Association of SH2 Domain Protein Tyrosine Phosphatases with the Epidermal Growth Factor Receptor in Human Tumor Cells

PHOSPHATIDIC ACID ACTIVATES RECEPTOR DEPHOSPHORYLATION BY PTP1C*

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The SH2 domain protein tyrosine phosphatases (PTPases) PTP1C and PTP1D were found associated with epidermal growth factor (EGF) receptor which was purified from A431 cell membranes by several steps of chromatography. Both PTPases also associated with the EGF receptor upon exposure of immunoprecipitated receptor to lysates of MCF7 mammary carcinoma cells. The associated PTPases had little activity toward the bound receptor when it was autophosphorylated in vitro. Receptor dephosphorylation could, however, be initiated by treatment of the receptor-PTPase complex with phosphatidic acid (PA). When autophosphorylated EGF receptor was exposed to lysates of PTP1C or PTP1D overexpressing 293 cells, the association of PTP1C but not of PTP1D was enhanced in the presence of PA. In intact A431 cells, an association of PTP1C and PTP1D with the EGF receptor was detectable by coimmunoprecipitation experiments. PA treatment reduced the phosphorylation state of ligand activated EGF receptors in A431 cells and in 293 cells overexpressing EGF receptors together with PTP1C but not in 293 cells overexpressing EGF receptors alone or together with PTP1D. We conclude that PTP1C but not PTP1D participates in dephosphorylation of activated EGF receptors. A possible role of PA for physiological modulation of EGF receptor signaling is discussed.

Growth factor receptors of the tyrosine kinase-type undergo rapid autophosphorylation upon ligand stimulation (1, 2). The autophosphorylation generates binding sites for SH2 domain containing intracellular proteins. Membrane recruitment of these molecules by binding to the autophosphorylated growth factor receptor is considered an important step for activation of several intracellular signaling pathways which ultimately lead to the cellular responses as cell division, cell differentiation, or cell locomotion. Dephosphorylation of the autophosphorylated growth factor receptors by PTPases presents a major mechanism of negative regulation of tyrosine kinase receptor signaling. Recently, it has become clear that PTPases comprise a large family of structurally very diverse molecules which can broadly be classified into cytosolic and transmembrane enzymes (see Refs. 7-10 for reviews). Different PTPases of both classes are capable of dephosphorylate autophosphorylated growth factor receptor in vitro (11-13) and when overexpressed together with the receptors in animal cells (14, 15). Also, attenuation of growth factor receptor signaling by overexpression of PTPases has been demonstrated (16). In particular, it could be shown that cellular transformation induced by overexpression of the growth factor receptors HER2/Neu or v-fms can be reverted by coexpression of the PTPases PTP1B or T-cell PTP (15, 17, 18). The currently available data suggest that, despite an only moderate substrate specificity of PTPases in vitro, the interaction of PTPases with phosphorylated substrates in intact cells is rather specific and in addition finely tuned by various mechanisms regulating the activity of the PTPases, including phosphorylation of the PTPases at Ser/Thr or Tyr residues (14, 19-22). To date, little is known about the identity and regulation of PTPases which physiologically interact with specific growth factor receptors and thereby regulate the respective signaling pathways. Such knowledge is, however, highly desirable for understanding the regulation of tyrosine kinase receptor signaling in normal and tumor cells. Also, characterization of PTPases which specifically regulate certain signaling pathways will identify novel targets for pharmacological modulation of cell growth. It might be possible to identify such PTPases by virtue of their physical association with receptor tyrosine kinases. Obvious candidates for a physical association with autophosphorylated growth factor receptor are PTPases possessing SH2 domains. Two SH2 domain-containing PTPases have been identified so far and designated (among other names) PTP1C (SH-PTP1) and PTP1D (SH-PTP2, syp, SH-PTP3, PTP2C) (see Ref. 23 for review). Association of PTP1D to the cytoplasmic domains of PDGF receptor, HER2/Neu, the EGF receptor, and Kit/SCF receptor has been demonstrated when the PTPase and the respective receptors were transiently overexpressed in 293 cells (14). Association of PTP1D was also shown with PDGF and EGF receptors in fibroblasts (24), with PDGF receptors in epithelial cells (25), and with insulin receptors as well as the insulin receptor substrate-1 in vitro (26). Receptor association with PTP1D leads to its tyrosine phosphorylation and activation of

