Both SH2 Domains Are Involved in Interaction of SHP-1 with the Epidermal Growth Factor Receptor but Cannot Confer Receptor-directed Activity to SHP-1/SHP-2 Chimera*

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The previously demonstrated functional and physical interaction of the SH2 domain protein-tyrosine phosphatase SHP-1 with the epidermal growth factor (EGF) receptor (Tomic, S., Greiser, U., Lammers, R., Khartitonekova, A., Imyanitov, E., Ullrich, A., and Böhmer, F. D. (1995) J. Biol. Chem. 270, 21277–21284) was investigated with respect to the involved structural elements of SHP-1. Various mutants of SHP-1 were transiently expressed in 293 or COS-7 cells and analyzed for their capacity to associate with immobilized autophosphorylated EGF receptor in vitro and to dephosphorylate coexpressed EGF receptor in intact cells. Inactivating point mutation of the C-terminal SH2 domain reduced the association weakly, point mutation of the N-terminal SH2 domain reduced association strongly and the respective double mutation abolished association totally. The capacity of SHP-1 to dephosphorylate coexpressed EGF receptor was impaired by all point mutations. Truncation of the N-terminal or of both SH2 domains strongly reduced or abolished association, respectively, but the truncated SHP-1 derivatives still dephosphorylated coexpressed EGF receptor effectively.

Various chimeric protein-tyrosine phosphatases constructed from SHP-1 and the closely homologous SHP-2 dephosphorylated the EGF receptor when they contained the catalytic domain of SHP-1. As native SHP-2, the chimera lacked activity toward the receptor when they contained the catalytic domain of SHP-2, despite their capacity to associate with the receptor and to dephosphorylate an artificial phosphopeptide. We conclude that the differential interaction of SHP-1 and SHP-2 with the EGF receptor is due to the specificity of the respective catalytic domains rather than to the specificity of the SH2 domains. Functional interaction of native SHP-1 with the EGF receptor requires association mediated by both SH2 domains.

The phosphorylation level of activated growth factor receptors with endowed tyrosine kinase activity is the net result of tyrosine specific autophosphorylation (1) and a rapid dephosphorylation by phosphotyrosine-specific phosphatases (PTPs). Since important aspects of receptor signaling depend on receptor autophosphorylation, the dephosphorylation reaction is believed to attenuate the receptor signal. Therefore, the identification and characterization of the PTPs involved in growth factor receptor dephosphorylation is of high interest. The SH2 domains containing PTPs SHP-1 and SHP-2 (2, 3) have been shown to interact with multiple growth factor receptors and to modulate their signaling activity. Transient coexpression of SHP-1 with different tyrosine kinase receptors results in complete or partial dephosphorylation of PDGFα- and β-receptor, insulin-like growth receptor-1 receptor, Kit/SCF receptor, insulin receptor, EGF receptor, and HER2 (4). SHP-2 has little activity with respect to dephosphorylation of associated receptors (4, 5) and rather seems to mediate a receptor signal via as yet not fully understood mechanisms (6–9). One important pathway seems to involve tyrosine phosphorylation of receptor-bound SHP-2 at the C terminus, which leads to subsequent association of Grb2 and activation of the Sos/Ras/Raf/MAPK-signaling cascade (10, 11). Depending on the cellular context, however, SHP-2 may be involved in silencing of certain growth factor receptor signals, as demonstrated for the membrane ruffling response to PDGF-BB in stably PDGF receptor expressing 293 cells (12). Also, SHP-2 has been shown to dephosphorylate the autophosphorylated PDGFβ-receptor preferentially at phosphotyrosines 751 and 771 in vitro (13).

