The Tyrosine Phosphatase SHP-1 Associates with the sst2 Somatostatin Receptor and Is an Essential Component of sst2-mediated Inhibitory Growth Signaling*

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Activation of the somatostatin receptor sst2, a member of the G protein-coupled receptor family, results in the stimulation of a protein-tyrosine phosphatase activity involved in the sst2-mediated growth inhibitory signal. Here, we report that SHP-1, a cytoplasmic protein-tyrosine phosphatase containing two Src homology 2 domains constitutively associated with sst2 as evidenced by coprecipitation of SHP-1 protein with sst2, in Chinese hamster ovary cells coexpressing sst2 and SHP-1. Activation of sst2 by somatostatin resulted in a rapid dissociation of SHP-1 from sst2 accompanied by an increase of SHP-1 activity. SHP-1 was phosphorylated on tyrosine in control cells and somatostatin induced a rapid and transient dephosphorylation on tyrosine residues of the enzyme. Stimulation of SHP-1 activity by somatostatin was abolished by pertussis toxin pretreatment of cells. Giα3 was specifically immunoprecipitated by anti-sst2 and anti-SHP-1 antibodies, and somatostatin induced a rapid dissociation of Giα3 from sst2, suggesting that Giα3 may be involved in the sst2-SHP-1 complexes. Finally, somatostatin inhibited the proliferation of cells coexpressing sst2 and SHP-1, and this effect was suppressed in cells coexpressing sst2 and the catalytic inactive SHP-1 (C458S mutant). Our data identify SHP-1 as the tyrosine phosphatase associated with sst2 and demonstrate that this enzyme may be an initial key transducer of the antimitogenic signaling mediated by sst2.

Somatostatin is a widely distributed inhibitory hormone that exhibits various biological effects, including neurotransmission, inhibition of exocrine and endocrine secretions, and cell proliferation. The diverse biological effects of somatostatin are mediated through somatostatin receptors that are coupled to a variety of signal transduction pathways including adenylate cyclase, ionic conductance channels, and protein phosphatases (1, 2). Recently five somatostatin receptors have been cloned. They belong to the family of G protein-coupled receptors and can couple to diverse signal transduction pathways (3–7).

The ability of somatostatin and its stable analogues to promote inhibition of normal and tumor cell growth has been known for many years (8, 9). In pancreatic tumor cells, we and others have previously shown that somatostatin and analogues antagonize the mitogenic effect of growth factors acting on tyrosine kinase receptors such as epidermal growth factor (9, 10). Although the molecular events leading to the inhibition of cell proliferation are still poorly understood, it has been shown that, after binding to somatostatin receptors, somatostatin analogues cause a rapid stimulation of a membrane protein-tyrosine phosphatase (PTPase)1 activity and dephosphorylate phosphorylated epidermal growth factor receptors (9, 11, 12) suggesting that a PTPase may participate in the somatostatin-induced inhibition of growth factor-mediated mitogenic signal. Recently, the expression of the sst2 somatostatin receptor subtype in NIH3T3 and Chinese hamster ovary (CHO) cells led us to the demonstration of the direct involvement of sst2 in both the antiproliferative effect of somatostatin and its stimulatory effect on PTPase activity (13, 14). Incubation of cells expressing sst2 with the PTPase inhibitor, vanadate, prevented both effects suggesting that a PTPase may be implicated in the negative growth signal induced by activation of sst2. In addition, we demonstrated that a PTPase of 70 kDa, identified as SHP-1, copurified with membrane somatostatin receptors (15) from pancreatic acinar cells that highly expressed sst2 receptor subtype (16). Taken together, these results suggest that SHP-1 may be a candidate for sst2-mediated early signaling events.

SHP-1 (also named SHPTP-1, SHP, or HCP) is a non-transmembrane PTPase that contains two Src homology 2 (SH2) domains involved in its association with multiple signaling molecules (17–20). SHP-1 associates in vivo with activated growth factor tyrosine kinase receptors such as epidermal growth factor receptor (21). SHP-1 has been shown to associate with activated cytokine receptors, interleukin-3 receptor β chain (22), erythropoietin receptor (23), interferon-α/β receptor (24), and also with B cell FcRIIB receptor (25). As a result of these interactions, SHP-1 becomes tyrosine-phosphorylated by these factors (22, 26, 27). SHP-1 is also tyrosine-phosphorylated in response to activation of G protein-coupled receptors (28, 29).