trophoresis; MES, 2-(N-morpholino)-ethanesulfonic acid; DMEM, Dulbecco’s modified Eagle’s medium; PIPES, 3-(3-cholamidopropyl)-dimethylammonio)-1-propane sulfonate; EGF, epidermal growth factor; PA, phosphatidic acid; PAGE, polyacrylamide gel electrophoresis; MES, 2-(N-morpholino)-ethanesulfonic acid; DMEM, Dulbecco’s modified Eagle’s medium; PIPES, 3-(3-cholamidopropyl)-dimethylammonio)-1-propane sulfonate; DTT, dithiothreitol; PDGF, platelet-derived growth factor, CMV, cytomegalovirus.
the PTPase activity (14, 24, 27). Although PTP1D is capable of dephosphorylating in vitro tyrosine-phosphorylated peptides corresponding to growth factor receptor and/or tyrosine kinase sites (28), it has little activity on coexpressed or coimmunoprecipitated receptors (14, 24), with the possible exception of Kit/SCF receptor (14). For PTP1C, physical association with autophosphorylated growth factor receptors has also been demonstrated for ligand-stimulated Kit/SCF receptor in hematopoietic cells (29) and with the cytoplasmic domain of HER2 in a transient overexpression system (14). Transient coexpression of PTP1C with different tyrosine kinase receptors results in complete or partial dephosphorylation of PDGFR and β receptors, insulin-like growth factor-I receptor, Kit/SCF receptor, insulin receptor, EGF receptor, and HER2 (14). A role of PTP1C for negative regulation of tyrosine kinase receptor signaling in hematopoietic cells has been proposed in relation to various proliferation abnormalities observed in mice carrying a defective PTP1C gene (23, 30). Recently, association of PTP1C with the erythropoietin receptor (31) and with the FcγRIIB1 receptor in B lymphocytes (32) and a role for PTP1C in negative regulation of erythropoiesis and B cell signaling, respectively, have been demonstrated. Both PTP1C and PTP1D are phosphorylated and possibly regulated by Ser/Thr specific kinases (33, 34), and PTP1C is potently activated in vitro by anionic phospholipids (35) including PA. Various tyrosine kinases are capable of phospho-rylating the PTspecific domain of PTP1C in vitro, and it becomes tyrosine phos-phorylated in A431 cells in response to EGF treatment (36). It is not known if these mechanisms are important for the regu-lation of the activity of the SH2 domain PTases toward growth factor receptors or other substrates in intact cells.

Here we demonstrate that PTP1D and PTP1C associate with EGF receptors in human epithelial tumor cells. The phospha-tases can be activated by PA to dephosphorylate the associated autophosphorylated receptor in vitro. PA stimulates associ-ation of PTP1C but not PTP1D to the EGF receptor. Phospha-tase activity toward the EGF receptor can be activated by PA in intact A431 cells, and the activity of PTP1C toward EGF receptor, when transiently coexpressed in 293 cells, can be en-hanced by PA. Our data suggest that PTP1C plays a role in EGF receptor dephosphorylation and that PA might be a phys-iological modulator of PTP1C activity.