SHP-2 and SHP-1 enzyme activity is negatively controlled by the N-terminal SH2 domain. This inhibition involves an intra- or intermolecular interaction of the SH2 domain with the C-terminal part of the PTP the detailed mechanism of which is yet unknown (14–18). Activation of SHP-1 and SHP-2 toward macromolecular substrates requires occupation of the SH2 domains, a reaction that can be mimicked in vitro by synthetic phosphopeptides (19, 20). An overlapping but not identical substrate specificity of SHP-1 and SHP-2 has been demonstrated with artificial peptide substrates in vitro (21). With some low molecular weight substrates in vitro, in particular p-nitrophenylphosphate at acidic pH, SHP-1 exhibits a higher intrinsic activity than SHP-2 (22, 23).

SHP-1 seems to be involved in negative regulation of receptor signaling and in receptor dephosphorylation in hematopoietic cells, as has been shown for Kit/SCF receptor (24) and CSF-1 receptor (25). Also, receptors without intrinsic tyrosine kinase activity including the erythropoietin receptor (26), the
interleukin-3 receptor (27) as well as the FcγIIb receptor in B cells (28) are subject to negative regulation by SHP-1. Interaction of the erythropoietin receptor with SHP-1 is mediated by the N-terminal SH2 domain of the PTP recognizing the sequence PHLK(P)Y429LYLV in the phosphorylated cytoplasmic domain of the receptor. Recruitment of SHP-1 to the erythropoietin receptor leads to strong elevation of its PTP activity and dephosphorylation of the receptor-associated tyrosine kinase JAK-2 and possibly other tyrosine phosphorylated targets in the proximity of the receptor. In turn, the erythropoietin signal is attenuated (26). Interaction of SHP-1 with the FcγIIb receptor is mediated via the C-terminal SH2 domain of the PTP, recognizing the phosphopeptide sequence ENL(P)YEGLN (28). For the CSF-1 receptor, no direct association of SHP-1 has been detected (24). In this case, the interaction leading to negative regulation might be mediated by the adapter protein Grb2 and another as yet unidentified, Mf 130,000 adapter protein (25). Comparatively little is known about the physiological substrates of SHP-2. SHP-2 potently dephosphorylates its own C terminus, and therefore one function of its PTP activity might be to regulate its own phosphorylation state and thus signaling activity mediated by the C-terminal Grb2 association (29). Recently, the Drosophila protein daughter of sevenless (DOS) has been identified as a target of corkscrew, the Drosophila homolog of SHP-2 (30). It has, therefore, been speculated that mammalian homologs of DOS do exist and possibly present prefer cellular substrates for SHP-2 (30). Apart from hematopoietic cells, SHP-1 is also expressed in epithelial cells (31, 32) and might be important for modulation of resident receptors in these cells. In an attempt to identify PTPs involved in negative regulation of the EGF receptor, we characterized PTP activity copurifying with the receptor from A431 human hematopoietic carcinoma cells (5). Both SHP-1 and SHP-2 were present in the receptor preparations and also associated with the EGF receptor in vivo and in intact A431 cells. We found that SHP-1 avidly dephosphorylates autophosphorylated EGF receptor in vitro and upon transient coexpression with the receptor in 293 cells and presumably contributes to EGF receptor dephosphorylation in A431 cells. SHP-2 had no detectable activity toward the receptor (5). To better understand the SHP-1/EGF receptor interaction and the structural basis for the strikingly different activity of SHP-1 and SHP-2 toward the receptor, we generated a series of SHP-1 mutants and chimeric PTPs from SHP-1 and SHP-2 and analyzed them with respect to association with the EGF receptor and receptor dephosphorylation. We find that both SH2 domains of SHP-1 are important for interaction with the receptor and that the capacity for receptor dephosphorylation is determined by the catalytic domain.

EXPERIMENTAL PROCEDURES

Constructs for PTP Expression, General—The SHP-1 cDNA used throughout this study was obtained by standard procedures from an SK-BR3 cDNA library and corresponds to the 597-amino acid splice variant of SHP-1, which is predominantly expressed in epithelial cells (32). The N-terminal sequence and numbering is identical to the one depicted in Shen et al. (31). Thus, Arg-32 and Arg-136 in our SHP-1 variant correspond to Arg-30 and Arg-136, respectively, of hematopoietic SHP-1 (33). All PCR reactions were carried out with Pfu polymerase (Stratagene, Heidelberg) for 25 cycles. All cloning operations were carried out with the respective DNAs in plBluescript KS(--) (Stratagene, Heidelberg). The final constructs were verified by DNA sequencing (77 sequencing kit, Pharmacia, Uppsala). As an additional step, the cDNA sequences were subsequently subcloned into the eucaryotic expression vector pRK5 RS (34).