Recent studies have suggested a role of SHP-1 in terminating growth factor mitogenic signals by dephosphorylating critical molecules. SHP-1 dephosphorylates a variety of protein-tyrosine kinase receptors when coexpressed in 293 cells (30) and has been shown to down-regulate interleukin-3-induced tyro-

1 The abbreviations used are: PTPase, protein-tyrosine phosphatase; CHO, Chinese hamster ovary; sst, somatostatin receptor; MEM, modified Eagle’s medium; FCS, fetal calf serum; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; SMS, somatostatin analogue SMS 201-995.

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sine phosphorylation and mitogenesis in hematopoietic cells (22). Association of SHP-1 with the erythropoietin receptor causes inactivation of Janus kinase 2 and termination of erythropoietin-induced proliferation signal (23). Similarly, association of SHP-1 with interferon-α/β receptor negatively regulates interferon-α/β-induced Janus kinase 1/Stat1 signaling pathway (24). SHP-1 has been also implicated as the mediator used by the FcγRIIB1 receptor in B lymphocytes to turn off B cell antigen receptor signaling (25). The role of SHP-1 in the negative regulation of hematopoiesis is consistent with the various hematopoietic abnormalities and hypersensitivity to growth factors in mice carrying the lethal mutation in the SHP-1 gene that results in motheaten phenotype (31, 32).

If one of the role of SHP-1 is a negative regulation of growth factor signaling, one might therefore speculate that SHP-1 could be activated by factors that negatively regulate cell growth such as somatostatin. In this study, the role of SHP-1 in signal transduction pathway of the G protein-coupled sst2 somatostatin receptor was investigated. Our results provide evidence of physical and functional association of SHP-1 with sst2 and demonstrate that somatostatin is a physiological modulator of SHP-1 that may be required in somatostatin-induced tyrosine phosphatase activation and antiproliferative signals initiated by sst2.

**EXPERIMENTAL PROCEDURES**

**Materials**—SMS 201–995 (SMS) and somatostatin were generous gifts of Dr. C. Bruns (Sandoz, Basel, Switzerland) and Dr. L. Moroder (Munich, Germany), respectively. [Tyr3]somatostatin was purchased from Bachem. |32P|ATP (3,000 Ci/mmol) was purchased from Iso- tophich (France). Enhanced chemiluminescence (ECL) immunodetection system and Hybond ECL nitrocellulose membrane were from Amer- sherm Corp. Poly(Glu, Tyr), cholesterol hemisuccinate, genetin (G418), and Sepharose-protein A beads were from Sigma. Minimal essential medium (MEM), fetal calf serum (FCS), and Lipoftect reagent were from Life Technologies, Inc. CHAPS was from Serva.

**Construction and Expression of sst2-SHP-1 and sst2-SHP-1 (Mutant) in CHO Cells**—The 2.1-kilobase HindIII/Norf fragment of mouse sst1 cDNA (Dr. M. L. Thomas, Howard Hughes Medical Institute, Washing- ton University, St. Louis, MO) was subcloned into the expression vector pCDNA (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen). CHO (DG44 variant) cells were grown to 50% confluency in 60-mm diameter dishes and were cotransfected using pcDNAneo vector (Invitrogen).

**Antibodies**—Polyclonal anti-sst2 antibodies were generated in rabbits immunized with a peptide corresponding to amino acid residues 191–206 of mouse sst2 as described previously (34). The monoclonal anti-SHP-1 antibodies were obtained from Transduction Laboratories (Medgene, France). Monoclonal antibodies raised against phosphoty- rosine (PY-20) were purchased from Santa Cruz Biotechnology (Tebu, France). |32P|ATP was phosphorylated with |32P|ATP as described elsewhere (13). The reaction was initiated by the addition of 30,000 cpm of |32P|-labeled poly(Glu, Tyr) and allowed to proceed for 10 min at 30 °C as described previously (13). PTPase activity was expressed in picomoles of inorganic phosphate released per min at 30 °C from radiolabeled substrate.

**Statistical Analysis**—Statistical comparison between SMS-treated and nontreated cells was performed using Student's paired t test.