**EXPERIMENTAL PROCEDURES**

**Purification of EGF Receptors from A431 Cells—Preparation of A431 cell membranes and isolation of EGF receptors were performed according to Akiyama et al. (37) with some modifications. In brief, A431 human epidermoid carcinoma cells (CRL 1555, obtained from the American Type Culture Collection, Rockville, MD) were cultured in DMEM (Life Technologies, Inc.) supplemented with 4.5 g of glucose, 2 mM glutamine, 7.5% fetal calf serum, and antibiotics. Cells were grown to near confluence on 625-cm² plates (Nunc). The medium was decanted, the cells were resuspended in 20 mM PIPES, pH 7.2, and stored at 80°C. Naphosphorylation mixture containing 20 mM HEPES, pH 7.5, 0.01% Brij 35, 0.1 mg/ml bovine serum albumin, 0.2% mercaptoethanol, 20 mM MgCl₂, 4 mM MnCl₂, 100 μM ATP, and 20 μCl of [γ-32P]ATP. The labeling mixture was applied to a 0.5-ml column of Dowex 1X-8, equilibrated in 30% acetic acid, and the 32P-labeled Raytide was eluted with acetic acid and stored at −80°C. For the assay, an aliquot was diluted in a Speedvac evaporator and redissolved in PTase assay buffer containing 50 mM MES, pH 6.0, and 5 mM DTT. For the assay, 20 μl of column fractions were diluted with 30 μl of assay buffer and incubated with 200 ng of monoclonal antibody 108 for 30 min at 37°C. The reaction was stopped, and released [32P]phosphate was measured as described by Po et al. (38). To detect PTase activity in immunoprecipitates, phosphorylated EGF receptor was prepared as follows. EGF receptors from 293 cell lysates were adsorbed to lentil lectin-Sepharose (Pharmacia) (200 μl of beads per 1 ml of 293 cell lysate). The beads were washed five times with washing buffer and once with 10 mM HEPES, pH 7.5. Then a phosphorylation mixture containing 20 mM HEPES, pH 7.5, 15 mM MnCl₂, 200 ng of EGF, 200 μM sodium orthovanadate, 5 μM ATP, and 50 μCl of [γ-32P]ATP was added. After incubation for 30 min at 25°C the beads were washed five times with washing buffer, and the 32P-phosphorylated EGF receptor was eluted with 600 μl of 0.3 M mannose in washing buffer. To measure PTase activity, MCF-7 cell lysate-exposed immunoprecipitates (10 μl) were mixed with 5 μl of buffer containing 200 mM sodium acetate, pH 5.5, 40 mM DTT, 4 mg/ml bovine serum albumin, 0.4% CHAPS, and 5 μl of 32P-phosphorylated EGF receptor (10,000–50,000 cpm) and incubated at 25°C for 2 h. The reaction was stopped, and released [32P]phosphate was measured as described above.

**Receptor Autophosphorylation and Diphosphorylation Experiments**—EGF receptor-containing samples were incubated in the presence of 1.2 μg/ml EGF, 50 mM HEPES, pH 7.5, and 3 mM MnCl₂ (final concentrations) for 20 min on ice. In some cases incubation was done in the presence of 50 μM PA (Sigma). To detect receptor autophosphorylation the samples were incubated on ice in the presence of [γ-32P]ATP. After 5 min an ATP sample buffer was added, and the samples were analyzed by SDS-PAGE and autoradiography. For diphosphorylation experiments, re-
EGF Receptor-associated PTPases

RESULTS

Association of Protein-tyrosine Phosphatase(s) with the EGF Receptor and Identification as PTP1C and PTP1D—EGF receptor was purified from A431 cell membranes according to the procedure of Akiyama et al. (37) involving preparation of a crude membrane fraction, membrane solubilization in Triton X-100, affinity chromatography on wheat germ agglutinin, and tyrosine-Phosphatase chromatography. We consistently observed copurification of PTPase activity with the EGF receptor when assayed with phosphorylated Raytide as a substrate (not shown). To further characterize the EGF receptor-associated PTPases the peak fractions from the tyrosine-Phosphatase column were subjected to a further chromatographic step employing a high resolution sizing column (Fig. 1A). Again, much of the PTPase activity coeluted with the EGF receptor (fractions 9–11) in this fractionation. A second peak and a third peak of PTPase activity eluted later (fractions 12 and 13 and 15–17, respectively) and might represent the same PTPase(s) which gradually dissociate from the receptor (see below). To test the possible involvement of SH2 domains in the association, the PTPaseEGF receptor complex was treated with phenylphosphate before application to the sizing column. Under these conditions, part of the PTPase activity still coeluted with the EGF receptor (fractions 9–11), whereas the majority of PTPase activity eluted from the column now at apparent lower molecular mass (fractions 12 and 13 and fractions 14–17) suggesting a partial disruption of the PTPaseEGF receptor complex.