Point and Deletion Mutants of SHP-1—The Arg-32 32 Lys (R32K) mutation in the N-terminal SH2 domain was generated by PCR-based mutagenesis, using the primers 1C-E, 5'-AGA TGG AGC ATT CCT ACG AAG AGA TGC TAT GCT CC-3'; RKNSH2, 5'-CAC GGA AGC ATT CCT GCT GAT AAG CCC AGT CGC AAG AAG C-3'; RKNSH2r, 5'-AAATG CTT TGG GCC CCT CTT GCC TGG CTT CAG CAG-3'; and 5'-GCA TGG AGC ATT CCT GCT GAT AAG CCC AGT CGC AAG AAG C-3'. The two PCR fragments obtained with the primer pairs 1C-E/RKNSH2 and RKNSH2/C-HCS) were cloned into plBluescript, reisolated, and thereafter ligated together. Before digestion of this construct yielded a fragment containing the mutated sequence which was used to replace the normal corresponding part in SHP-1 to yield SHP-1 R138K. Exchange of this fragment in SHP-1 R32K yielded the double point mutant SHP-1 R132K, R138K. To obtain SHP-1 A132H, SHP-1 DNA was digested with BstEII and EcoRI. This treatment removed all DNA 5' of the codon for Met-172, leaving Met-172 intact. To obtain SHP-1 32 Lys SH2, SHP-1 DNA was digested with BamHI and XhoI, and the ends were filled with Pfu polymerase. This fragment was ligated into EcoRV-digested pRK5 RS. Ligation of the filled BamHI 5'-end of the SHP-1 fragment to the 5'-end of EcoRV-digested pRK5 RS generated a frame with the remaining sequence of SHP-1 (amino acids 56–597). SHP-1 A132H SH2, R138K was obtained by the identical procedure from SHP-1 R138K.