**RESULTS**

**SHP-1 Associates with sst2 in Resting CHO/sst2-SHP-1 Cells**—We had previously reported that, in CHO cells expressing sst2 somatostatin receptors, somatostatin analogues stim- ulated PTPase activity (14). Furthermore, in pancreatic cells that highly expressed endogenous sst2, SHP-1 copurified with somatostatin receptors (15). To investigate whether SHP-1 inter- acts with sst2, we stably coexpressed SHP-1 and sst2 in CHO cells. CHO/sst2-SHP-1 clones expressed sst2 as a protein of 95 kDa detected by immunoblotting as previously reported (34) and SHP-1 as a protein of 68 kDa, whereas these proteins were barely detectable in wild CHO cells (Fig. 1). sst2 immu- noprecipitates prepared from CHO/sst2-SHP-1 cells were exam- ined by immunoblotting for the presence of SHP-1. As shown in Fig. 2, the 68-kDa SHP-1 protein was coprecipitated with the 95-kDa sst2 protein. Similarly, SHP-1 immunoprecipi- tation resulted in the coimmunoprecipitation of SHP-1 and sst2. These protein bands were not seen when immunoprecipi- tations were performed in the presence of preimmune serum instead of immune serum. Quantification of immunoblots re-
7.5% SDS-PAGE and subjected to sequential immunoblotting (blot). Arrows indicate the position of sst2 and SHP-1. Size markers (kDa) are with anti-sst2 or anti-SHP-1 antibodies or preimmune serum (PI). SHP-1 (lanes 2 and 3) or monoclonal anti-SHP-1 antibody (lanes 4 and 5) or preimmune serum (PI) (lane 1). The position of molecular mass markers are shown on the side and are indicated in kilodaltons. Arrows indicate the positions of SHP-1 and sst2.

revealed that the amount of sst2 present in the SHP-1 immunoprecipitates (lane ip SHP-1-blot sst2) represents approximately 17–20% of immunoprecipitated sst2 (ip sst2-blot sst2). Taking into account the efficiency of sst2 immunoprecipitation, this represents about 5% of total cellular sst2. From these results, we conclude that SHP-1 physically associates with sst2 and that sst2-SHP-1 complexes are preformed in resting cells.

Somatostatin Promotes Dissociation of sst2-SHP-1 Complexes and Induces Activation and Tyrosine Dephosphorylation of SHP-1—To determine whether the interaction of SHP-1 with sst2 was affected by somatostatin treatment, CHO/sst2-SHP-1 cells were incubated in the presence of the somatostatin analogue, SMS, for various times prior to solubilization and immunoprecipitation with anti-sst2 antibodies. The amount of SHP-1-associated with sst2 was then analyzed by immunoblotting with anti-SHP-1 antibodies and the blots were reprobed with anti-sst2 antibodies or preimmune serum (PI). Arrows indicate the position of sst2 and SHP-1. Size markers (kDa) are indicated to the left of the immunoblot.

We further investigated the effect of SMS on SHP-1 activity in CHO/sst2-SHP-1 cells. Cells were incubated in the presence of 1 nm SMS for various times, after which they were solubilized, and SHP-1 activity was measured in SHP-1 immunoprecipitates. As shown in Fig. 4, SHP-1 activity was increased upon treatment with SMS. The stimulation of SHP-1 activity was maximal after 30 s of SMS treatment and slightly declined up to 10 min.

We then examined the effect of SMS treatment on the level of tyrosine phosphorylation of SHP-1. SMS-treated or untreated cells were subjected to immunoprecipitation with anti-SHP-1 antibodies and immunoblotted with either anti-phosphotyrosine or anti-SHP-1 antibodies. The immunoblot revealed that SHP-1 was tyrosine phosphorylated in untreated cells and that SMS induced a rapid and transient dephosphorylation of SHP-1 (Fig. 5). The dephosphorylation of SHP-1 was observed as early as 30 s after SMS treatment, was maximal at 1 min and declined subsequently until control levels. All these results indicate that SMS induced a transient increase and a subsequent dissociation of preformed sst2-SHP-1 complexes, which was associated with the stimulation and the transient dephos-
phorylation of the enzyme.