In an alternative approach, EGF receptors were immunoprecipitated from lysates of 293 cells which overexpressed the receptor. Then, the immunoprecipitates were incubated with lysates of MCF7 human mammary carcinoma cells, thereafter washed extensively and checked for associated PTPase activity using autophosphorylated EGF receptor as a substrate. As shown in Fig. 1B, PTPase activity was associated with the immunoprecipitated receptors. The extent of PTPase association was dependent on the pretreatment of the immunoprecipitated receptors. Only little PTPase activity associated in the absence of any pretreatment. A clear PTPase association was, however, detectable by allowing autophosphorylation of the receptors in the presence of ATP and even more in the presence of ATP5S before exposing it to the cells lysates. The associated PTPase activity was almost completely inhibited by sodium orthovanadate (Fig. 1B). When immunoprecipitated autophosphorylated EGF receptor was exposed to MCF7 lysate in the presence of phenylphosphate, the amount of receptor-associated PTPase activity was greatly reduced (Fig. 1B), again indicating the involvement of SH2 domains in the receptor-PTPase association. We therefore analyzed the EGF receptor preparations containing PTPase activity for the presence of SH2 domain PTPases by immunoblotting. Clearly, PTP1C and PTP1D were detectable by immunoblotting with specific antibodies in peak fractions of the column-purified EGF receptor (Fig. 2) as well as associated from MCF7 cell lysates to immunoprecipitated EGF receptor (not shown). To determine whether a PTPase-EGF receptor-association also occurs in intact A431 cells, A431 cell lysates were subjected to immunoprecipitation with or without EGF receptor antibodies. As shown in Fig. 2, PTPase activity associated in the presence of anti-PTP1C and anti-PTP1D antibodies, and the immunoprecipitates were analyzed by autophosphorylation in the presence of [γ-32P]ATP and EGF, followed by SDS-PAGE and autoradiography. When lysates of serum-deprived but not EGF-stimulated cells were subjected to immunoprecipitation, weak autophosphorylation signals at the expected size of EGF receptor were observed. These signals were not seen when nonspecific IgG was employed for the immunoprecipitation (Fig. 3, lane 3). When the A431 cells were stimu-
EGF Receptor-associated PTPases

Fig. 1. Association of PTPase activity with the EGF receptor (EGFR). A, copurification of EGF receptor and PTPase activity from A431 cell membranes. A particulate fraction from A431 cells was extracted with detergent and subjected to affinity chromatography on wheat germ agglutinin as described under "Experimental Procedures." The glycoprotein fraction eluted from the wheat germ agglutinin column was then chromatographed over a tyrosine-Sepharose column as described by Akiyama et al. (37). Fractions were monitored for the presence of EGF receptor by an autophosphorylation assay. The peak fractions were concentrated and either directly subjected to chromatography on a Superdex 200HRC sizing column (squares) or incubated with 20 mM phenylphosphate and subsequently separated on the same column (triangles). The points represent the PTPase activity toward 32P-labeled Raytide. The elution position of EGF receptor (hatched area) was detected by autophosphorylation assays and found to be identical (fractions 9–11) under both conditions. B, binding of PTPase to immunoprecipitated EGF receptor. EGF receptor was immunoprecipitated with anti-EGF receptor antibodies 108 from cell lysates of EGF receptor overexpressing 293 cells, as described under "Experimental Procedures." The precipitates were incubated in the absence of any agents (1) or in the presence of ATP (2) or ATP-γ-S (3–5), washed, and exposed to lysates of MCF7 human mammary carcinoma cells in the presence (4) or absence (1–3, 5) of 20 mM phenylphosphate (PhP). After extensive washing, the receptor-associated PTPase activity was measured using exogenously added 32P-labeled Raytide, as described under "Experimental Procedures," in the absence (lanes 1–4) or presence (lane 5) of 1 mM sodium orthovanadate (V). The results are expressed in arbitrary units.

Association of PTPase activity with the EGF receptor

Stimulation of the EGF receptor by PA and involvement of PTP1C—We then tested the capability of the EGF receptor-associated PTPases to dephosphorylate the autophosphorylated receptor. In these experiments, peak fractions of the column-purified EGF receptor containing the associated PTPases 1C and 1D were allowed to autophosphorylate in the presence of [γ-32P]ATP. The kinase reaction was quenched by addition of EDTA, and after different time points aliquots of the receptor were analyzed for radioactivity by SDS-PAGE and autoradiography. Only little dephosphorylation of the receptors occurred under these conditions (Fig. 4A), indicating that the receptor-associated PTPases despite their activity against exogenous substrate (see above) were unable to use the bound receptor as a substrate. A complete failure to dephosphorylate the bound receptor was also observed in case of PTPase(s) associated with immunoprecipitated receptor from MCF7 cell lysates (not shown), although exogenously added autophosphorylated EGF receptor was readily dephosphorylated. These findings were unexpected, since previous transient coexpression experiments suggested the capability of PTP1C to dephosphorylate EGF receptors in intact cells (14). We therefore asked if, under certain circumstances, the bound PTPases could be activated to use the bound receptor as a substrate. As potential activators we considered phospholipids, since three findings point to a modulatory role of membrane lipids for PTPase activity. 1) The activity of PTPases to dephosphorylate autophosphorylated receptors in cell membranes is impaired by detergents (5, 6). 2) Various acidic phospholipids have been shown to enhance PTP1C activity for artificial substrates in vitro (35). 3) PA enhances EGF receptor dephosphorylation in A431 cell membranes, whereas other lipids including other acidic phospholipids have no or inhibitory effects on EGF receptor dephosphorylation. Indeed, when the dephosphorylation reaction of column-purified EGF receptor was carried out in the presence of PA, a clear decrease of radioactivity in the receptor could now be observed (Fig. 4B). This activity was due to the associated PTPases, because the dephosphorylation could be completely blocked with 50 μM pervanadate, and activation by PA was specific, since palmitic acid, when applied in the same concentration, had much less effect on the receptor dephosphorylation (data not shown).