Construction of SHP-1/SHP-2 Chimera—The chimera are composed of SH2 domains (SH2), the "hinge" part (hg), and the catalytic part (CAT) derived from SHP-1 or SHP-2 and are designated as depicted in Fig. 1, using raised "1" or "2" to indicate the origin of the respective domain. Chimera SH21hg2CAT2, SH22hg1CAT1, SH2hg1CAT, and SH2hg2CAT2 were obtained by PCR amplification of SHP-1 and SHP-2 fragments with suitable cloning sites at one end and EcoRI sites at the internal ends intended for the fusion. The individual fragments were initially separately cloned into plBluescript KS and subsequently joined. Employing this method, an additional Glu-Phe dipeptide sequence corresponding to the EcoRI site was introduced at the fusion site of these chimeric molecules. SH21hg2CAT was constructed using the primers 1C, 5'-AGA TCG TCC CCT ACA GAG AGA TGC TGT CC-3', and 5'-GCC GAA TTC TTC GCT TGG CCG CCC-3', and 5'-GCC GAA TTC TTC GCT TGG CCG CCC-3', to amplify an SH2 fragment with a 5' HindIII and a 3' EcoRI site from SHP-1 DNA. The CAT fragment 5'-GCC GAA TTC GTC TAC CTG CGG CAG CCG TAC-3' and 5'-CCG GAA TTC CTT AAC ACG ACT AGC AGC AGC AGC ACT-3' was digested with HindIII and a 3' XhoI site was generated using the primers 6D, 5'-GCC GAA TTC AAA AAT AAG TAT AAA AAC AAC ATC TGG CCC-3', and 4D, 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3'. Both fragments were cloned in plBluescript KS, joined at the EcoRI site, and subcloned in pRK5 RS. SH21hg2CAT was assembled in the same way from the primers 5'-HindIII, primers 1D, 5'-AGA TCA AGC TGG ATG AGA GAG ACA TGA CAT CGC GG-3' and 5D, 5'-GCC GAA TTC ATT GTT ATT GTT TCT TGT CCT ACC-3', to amplify a SHP-1 fragment with a 5' HindIII and a 3' EcoRI site from SHP-1 DNA. The CAT fragment 5'-GCC GAA TTC GTC TAC CTG CGG CAG CCG TAC-3' and 5'-GCC GAA TTC CTT AAC ACG ACT AGC AGC AGC ACT-3' was digested with HindIII and a 3' XhoI site was generated using the primers 6D, 5'-GCC GAA TTC AAA AAT AAG TAT AAA AAC AAC ATC TGG CCC-3', and 4D, 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3', and 5'-CTA GTC TAG ATC TAG TGA AAT AAC TCT GCT GTT GC-3'. The two PCR fragments generated with the primer pairs 1C-E/RKNSH2 and RKNSH2/C-HCS) were cloned into plBluescript, reisolated, and thereafter ligated together. Before digestion of this construct yielded a fragment containing the mutated sequence which was used to replace the normal corresponding part in SHP-1 to yield SHP-1 R138K. Exchange of this fragment in SHP-1 R32K yielded the double point mutant SHP-1 R132K, R138K. To obtain SHP-1 A132H, SHP-1 DNA was digested with BstEII and EcoRI. This treatment removed all DNA 5' of the codon for Met-172, leaving Met-172 intact. To obtain SHP-1 32 Lys SH2, SHP-1 DNA was digested with BamHI and XhoI, and the ends were filled with Pfu polymerase. This fragment was ligated into EcoRV-digested pRK5 RS. Ligation of the filled BamHI 5'-end of the SHP-1 fragment to the 5'-end of EcoRV-digested pRK5 RS generated a frame with the remaining sequence of SHP-1 (amino acids 56–597). SHP-1 A132H SH2, R138K was obtained by the identical procedure from SHP-1 R138K.

To obtain the chimera SHP-1 tail 5', an existing Plel site in the DNA
corresponding to the highly conserved amino acid sequence VHCSAG (SHP-1 AS 453–458 or SHP-2 AS 457–462) was used. A DNA fragment corresponding to the last 135 amino acids of SHP-2 was excised with PstI from the previously generated CAT² DNA and used to replace the respective fragment in SHP-1 DNA.

**Transfections**—Transfections were carried out using the cytomegalovirus promotor-driven expression vector pRK5 RS, 293 human embryonal kidney cells, or COS-7 cells (German Collection of Microorganisms and Cell Cultures, DSM ACC 60) by a Ca²⁺ precipitation method, as described previously (34).

**PTP Assays**—293 cells were transiently transfected with the various PTP constructs. Cell lysates were obtained in the absence of PTP inhibitors and directly assayed for PTP activity using [³²P]Raytide as a substrate as described previously (5). The amount of lysate protein was titrated in each assay to fall into the linear range of the method. Data from different assay series were normalized by computing the ratio from the activity in lysates of PTP expression plasmid-transfected cells and the activity in the lysate of cells mock-transfected in parallel.

**Antibodies**—Anti-SHP-1 CT was obtained from Santa Cruz Biotechnology (Santa Cruz, CA; catalogue no. sc-287). Anti-SHP-1 SH2 polyclonal rabbit antiserum was described by D'Ambrosio et al. (28) and kindly provided by Dr. J. C. Cambier (Denver, CO). Monoclonal anti-SHP-2 SH2 and polyclonal anti-phosphotyrosine antibodies were from Transduction Laboratories (Lexington, KY; catalogue nos. P17420 and P11230, respectively). The EGF receptor was detected with a polyclonal anti-EGF receptor antibody from Santa Cruz Biotechnology (catalogue no. sc-03) or in some experiments with a monoclonal antibody from Transduction Laboratories (catalogue no. E12020). For association assays, the monoclonal anti-EGF receptor antibody mab425, which is directed against the extracellular domain of the receptor, was used. The latter antibody was kindly provided by Dr. A. Luckenbach (Merck, Darmstadt).