Association of sst2 with SHP-1 Involves the Pertussis Toxin-sensitive G Protein, G\textsubscript{i3}—In CHO cells expressing sst2, we previously reported that stimulation of PTPase activity by somatostatin was suppressed by pretreatment of cells with pertussis toxin indicating that a pertussis toxin sensitive G protein was involved in this effect (13). Furthermore, in these cells, sst2 has been demonstrated to be coupled to pertussis toxin-sensitive G proteins, G\textsubscript{a3} and G\textsubscript{a2}, but not to G\textsubscript{i1} and G\textsubscript{i2} (36). Preincubation of CHO/sst2-SHP-1 cells with pertussis toxin for 18 h at 100 ng/ml abolished the stimulatory effect of

\[\text{SMS on SHP-1 activity in SHP-1 immunoprecipitates (not shown) suggesting that sst2-coupled activation of SHP-1 was mediated by a pertussis toxin-sensitive G protein. To identify the G protein involved in the sst2-SHP-1 complexes, SHP-1 and sst2 immunoprecipitates from CHO/sst2-SHP-1 cells were analyzed by immunoblotting with antibodies directed against G\textsubscript{a1}, G\textsubscript{a2}, G\textsubscript{a3}, and G\textsubscript{i3}. G\textsubscript{i3} was immunoprecipitated with anti-sst2 antibodies as well as anti-SHP-1 antibodies, suggesting that G\textsubscript{i3} was present in the sst2-SHP-1 complexes. In contrast, G\textsubscript{i1}, G\textsubscript{i2}, and G\textsubscript{i3} were never detected in the sst2-SHP-1 immunoprecipitates (Fig. 6). In addition PTPase activity can be immunoprecipitated by anti-G\textsubscript{i3} antibodies but not by G\textsubscript{i2} antibodies (not shown). All these results argue in favor of a role for G\textsubscript{i3} in the formation of the sst2-SHP-1 complexes.}

To investigate whether the association of G\textsubscript{i3} with sst2 can be modified by somatostatin, CHO/sst2-SHP-1 cells were treated for various times with 1 nM SMS and G\textsubscript{i3} was identified in sst2 and SHP-1 immunoprecipitates. As observed in Fig. 6, SMS treatment induced a transient increase at 30 s of the amount of G\textsubscript{i3} immunoprecipitated either by anti-sst2 or anti-SHP-1 antibodies which was followed by a rapid decrease of the amount of immunodetected G\textsubscript{i3}. Only 30% of sst2-associated G\textsubscript{i3} was detected after 10 min of SMS treatment. The time course of G\textsubscript{i3} dissociation from sst2 and SHP-1 paralleled that of dissociation of SHP-1 from sst2, suggesting that these events can be linked.

**Activation of SHP-1 by Somatostatin Is Involved in the Antiproliferative Signal Mediated by sst2**—To obtain direct evidence of the role of SHP-1 in the inhibitory effect of somatostatin on cell proliferation, we generated a catalytically inactive SHP-1 by introducing a point mutation into the conserved catalytic residue, cysteine 453, which is crucial for catalytic activity of the enzyme. Cys\textsuperscript{453} was mutated to Ser. This mutation completely abolished the phosphatase activity of the SHP-1 mutant transiently expressed in COS-7 cells.\textsuperscript{2} We stably coexpressed the SHP-1 mutant and sst2 in CHO cells and we selected clones (CHO/sst2-SHP-1(C453S)) that expressed the

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\[\text{Fig. 5. Time-dependent tyrosine dephosphorylation of SHP-1 in response to SMS in CHO/sst2-SHP-1 cells. } A, \text{CHO/sst2-SHP-1 cells were cultured for 24 h in aMEM containing 10% FCS and in serum-free aMEM overnight. Cells were incubated for indicated times at 37 °C with 1 nM SMS and solubilized with 1.5% CHAPS. Cell lysates were subjected to immunoprecipitation (ip) with anti-SHP-1 (SHP-1) antibodies. Immunoprecipitates were resolved by a 7.5% SDS-PAGE and analyzed by immunoblotting with anti-phosphotyrosine antibodies (blot P-Tyr). The same filter was then reprobed with anti-sst2 antibodies, anti-Gi antibodies (not shown). All these results argue in favor of a role for G\textsubscript{i3} in the formation of the sst2-SHP-1 complexes.}