We next wished to determine whether an activation of phosphatase(s) for the autophosphorylated EGF receptor by PA might occur in intact cells and further to differentiate whether PTP1C or PTP1D (both present in the investigated receptor preparations) might mediate the effect of PA. To this end, PTP1C and PTP1D were transiently coexpressed with the EGF receptor in 293 cells. The cells were left untreated or were treated with PA, then stimulated with EGF. Subsequently the phosphorylation state of the EGF receptors was analyzed by immunoblotting with antiphosphotyrosine antibodies. When EGF receptor was expressed in the absence of PTPase, no effect of PA treatment on the phosphorylation state of the receptor was seen (Fig. 5, upper panel as indicated). As described earlier (14), expression of PTP1D had no effect on the phosphorylation state of coexpressed EGF receptors (Fig. 5, upper panel), and PA treatment of cells expressing EGF receptors and PTP1D had no effect (Fig. 5, upper panel). In contrast, PTP1C expression reduced the phosphorylation state of coexpressed EGF receptors to 32% of the respective control. When cells overexpressing EGF receptors and PTP1C were in addition treated with PA, the extent of EGF receptor phosphorylation was further reduced after 1 h of treatment to 21% and even further.

2. U. Greiser, unpublished results.
after 24 h of PA treatment to 13%. Control experiments (Fig. 5, lower panels) confirmed that comparable amounts of EGF receptor and the respective PTPases entered the analysis in all cases. We conclude that PA treatment enhances the EGF receptor-directed activity of PTP1C in this system.

We further considered whether PA would also modulate the EGF receptor phosphorylation state in intact A431 cells. Cells were pretreated for different length of time with PA or left untreated, then stimulated with EGF and analyzed for the phosphotyrosine content of the EGF receptors. We consistently found that PA treatment for 1 h reduced the phosphotyrosine content of the EGF receptors (Fig. 6A). A similar reduction after 15 min and 24 h of treatment was observed occasionally but not in all experiments (not shown). To check the involvement of PTPases in this reduction of receptor phosphorylation, parallel cultures were treated with 50 μM pervanadate prior to EGF stimulation. No differences in the phosphorylation state between samples from PA-treated or nontreated cells were seen in presence of pervanadate (Fig. 6B). These data suggest that the reduction in phosphotyrosine content of EGF receptors induced by PA in A431 cells is mediated by PTPases.

PA Promotes Association of PTP1C with the Autophosphorylated EGF Receptor—As a first attempt to understand the mechanism underlying the observed activation of PTP1C, we investigated the effect of PA on the association of PTP1C and PTP1D with the autophosphorylated EGF receptor in vitro. EGF receptor was immunoprecipitated from lysates of receptor

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**FIG. 2.** Detection of PTP1D and PTP1C in column-purified EGF receptor (EGFR) preparations. EGF receptor was purified from A431 cell membranes, as described under “Experimental Procedures,” to the stage of tyrosine-Sepharose chromatography. Peak fractions were highly concentrated (Microcon) and subjected to SDS-PAGE and immunoblotting with monoclonal anti-PTP1D or PTP1C antibodies as indicated. The amount of sample applied per lane corresponds to EGF receptor isolated from about 5 × 10⁶ A431 cells (lanes 3 and 4). For reference, aliquots of lysates of 293 cells overexpressing either PTP1C (lane 1) or PTP1D (lane 6) were also analyzed. Only SDS-PAGE sample buffer was loaded in lanes 2 and 5. The positions of the phosphatases and of molecular mass markers are indicated.