**Association of PTP Derivatives with Autophosphorylated EGF Receptor**—Mab425 anti-EGF receptor antibodies were covalently coupled to
protein A-Sepharose beads (Pharmacia, Uppsala). For this, 1 mg antibody was incubated with 0.6 ml of phosphate-buffered saline-washed protein A-Sepharose for 1 h at 4 °C with end-over-end rotation. The beads were washed three times with phosphate-buffered saline and twice with 0.1 M sodium borate, pH 9.0 (borate buffer), and the volume was adjusted to 1.2 ml with borate buffer. Dimethyl pimelimidate (Sigma) was added to a final concentration of 20 mM, and the mixture was incubated for 30 min at room temperature. The reaction was quenched by two washes with 0.2 M ethanolamine, pH 8.0, and subsequent incubation in this solution overnight at 4 °C. The beads were sequentially washed with 50 mM glycine, pH 3.0, 1 M Tris-HCl, pH 7.4, and twice with phosphate-buffered saline. The association assays were carried out using these beads (“mab425 beads”) as described previously (5). In brief, 20 μl of beads (1:1 suspension) were incubated with lysates of EGF receptor overexpressing or mock transfected 293 cells in the presence or absence of ATP and kinase buffer (see the figure legends). The beads were subsequently washed and exposed to PTP inhibitor-containing lysates of 293 cells overexpressing the different PTP constructs for 1 h at 4 °C. For the direct comparisons made in case of the SHP-1 SH2 domain mutants, the same amounts of expressed PTP were included in the incubations. Thereafter, the beads were washed, and the amount of bound PTP was evaluated by SDS-polyacrylamide gel electrophoresis and immunoblotting.

**Results**

**Effects of SHP-1 SH2 Domain Mutations on the Association of SHP-1 SH2 Domain Mutants with Autophosphorylated EGF Receptor in Vitro.** Lysates of EGF receptor overexpressing 293 cells were incubated in the presence of EGF, ATP and kinase buffer to allow autophosphorylation of EGF receptors (R), control incubations were performed in the absence of either agent (C). The treated lysates were exposed to anti-EGF receptor mab425 beads (see “Experimental Procedures”) to immobilize the EGF receptors. The beads were washed and subsequently incubated with lysates of 293 cells overexpressing SHP-1 SH2 domain mutants, as indicated. Identical amounts of the different SHP-1 mutants were included in the incubation reactions. Bound SHP-1 mutants were visualized by SDS-polyacrylamide gel electrophoresis and immunoblotting using anti-SHP-1 CT antibody. Note that this antibody recognizes the C-terminal end of SHP-1 and therefore reacts with all mutants with the same sensitivity.

**Fig. 2.** Association of SHP-1 SH2 domain mutants with autophosphorylated EGF receptor in vitro. Lysates of EGF receptor overexpressing 293 cells were incubated in the presence of EGF, ATP and kinase buffer to allow autophosphorylation of EGF receptors (R), control incubations were performed in the absence of either agent (C). The treated lysates were exposed to anti-EGF receptor mab425 beads (see “Experimental Procedures”) to immobilize the EGF receptors. The beads were washed and subsequently incubated with lysates of 293 cells overexpressing SHP-1 SH2 domain mutants, as indicated. Identical amounts of the different SHP-1 mutants were included in the incubation reactions. Bound SHP-1 mutants were visualized by SDS-polyacrylamide gel electrophoresis and immunoblotting using anti-SHP-1 CT antibody. Note that this antibody recognizes the C-terminal end of SHP-1 and therefore reacts with all mutants with the same sensitivity.

