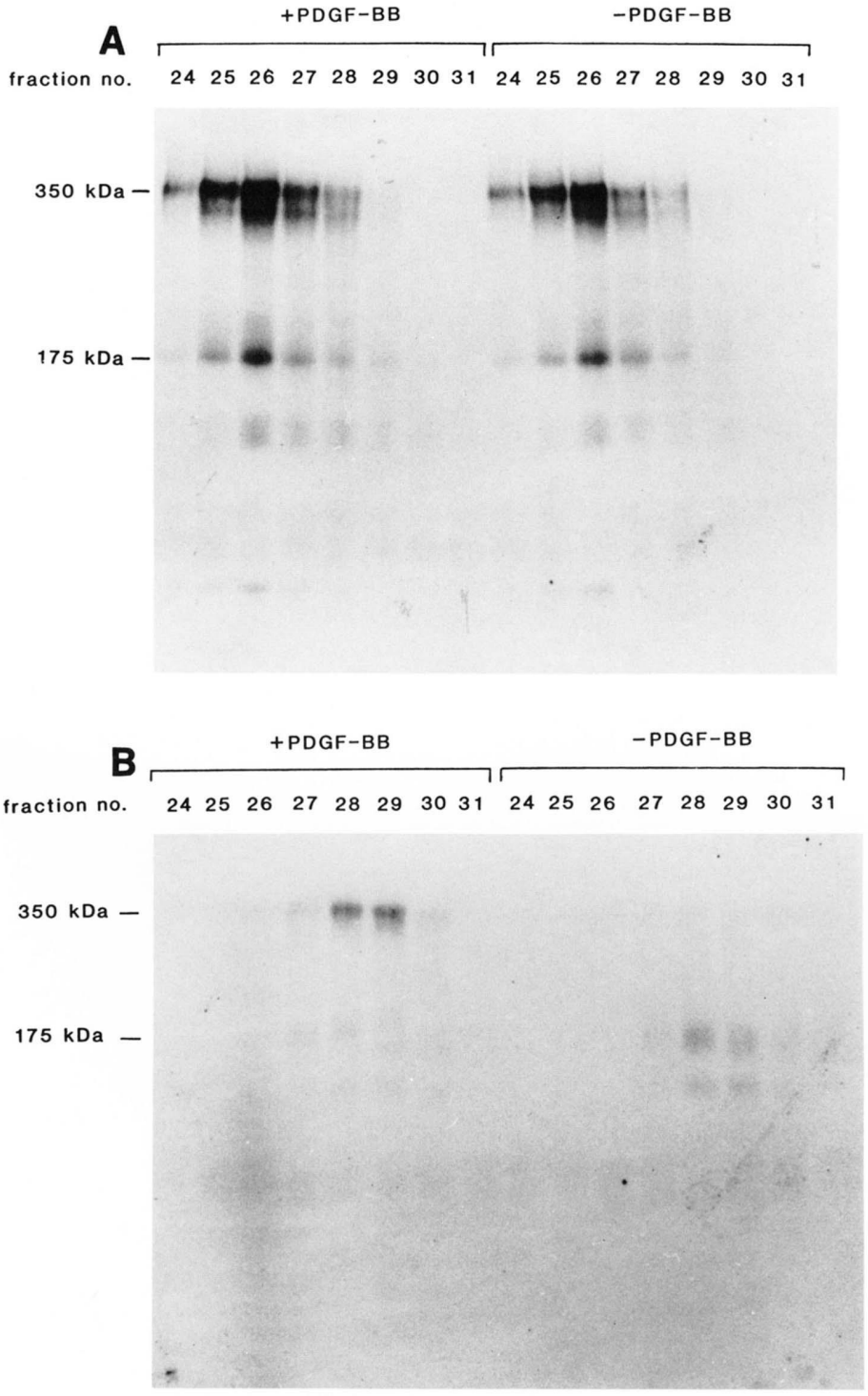

FIG. 8. **Kinetic properties of B-type PDGF receptor dimers and monomers.** Purified receptor was incubated in the absence (*B*) or presence (*A*) of PDGF-BB and chromatographed on the Superose 6 column. Individual fractions were concentrated 10-fold and incubated with [^{32}P]ATP in the absence or presence of PDGF-BB. Samples were analyzed, after cross-linking with DSS, by SDS-gel electrophoresis and autoradiography.

sample incubated in the presence of PDGF-BB eluted in fractions 25–27, corresponding to the elution position of thyroglobulin; when analyzed by SDS-gel electrophoresis and autoradiography after cross-linking with DSS, all of it appeared as receptor dimers (Fig. 8*A*). Furthermore, addition of PDGF-BB to the gel chromatography fractions had no effect on autophosphorylation or dimerization (Fig. 8*A*). The sample that was incubated in the absence of PDGF-BB prior to gel chromatography gave essentially all receptor activity later, in fractions 28 and 29, corresponding to the elution position of ferritin. When these fractions were incubated with [^{32}P]ATP in the absence of PDGF-BB, only background autophosphorylation was detected, and all receptors appeared as monomers after analysis by SDS-gel electrophoresis and autoradiography after cross-linking (Fig. 8*B*). As expected, addition of PDGF-BB to these fractions induced receptor autophosphorylation and dimerization (Fig. 8*B*).