**FIG. 3.** Detection of EGF receptor (EGFR) in anti-PTP1C and anti-PTP1D immunoprecipitates. Serum-deprived subconfluent cultures of A431 cells were left unstimulated (lanes 1–3) or were stimulated with 100 ng/ml EGF for 10 min at room temperature (lanes 4–6). Cell lysates were prepared and subjected to immunoprecipitation with anti-PTP1C antibodies (lanes 1 and 4), anti-PTP1D antibodies (lanes 2 and 5) or nonspecific IgG (lanes 3 and 6). Subsequently, the samples were incubated on ice in the presence of [γ-³²P]ATP, as described under “Experimental Procedures,” and then analyzed by SDS-PAGE and autoradiography.

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**FIG. 4.** PA activates EGF receptor (EGFR) dephosphorylation by the receptor-associated PTPase(s). Column-purified EGF receptor was incubated in the absence (A) or presence of 50 μg/ml PA (B) for 20 min on ice. Then, the receptor was autophosphorylated, as described under “Experimental Procedures,” the phosphorylation was quenched by addition of EDTA, the samples were transferred to 37 °C, and the radioactivity in the receptor was monitored at the indicated time points by SDS-PAGE and autoradiography. Numbers underneath the lanes represent the percentage of radioactivity (mean of the duplicates, 100% at time point 0 min) as obtained by densitometric quantification.

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overexpressing 293 cells with anti-EGF receptor antibodies (Ab425 coupled to Affi-Prep Hz beads) and autophosphorylated. Thereafter, the receptor precipitates or respective control precipitates were exposed to lysates of 293 cells either overexpressing PTP1C or PTP1D, in the presence or absence of PA. The amount of receptor-associated PTPase was then analyzed by immunoblotting with the respective PTPase-specific antibodies. In this experimental setting, binding of PTP1C and PTP1D to the precipitates which depended on the presence of immunoprecipitated autophosphorylated EGF receptor was clearly detectable (Fig. 7, lanes 2 and 6, respectively, versus lanes 1 and 5). The presence of PA increased the amount of receptor-associated PTP1C strongly (Fig. 7, lane 4 versus lanes 2 and 3). No effect of PA on the association of PTP1D to the precipitated EGF receptor could be observed (Fig. 7, lane 8 versus lanes 6 and 7).

**DISCUSSION**

The activated and autophosphorylated receptor for EGF is subject to a rapid dephosphorylation by PTPases (3, 6). Receptor dephosphorylation is believed to present a major mechanism of negative regulation of receptor function, and the identification of the involved PTPases is therefore important for understanding of EGF receptor signaling. Also, since mitogenic signaling of EGF receptors and related receptors has been implicated in unwanted growth of certain human tumors, characterization of the receptor dephosphorylation mechanisms might be of significance for a pharmacological modulation of receptor signaling. As one approach to characterize such PTPases we tried to identify those which are capable of physically associating with EGF receptors. PTPase activity which associated from MCF7 cell lysates to immunoprecipitated EGF receptors and PTPase activity which was detectable in EGF receptor preparations obtained with several purification steps from A431 cell membranes could at least in part be attributed to receptor-associated PTP1C and PTP1D. We could also demonstrate the capability of PTP1D and PTP1C to interact with the EGF receptor in intact A431 cells. A physiological interaction of these PTPases with EGF receptors has been predicted from in vitro experiments and from data obtained in cells overexpressing the phosphatases (14, 24). Here we show that an interaction of PTP1C and PTP1D with EGF receptors is detectable in nontransfected cells, i.e. the human tumor cell line A431. As expected, the association seems largely to depend on the interaction of the PTPase SH2 domains with the receptors, since significantly more PTPase could be bound to activated phosphorylated receptors, and the association was largely abrogated by phenylphosphate. Although the displace-
PTP1C has been shown to be involved in negative regulation of back regulation of EGF receptor signaling. A role for the cellular phospholipids largely via a pathway involving phosphorylation of diacylglycerol (40,41). One might therefore speculate that the activation of the receptor-dependent PTPase activity. PTP1C and PTP1D, other, not yet identified, PTPases contribute to the receptor-associated PTPase activity. Despite the activity of the EGF receptor-bound PTPases against exogenous substrates, little activity against the bound receptor was detectable in vitro. In the presence of PA, however, dephosphorylation activity could be observed. PA also enhanced EGF receptor dephosphorylation in intact A431 cells and in 293 cells transiently coexpressing EGF receptors and PTP1C, but not in 293 cells expressing EGF receptors alone or EGF receptors and PTP1D. Taken together, these data suggest that PTP1C but not PTP1D is capable of dephosphorylating EGF receptors and that this activity can be enhanced by PA. EGF stimulation of A431 cells has been shown to increase cellular PA levels up to 0.17% of the total cellular phospholipids largely via a pathway involving phosphorylation of diacylglycerol (40,41). One might therefore speculate that the activation of the receptor-dephosphorylating PTPase PTP1C by PA could present part of a negative feedback regulation of EGF receptor signaling. A role for PTP1C in negative control of receptor activity has also been suggested in case of Kit/SCF receptor (24, 30), and recently PTP1C has been shown to be involved in negative regulation of erythropoietin receptor (31) and FcRIIB1 receptor signaling (32).