Effects of SHP-1 SH2 Domain Mutations on the Capacity to Dephosphorylate Coexpressed EGF Receptor—All SH2 domain mutants of SHP-1 exhibited catalytic activity. Lysates of 293 cells overexpressing SHP-1 or the various mutants displayed 1.4–2.2-fold PTP activity compared with mock-transfected cells when assayed toward the synthetic peptide substrate [32P]Ray-tide (not shown). To analyze the effect of SH2 domain mutations on the functional interaction of SHP-1 with the EGF receptor, the various SHP-1 mutants were coexpressed with the EGF receptor in 293 cells and analyzed for their association with immobilized, autophosphorylated EGF receptor. As shown in Fig. 2 (lane 4 versus lane 2), mutation of the N-terminal SH2 domain (SHP-1 R32K) strongly reduced the amount of EGF receptor-bound SHP-1, however, some association was still detectable. Very similarly, truncation of the N-terminal SH2 domain reduced but not abolished the association (Fig. 2, lane 10). The corresponding inactivating mutation of the C-terminal SH2 domain (SHP-1 R138K) reduced the amount of associated SHP-1 weakly but reproducibly (Fig. 2, lane 6). In four experiments the signal of associated SHP-1 R138K was 73 ± 4% compared to native SHP-1 (100%) as revealed by densitometric analysis. The double point mutant SHP-1 R32K,R138K or SHP-1 lacking both SH2 domains (SHP-1-ΔSH2) were completely unable to associate with the EGFR receptor (Fig. 2, lanes 8 and 12). Taken together, the data suggest that both SH2 domains are important for the association of SHP-1 with the EGF receptor; however, the C-terminal SH2 domain contributes to a lesser extent to the association than the N-terminal SH2 domain.

**Effects of SHP-1 SH2 Domain Mutations on the Capacity to Dephosphorylate Coexpressed EGF Receptor—** All SH2 domain mutants of SHP-1 exhibited catalytic activity. Lysates of 293 cells overexpressing SHP-1 or the various mutants displayed 1.4–2.2-fold PTP activity compared with mock-transfected cells when assayed toward the synthetic peptide substrate [32P]Ray-tide (not shown). To analyze the effect of SH2 domain mutations on the functional interaction of SHP-1 with the EGF receptor, the various SHP-1 mutants were coexpressed with the EGF receptor in 293 cells. Subsequently, the cells were stimulated with EGF, and the autophosphorylation level of the EGF receptor coexpressed with the PTP mutants was compared to that of cells expressing EGF receptor in the absence of PTP or in the presence of SHP-1. As shown in Fig. 3A (lane 3 versus lane 2) and demonstrated before (4, 5), coexpression of EGF receptor with native SHP-1 clearly reduced the receptor autophosphorylation level, indicative of receptor dephosphoryl-
Inactivating point mutations in either the N-terminal or the C-terminal or both SH2 domains, strongly impaired the capacity of the respective SHP-1 mutants to dephosphorylate the EGF receptor (Fig. 3A, lanes 4, 6, and 5, respectively) although some residual activity cannot be excluded. Similar results were obtained, when the SHP-1 SH2 domain point mutants were expressed in COS-7 cells, and phosphorylation levels of stimulated endogenous EGF receptors were analyzed. Compared to the reduction of the phosphorylation level caused by expression of SHP-1 (29 ± 18% of control) SHP-1 R32K, SHP-1 R32K,R138K, and SHP-1 R138K reduced the receptor phosphorylation to 54 ± 24, 58 ± 20, and 58 ± 10%, respectively (means of four identical experiments). Thus, in the full-length SHP-1, both SH2 domains seem important for the capacity to dephosphorylate coexpressed EGF receptor.

SH2 domain truncation mutants were likewise analyzed for their activity in the same assay (Fig. 3B). Despite their impaired association with the EGF receptor demonstrated above, SHP-1ΔN-SH2 and SHP-1ΔSH2, exhibited activity toward the coexpressed receptor which was comparable to that of the native SHP-1.