DISCUSSION

We show in this paper that PDGF-BB induces dimerization of the B-type PDGF receptor. The dimerization is closely associated with activation of the receptor kinase but is not dependent on phosphorylation reactions, since dimerization

occurred also in the absence of ATP. The dimerization is dose-dependent and is optimal at about 0.5 μg/ml PDGF-BB; at higher concentrations of PDGF-BB, receptor dimerization and kinase activation decrease. This indicates that the dimeric PDGF-BB molecule binds two receptors. The complex of one PDGF-BB molecule and two receptors is stable and does not dissociate during nondenaturating electrophoresis or gel chromatography.

The interpretation of the data of the present work is schematically illustrated in Fig. 9. It is conceivable that receptors occur in an equilibrium between monomers and dimers. In the absence of ligand, the equilibrium favors the monomeric configuration, whereas at optimal ligand concentration the equilibrium favors the dimeric configuration. Whether PDGF-BB preferentially binds first to one receptor and then rapidly to another, or binds to preexisting receptor dimers, remains to be elucidated. That complexes of one ligand and one receptor exist is most likely, since increasing the concentration of PDGF-BB over 0.5 μg/ml was found to lead to increasing monomerization of the receptor (Fig. 3). Interestingly, the receptor kinase was found to be inactive in the monomeric complex, in spite of the fact that the ligand most likely is bound to the receptor (Fig. 9).

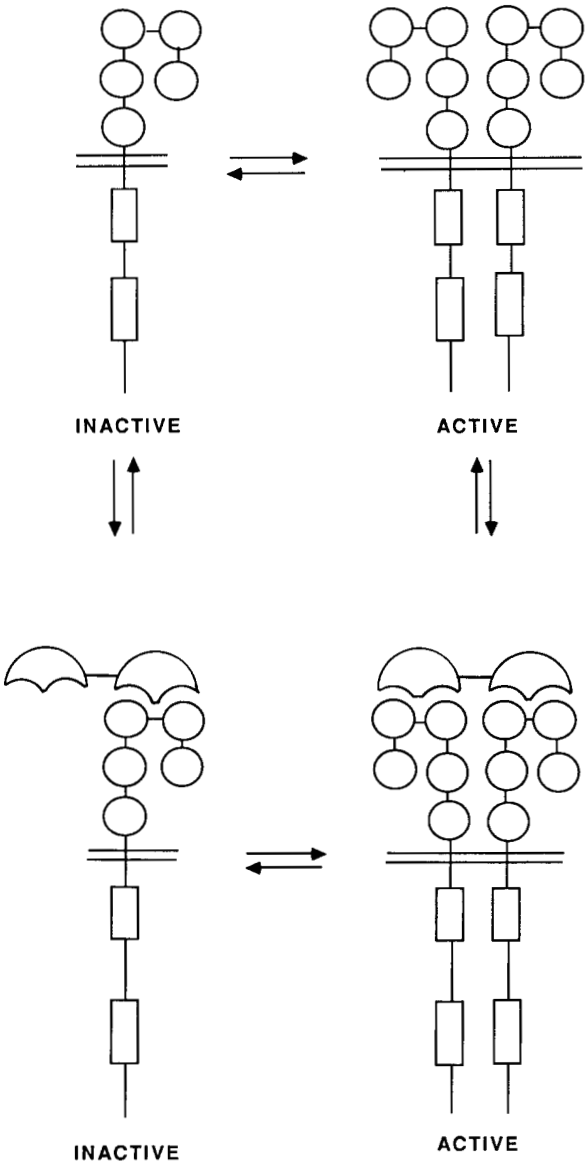

FIG. 9. **Schematic representation of B-type PDGF receptor monomers and dimers.** The external part of the receptor consists of five immunoglobulin-like domains (*circles*), and the internal part consists of a tyrosine kinase domain with an insert sequence (*rectangles*). It is conceivable that the receptor occurs in an equilibrium between monomers and dimers. In the absence of ligand (*upper right* and *left*) the equilibrium favors the monomeric configuration, whereas in the presence of an optimal concentration of ligand (*lower right*) the equilibrium favors a dimeric configuration. Ligand bound to monomeric receptor (*lower left*) can occur as an intermediate in the formation of a complex of ligand and two receptors, or in the presence of very high concentrations of ligand (see text).