The activation of PTP1C by various acidic phospholipids in vitro against artificial substrates has previously been shown by Zhao et al. (35). The data in this study suggest that the lipid-PTP1C interaction leading to PTP1C activation is direct and involves a conformational change of the phosphatase which facilitates proteolytic degradation (35). When we examined the effect of PA on the association of both SH2 domain PTPases to the autophosphorylated EGF receptor in vitro, association of PTP1C, but not PTP1D, was increased in the presence of PA. It is possible that the conformational change of PTP1C which is induced by PA leads to the increased association with the EGF receptor and also forms the basis for activation toward the receptor as observed in our dephosphorylation experiments. Recently, a structural model for PTP1C was proposed (42) based on the kinetic analysis of differently truncated enzyme variants and enzyme activation by phosphorylated peptides which interact with the PTP1C SH2 domains. According to this model, the PTP1C SH2 domains are capable of interacting with the PTP1C C terminus in a phosphorylation-dependent manner and thereby drive the PTPase domain in an inactive conformation (42). One could speculate that PA has the capacity to disrupt this intramolecular interaction. Further experiments are required to clarify the activation mechanism.

In our experiments no evidence for a role of EGF receptor-associated PTP1C in receptor dephosphorylation was obtained. PTP1D has previously been demonstrated to mediate mitogenic signals of the receptors for PDGF, insulin, EGF, and insulin-like growth factor-I (43–45) and a positive role of PTP1D in tyrosine kinase receptor signaling is also likely from its homology to Drosophila csu (14, 25, 46). The association of PTP1D with EGF receptors in human tumor cells therefore defines an interesting target for interference with EGF-driven tumor cell growth.