\[\text{Fig. 6. Identification of the G protein subunits in sst2 and SHP-1 immunoprecipitates from CHO/sst2-SHP-1 cells. } \text{CHO/sst2-SHP-1 cells were cultured for 24 h in aMEM containing 10% FCS and in serum-free aMEM overnight. Cells were incubated at 37 °C for the indicated times with 1 nM SMS and solubilized with 1.5% CHAPS. Cell lysates were subjected to immunoprecipitation (ip) with anti-sst2 (sst2) (A) or anti-SHP-1 (SHP-1) (B) antibodies. Immunoprecipitates were resolved by a 7.5% SDS-PAGE and analyzed by immunoblotting with antibodies directed against G\textsubscript{a3} and sst2 (A) or SHP-1 (B). C and D, Immunoblots were densitometrically analyzed, and data were plotted as a percentage of control values obtained from cells at time 0. Data are presented as means ± S.E. of three separate experiments. (Statistical comparison between treated and untreated cells, } p < 0.05.\text{) E, Cell lysates were subjected to immunoprecipitation with anti-sst2 (sst2) or anti-SHP-1 (SHP-1) antibodies. Immunoprecipitates were sequentially analyzed by immunoblotting with antibodies directed against G\textsubscript{a1, a2}, G\textsubscript{i1, i2, i3}, sst2, SHP-1, or preimmune serum (PI).}

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\[\text{\textsuperscript{2} C. Nahmias, unpublished results.}\]
two proteins at a similar level with that observed in CHO/sst2-SHP-1 cells as demonstrated by immunoblotting (Fig. 7). CHO/sst2-SHP-1(C453S) cells were incubated with SMS after which SHP-1 was immunoprecipitated with anti-SHP-1 antibodies, and PTPase activity was measured. SMS no more stimulated SHP-1 activity in cells expressing the SHP-1 mutant (Fig. 8). Expression of sst2 in CHO cells did not modify the serum-stimulated cell growth whereas coexpression of sst2 and SHP-1 inhibited by 39 ± 6% (n = 3) serum-activated cell growth after 3 days of culture when compared with control cells. These results are consistent with the negative role of SHP-1 on CHO cell growth (37). Incubation of wild CHO cells for 24 h in the presence of 1 nM SMS did not modify the serum-stimulated cell growth, whereas SMS inhibited by 36% the growth of CHO/sst2-SHP-1 cells (Fig. 9), in agreement with the growth inhibitory effect of somatostatin analogues in cells expressing sst2 (13, 14). In contrast, the SMS-induced inhibition of cell proliferation was abolished in CHO cells coexpressing sst2 and the SHP-1 mutant. This indicates that the expression of the catalytic inactive SHP-1 blocks the negative regulation of cell growth induced by SMS and demonstrates that SHP-1 may play an important role in the transduction of the negative growth signal promoted by activation of sst2.

**DISCUSSION**

Somatostatin acts as a growth inhibitory factor in a variety of normal and tumor cells (2, 9, 10). We and others have demonstrated that somatostatin and analogues induce the stimulation of a membrane PTPase, which may be involved in the inhibitory effect of these peptides on cell proliferation (9, 11, 12, 38). More recently, we have established the role of the somatostatin receptor sst2 in mediating the stimulatory effect of somatostatin on PTPase activity and its negative effect on cell growth (13, 14). The identification of involved PTPase is therefore important for the understanding of the negative growth signal transduction pathway promoted by sst2. In the present study, we have shown that the phosphotyrosine phosphatase SHP-1 associates with the sst2 somatostatin receptor subtype and established that SHP-1 is involved in the growth inhibitory signal transduction pathway of sst2.

SHP-1 is a cytoplasmic PTPase containing two SH2 domains that enable it to bind specific tyrosine residues of phosphorylated proteins. SHP-1 has been reported to associate with a variety of activated growth factor tyrosine kinase receptors, cytokine receptors, and also with the B cell FcRIB receptor (21–25). We have established that SHP-1 associates with another class of receptor, the G protein-coupled receptor sst2. Results from coprecipitation of SHP-1 with sst2 in CHO cells coexpressing sst2 and SHP-1 provide evidence that SHP-1 associates with sst2 in basal conditions. Specific components mediating this association remain to be determined. However, the demonstration that the G_{i/o} subunit, which is known to couple sst2 receptors at the resting level (36), is present in the sst2-SHP-1 complex and can immunoprecipitate PTPase activity strongly suggests that G_{i/o} could achieve a direct coupling between sst2 and SHP-1. Such a receptor-G protein coupling which is evident in overexpression system (39) has been recently reported for the 5-HT_{1A} receptor which interacts with G_{i/o} in the absence of agonist (40). The molecular base of interaction between G_{i/o} and SHP-1 is not known, but preliminary results suggest that G_{i/o} could be tyrosyl-phosphorylated and therefore interact with the SHP-1 SH2 domains (3). However, our results do not preclude the possibility that another protein may contribute to the interaction of SHP-1 with sst2.