To evaluate the possible role of the C-terminal SH2 domain for the activity of SHP-1ΔN-SH2, its derivative with inactivated C-terminal SH2 domain, SHP-1ΔN-SH2,R138K (Fig. 1) was analyzed for activity. SHP-1ΔN-SH2,R138K was fully active with respect to dephosphorylation of coexpressed EGF receptor (not shown). However, expression of the mutant in both, 293 and COS-7 cells gave rise to a degradation product of similar size as SHP-1ΔSH2, complicating interpretation of this result (see “Discussion”).

Interaction of SHP-1/SHP-2 Chimera with the EGF Receptor—As demonstrated previously (5), SHP-1 and SHP-2 both associate with activated EGF receptor, however, only SHP-1 has the capacity for receptor dephosphorylation. The structural basis for this difference is unknown. Differential SH2 domain specificity, differential catalytic domain specificity (or both) and the structurally divergent C-terminal tails (see Fig. 1) might be important for the differential activity toward EGF receptor. We, therefore, generated a number of “swap” mutations leading to SHP-1/SHP-2 chimeric PTPs (Fig. 1) and analyzed their activity. All chimeric PTPs exhibited activity toward a synthetic phosphopeptide substrate: PTP activity in lysates of 293 cells overexpressing SHP-1, SHP-2 or the different PTP chimera was 1.3–3.1-fold elevated over the activity detectable in mock-transfected cells (not shown), demonstrating that the catalytic domains of the various constructs are functional. Also, all chimeric PTPs associated with autophosphorylated immobilized EGF receptor in vitro (Fig. 4), demonstrating the functionality of the SH2 domains. When coexpressed with EGF receptor in 293 cells, the chimeric molecules fell into two classes with respect to their activity toward the activated receptor: The constructs containing the SHP-2 catalytic domain with the SH2 domain of SHP-1 (with or without the “hinge” domain) failed to dephosphorylate the receptor (Fig. 5, lanes 5, 7 versus lane 1) as did native SHP-2 (lane 3). Thus, the SHP-1 SH2 domains cannot confer the capacity to dephosphorylate EGF receptor to SHP-2. All constructs with the catalytic domain of SHP-1, with the SH2 domains of SHP-2 (with or without hinge domain) or with the C-terminal tail of SHP-2 were active toward coexpressed EGF receptor (Fig. 5, lanes 4, 6, 9, and 11) similar to native SHP-1 (lane 2). Identical results were obtained when the activity of all chimeric PTPs was analyzed toward the endogenous EGF receptor in COS-7 cells (not shown).

**DISCUSSION**

The SH2 domain PTP SHP-1 associates with autophosphorylated EGF receptor and might contribute to EGF receptor dephosphorylation in epithelial cells. In the present study we investigated structural elements of SHP-1 for their importance in the SHP-1 EGF receptor interaction. Both the N-terminal and the C-terminal SH2 domain of SHP-1 contribute to the
rendering an association involving the C terminus unlikely in association of SHP-1 in the absence of intact SH2 domains, (37). For the EGF receptor, we observed very little if any via the C-terminal part to the insulin receptor has been shown under the understanding of the association mechanism. Binding of SHP-1 involved in the SHP-1 interaction are required for a further identification of the structural elements of the EGF receptor third components less likely. Clarifying this point as well as which makes an interaction mechanism involving unidentified diator for the interaction would be needed at abundant levels, rylation in the high level coexpression setting, a putative me-
SHP-1 detected in our assays. However, to mediate dephosphorylation in the high level coexpression setting, a putative me-
diator for the interaction would be needed at abundant levels, which makes an interaction mechanism involving unidentified third components less likely. Clarifying this point as well as identification of the structural elements of the EGF receptor involved in the SHP-1 interaction are required for a further understanding of the association mechanism. Binding of SHP-1 via the C-terminal part to the insulin receptor has been shown (37). For the EGF receptor, we observed very little if any association of SHP-1 in the absence of intact SH2 domains, rendering an association involving the C terminus unlikely in this case.