The present study has not given any proof of the existence of receptor dimers without ligand bound. It is possible, however, that dimeric complexes, transiently formed in the absence of ligand, account for the variable background autophosphorylation that is seen in the receptor preparations. If such complexes occur, they are instable and dissociate, *e.g.* during gel chromatography, or occur in such a low amount that they are not detected (Figs. 7 and 8). Indirect support for the notion that spontaneously formed dimeric receptor complexes account for the background activity seen comes from the experiment shown in Fig. 3; the rate of phosphorylation of the 170-kDa receptor in the absence of ligand is actually higher than in the presence of a very high concentration of PDGF-BB, which forces the receptors into monomeric configurations. Additional support for the notion that dimeric receptor complexes formed in the absence of ligand are active comes from experiments using a monoclonal antibody directed against the external domain of the receptor but not against the ligand-binding domain. This antibody was found to induce activation of the receptor kinase in a dose-dependent fashion, but Fab′ fragments were found to be without effect (15). That the monoclonal antibodies induced receptor dimerization was verified with covalent cross-linking with DSS (not shown). Taken together, the available data indicate that activation of the receptor kinase correlates with receptor dimerization rather than with ligand binding (Fig. 9).

The present work was done in a solubilized system using purified PDGF B-type receptor. In this system PDGF-BB specifically induced dimers; complexes of more than two receptors were not found, neither after cross-linking with DSS and analysis by SDS-gel electrophoresis and autoradiography (Figs. 1–3), nor in the V_0 fractions of the gel chromatogram (Fig. 7). Ligand binding to intact cells induces receptor clustering (30). It is possible that receptor dimerization is the first step in such clustering and that the dimeric receptor complexes subsequently interact with each other to form larger aggregates. Apparently, the forces involved in aggregation of dimeric receptors in intact cells are too weak to be demonstrated in a solubilized system. Alternatively, clustering is dependent on other cellular structures, *e.g.* the cytoskeleton. The K_d for binding of PDGF-BB to B-type receptors on intact human fibroblasts is about 0.5 nM, *i.e.* 15 ng/ml.[3] Considerably higher concentrations, about 100 ng/ml, were needed to get half-maximal activation and dimerization of the solubilized receptor (Figs. 2 and 3). In view of the observation that one PDGF-BB molecule binds to two receptors, it is likely that the binding occurs at higher affinity if the two receptors are anchored in the cell membrane and cooperate in the binding, as opposed to if they are solubilized.

Observations analogous to those described here for the B-type PDGF receptor have recently been reported for the EGF receptor. The Triton X-100 solubilized EGF receptor occurs as a mixture of monomers, dimers, and oligomers (31–34). Dimeric receptors have higher tyrosine kinase activity and higher affinity for EGF than monomeric receptors (32, 33).

Binding of EGF has been found to induce the formation of dimers and oligomers (32) (however, see also Ref. 34); this effect of EGF has been observed also in membranes (35) and in intact cells (36). These observations have led to an allosteric oligomerization model to explain how ligand binding to the extracellular domain activates the intracellular tyrosine kinase domain (37). Intramolecular mechanisms for the activation of the EGF receptor have, however, also been proposed. Such models are supported by some kinetic data (38) and by the fact that sphingosine activates the receptor in the absence of dimerization (35).

There is one principal difference in the effects of PDGF and EGF on dimerization of their receptors. EGF is a small monovalent molecule that most likely binds only to one receptor molecule. It is conceivable that ligand binding induces a conformational change in the EGF receptor that increases the affinity for other EGF receptor complexes. In contrast, the bivalent PDGF molecule has the capacity to simultaneously bind two receptors and thereby stabilize the dimerization. Consistent with this difference in mechanism, high concentrations of EGF have not been reported to induce monomerization of receptors, as has been described in this paper for PDGF (Fig. 3).

To date, more than a half-dozen growth factor receptors have been cloned; in each case the predicted amino acid sequence indicates a single transmembrane domain. Thus, the external ligand-binding domains of the receptors are separated from the intracellular effector domains by a single flexible polypeptide. The findings of the present work on PDGF, and previous work on EGF, suggest intermolecular mechanisms for signal transduction after ligand binding to the receptors. Although intramolecular mechanisms of signal transduction have not been excluded, *e.g.* involving a dislocation of the hydrophobic transmembrane domain that could transfer a conformational change from the ligand binding domain to the cytoplasmic kinase domain, it is possible that receptor dimerization is of general importance in signal transduction of growth factor receptors. The exact mechanism whereby two receptors in a dimer interact to activate the kinase remains to be elucidated.

The three isoforms of PDGF differ in their binding affinities to A- and B-type PDGF receptors. It is not known whether dimerization is also involved in activation of A-type receptors. An interesting possibility is that PDGF-AB and PDGF-BB, which bind to both A- and B-type receptors, can induce the formation of heterodimeric receptor complexes. Since there are indications that A- and B-type receptors mediate different cellular effects (8), one ligand would thus (via such a heterodimer complex) induce the activation of two different signals.

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