REFERENCES

1. Ulrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
2. Farquhar, W. J., Johnson, D. E., and Williams, L. T. (1993) Annu. Rev. Biochem. 62, 433–481
3. Searle, G., Cohen, S., and Garbers, D. L. (1982) Biochem. Biophys. Res. Comm. 107, 1104–1109
4. Brandt, D. L., Borstein, P., and Gallis, B. (1981) J. Biol. Chem. 259, 6611–6622
5. Faure, R., Biquarran, G., Bergeron, J. M., and Ponner, B. I. (1992) J. Biol. Chem. 267, 11215–11221
6. Böhm, F. D., Böhm, S. A., and Heldin, C. H. (1993) FEBS Lett. 331, 276–280
7. Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991) Science 252, 401–406
8. Charbonneau, H., and Tonks, N. K. (1992) Annu. Rev. Cell Biol. 8, 483–493
9. Pot, D. A., and Dixon, J. E. (1992) Biochem. Biophys. Acta 1136, 35–43
10. Brandt, D. L. (1992) Biochem. Biophys. Acta 1134, 63–77
11. Tonks, N. K., Diltz, C. D., and Fischer, E. H. (1988) J. Biol. Chem. 263, 6731–6737
12. Hatakeyama, N., Zhang, W. R., and Goldstein, B. J. (1992) Biochem. J. 284, 569–576
13. Zhang, W. R., Hashimoto, N., Ahmad, F., Ding, W. D., and Goldstein, B. J. (1994) Biochem. J. 302, 39–47
14. Vogel, W., Lammers, R., Huang, J., and Ulrich, A. (1993) Science 259, 1611–1614
15. Lammers, R., Bossemmeier, B., Cod, D. E., Tonks, N. K., Schlessinger, J., Fischer, E. H., and Ulrich, A. (1993) J. Biol. Chem. 268, 22456–22462
16. Mooney, R. A., Freund, G. G., Way, B. A., and Bordwell, K. L. (1992) J. Biol. Chem. 267, 23434–23446
17. Bouchard, P., Zhao, Z., Banville, D., Dumas, F., Fischer, E. H., and Shen, S. H. (1993) Cell 72, 759–764
18. Zander, N. F., Cod, D. E., Diltz, C. D., Rohrschneider, L. R., Krebs, E. G., and Fischer, E. H. (1993) Oncogene 8, 1175–1182
19. Brandt, D. L., and Pinault, F. M. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 6696–6700
20. Schingley, A. R., Pai, A. L., Johnson, K. A., Hill, D. E., and Erikson, R. L. (1993) Cell Growth & Diff. 4, 239–246
21. Flint, A. J., Gebbink, M. F., Franza, B. J., Hill, D. E., and Tonks, N. K. (1993) EMBO J. 12, 1937–1946
22. Garton, A. J., and Tonks, N. K. (1994) EMBO J. 13, 3763–3771
23. Feng, G. S., and Pawson, T. (1994) Trends Genet. 10, 54–58
24. Feng, G. S., Hui, C. C., and Pawson, T. (1993) Science 259, 1607–1611
25. Kallakassakas, A., Feng, G. S., Pawson, T., and Vailis, M. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 6939–6942
26. Ugi, S., Maegawa, H., Olsfie, J. M., Shigeta, Y., and Kashiwagi, A. (1994) FEBS Lett. 340, 216–220
27. Lechleider, R. J., Sugimoto, S., Bennett, A. M., Kadowish, A. S., Cooper, J. A., Shoelson, S. E., Walsh, C. T., and Neel, B. G. (1993) J. Biol. Chem. 268, 21478–21481
28. Sugimoto, S., Lechleider, R. J., Shoelson, S. E., Neel, B. G., and Walsh, C. T. (1993) J. Biol. Chem. 268, 22771–22776
29. Yi, T. L., Mui, A. L. F., Krystal, G., and Ihle, J. N. (1993) Mol. Cell. Biol. 13, 7577–7586
30. Kazmowski, M., Minaric, R. I., Feng, G. S., Shen, R., Pawson, T., and Siminovitch, K. A. (1993) J. Exp. Med. 178, 2157–2163
31. Klingmüller, U., Lorenz, U., Cantisley, C. L., Neel, B. G., and Lodish, H. F. (1996) Cell 85, 729–738
32. D’Ambrosio, D., Hipsen, K. L., Minskoff, S. A., Melman, I., Pani, G., Siminovitch, K. A., and Cambier, J. C. (1995) Science 268, 293–297
33. Polakis, P., Zhao, Z. H., Filloux, C., Fischer, E. H., and Van Obberghen, E. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 5002–5006
34. Zhao, Z. H., Shen, S. H., and Fischer, E. H. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 5007–5011
35. Zhao, Z. H., Shen, S. H., and Fischer, E. H. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 4251–4255
36. Bouchard, P., Zhao, Z., Barvville, D., Dumas, F., Fischer, E. H., and Shen, S. H. (1994) J. Biol. Chem. 269, 19885–19892
37. Akiyama, T., Kadokoa, T., and Ogawara, H. (1985) Biochem. Biophys. Res. Comm.
Commun. 131, 442–448
38. Pot, D. A., Woodford, T. A., Remboutsika, E., Haun, R. S., and Dixon, J. E. (1991) J. Biol. Chem. 266, 19688–19696
39. Pumiglia, K. M., Lau, L. F., Huang, C. K., Burroughs, S., and Feinstein, M. B. (1992) Biochem. J. 286, 441–449
40. Kaszkin, M., Fürstenberger, G., Richards, J., Seidler, L., and Kinzel, V. (1991) Cancer Res. 51, 4328–4335
41. Kaszkin, M., Richards, J., and Kinzel, V. (1992) Cancer Res. 52, 5627–5634
42. Pei, D. H., Lorenz, U., Klingmüller, U., Neel, B. G., and Walsh, C. T. (1994) Biochemistry 33, 15483–15493
43. Milarski, K. L., and Saltiel, A. R. (1994) J. Biol. Chem. 269, 21239–21243
44. Bennett, A. M., Tang, T. L., Sugimoto, S., Walsh, C. T., and Neel, B. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7335–7339
45. Xiao, S., Rose, D. W., Sasaoka, T., Maegawa, H., Burke, T. R., Roller, P. P., Shoelson, S. E., and Olefsky, J. M. (1994) J. Biol. Chem. 269, 21244–21248
46. Perkins, L. A., Larsen, I., and Perrimon, N. (1992) Cell 70, 225–236