Inactivating point mutations of both SH2 domains not only impaired association with the EGF receptor, but also impaired the capacity of SHP-1 to dephosphorylate the coexpressed receptor in intact cells. Due to inherent experimental errors, these assays yielded only qualitative results and did not allow to draw any conclusions about subtle differences between the effects of the different PTP mutants. Therefore, we were unfortunately unable to discern whether inactivation of the N-terminal, of the C-terminal SH2 domain, or of both contributes differently to the impairment of receptor-directed activity. In vitro studies with recombinant receptor and PTPs are intended to investigate such likely differences. Truncation of the SH2 domains likewise gradually abrogated association; however, the truncated SHP-1 molecules exhibited activity toward the receptor which was indistinguishable from that of native SHP-1 within the limitations of the experimental design. A mutant with deleted N-terminal SH2 domain and inactivated C-terminal SH2 domain (SHP-1ΔN-SH2,R138K) was likewise fully active, suggesting that the activation by N-terminal truncation overrides the contribution of the C-terminal SH2 domain to activation observed with the full-length SHP-1. However, degradation products resembling SHP-1ΔSH2 in cells overexpressing SHP-1ΔN-SH2,R138K could potentially contribute to the observed dephosphorylation and complicate interpretation of this result. A final clarification of this point has, therefore, to await analysis of activation of the purified recombinant mutant PTP by corresponding EGF receptor phosphopeptides.

Our data are in line with the current concept of SHP-1 activation. For the full-length enzyme, receptor association is required for activation. However, SHP-1 with truncated SH2 domains is constitutively active and can dephosphorylate the receptor in the absence of any association. The latter finding might partially be related to high level overexpression in our cell system. On the other hand, the intriguing implication arises that SHP-1, once activated by binding to an appropriate phosphoprotein could act on other substrates in proximity without the need of a direct association. Such type of interaction has been proposed for SHP-1 and the CD45 receptor (25). As shown previously (4, 5), SHP-2 exhibits no detectable activity toward coexpressed EGF receptor in 293 cells. This finding raised the interesting question, whether the differential activity of SHP-1 and SHP-2 is mediated by the SH2 domains, the catalytic domain, or the C terminus of the molecules, where the protein sequences are most divergent (Fig. 1). To address this question, we generated chimeric PTPs containing the SH2 domains of one PTP and the catalytic part of the other PTP or vice versa and also exchanged the C-terminal tail of SHP-1 for the one of SHP-2. Such a strategy had been proposed earlier by Sun and Tonks (38) to dissect structure-function relationships in this PTP family, but has to our knowledge not yet been applied. Clearly, the SH2 domains of SHP-1, although mediating association of the respective chimera (SH22hg2CAT1 and SH22hg2CAT2), are not sufficient to confer activity toward the EGF receptor. Vice versa, molecules with the SH2 domains of SHP-2 and the catalytic part of SHP-1 (SH22hg2CAT1, SH22hg2CAT2, SH22hg2CAT2, SH22hg2CAT2) are similarly active as native SHP-1 toward the receptor. In this setting it is possible that the SH2 domains of SHP-2 mediate receptor association (as shown) leading to subsequent activation of the chimeric PTPs. If this assumption is correct, the possibly distinct phosphopeptide specificity of the SHP-2 SH2 domains has no influence on the capacity of the chimera to dephosphorylate the receptor. Alternatively, the SH2 domains of SHP-2 might be unable to participate in a regulatory interaction with the C-terminal part of SHP-1, as occurs in native SHP-1, which would in turn lead to a constitutively active chimeric PTP in a similar manner as does truncation of the SH2 domains. Kinetic
investigation of the recombinant chimera is required to resolve this issue. Exchange of the C-terminal tail of SHP-1 for that of SHP-2 did not impair the activity toward the EGF receptor. Thus, the distinct C-terminal sequences are not important for the differential enzymatic activity of SHP-1 and SHP-2 in this system.

Taking all data together, the catalytic domain specificity seems to be the prime determinant for the potent activity of SHP-1 toward the EGF receptor, whereas the SH2 domains play a major role in activity regulation rather than in substrate targeting of SHP-1.

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