Furthermore, we demonstrated that the occupancy of sst2 promotes a rapid and transient increase (at 0.5 min) of the recruitment of SHP-1 and to a lesser extent that of G_{i/o} to sst2,
which is followed by a rapid dissociation of these molecules from sst2. The weak increase of the interaction between sst2 and either Ga12 or SHP-1 following sst2 stimulation may be related to the known transient nature of the association of receptors with G proteins driven by receptor occupancy (41). A similar dynamic interaction was recently observed with IL-8 receptors that interacted with Ga12 in a time-dependent manner, attaining a maximal level by 1 min and then declining until control levels after 10 min of IL-8 stimulation (42). Moreover, the observation that the interaction of sst2 with Ga12 and SHP-1 was decreased following sst2 activation indicates that somatostatin stimulates the dissociation of the majority of preformed complexes. We also demonstrated that somatostatin activation of sst2 leads to a rapid increase of SHP-1 activity, which is maximal at 0.5 min, maintained as a plateau until 3 min, and then decreased at 10 min. These results argue in favor of the role of SHP-1 in the early events of somatostatin action. One might speculate that, following exposure of cells to SMS, the transient increase of association of SHP-1 to sst2 via Ga12 was accompanied by an activating conformational change of the enzyme, the activation of the enzyme being able to result from the relief of autoinhibition by the SH2 domains (43). The fact that somatostatin-induced stimulation of the enzyme was inhibited by pertussis toxin emphasizes the implication of a Ga12-like protein in this effect and supports the idea that stimulation of SHP-1 by somatostatin results from activation of Ga12. Consistent with these results, we and others have previously reported that the activation of PTPase activity by somatostatin involved a pertussis toxin-sensitive G protein (11, 14). On the other hand, we found that somatostatin induces a rapid dissociation (1 min) of sst2-Ga12-SHP-1 complexes. When dissociation of the complexes start, the enzyme is still active. The dissociation may allow SHP-1 to associate with specific substrates, such as phosphorylated growth factor receptors and downstream molecules, with this probably leading to negative regulation of mitogenic signals by dephosphorylation.

We also demonstrated that, concomitantly with the increase of SHP-1 activity, somatostatin induces a transient dephosphorylation of SHP-1 on tyrosine residues that is maximal at 1 min and returns to basal level at 10 min. These results suggest that activated SHP-1 undergoes a rapid tyrosine autodephosphorylation due to the enzyme activation. This hypothesis is strengthened by the observation that SHP-1 was rapidly autodephosphorylated in vitro, whereas the catalytically inactive mutant SHP-1 was stably tyrosine-phosphorylated (44, 45). In contrast, SHP-1 has been reported to be tyrosine-phosphorylated in response to various growth factors and cytokines receptors as well as mitogenic factors acting on G protein-coupled receptors (22, 26, 27, 29, 46). The role of tyrosine phosphorylation/dephosphorylation of SHP-1 has to be elucidated. Different sites of SHP-1 tyrosine phosphorylation have been identified and may play specific role (44, 47).

SHP-1 was found to play a major role in negatively regulating signaling pathways. For instance, SHP-1 has also been demonstrated to terminate IL-3 and EPO growth signals, its recruitment to the activated receptors causing dephosphorylation and inactivation of specifically associated signaling molecules (22, 23). In CHO cells overexpressing the insulin receptor, a negative effect of overexpressed SHP-1 on cell proliferation has been also reported (37). The demonstration that the negative effect of somatostatin on CHO cell growth can be suppressed in cells coexpressing sst2 and the inactive SHP-1 mutant argues in favor of the role of SHP-1 in the negative growth signal induced by somatostatin-activated sst2. Further, the rapid activation of SHP-1 following somatostatin addition in CHO cells coexpressing sst2 and SHP-1 raises the possibility that activation of SHP-1 is the initiating step for sst2 signal transduction leading to inhibition of cell proliferation. These hypothesis are strengthened by the observation that rat pancreatic tumor AR42J cells are sensitive to the antiproliferative effect of somatostatin analogs and highly express sst2 receptors and SHP-1 (10, 16, 35). Examining SHP-1 expression in cells sensitive to somatostatin analogs would add significantly to these conclusions. It is well known that growth factors transduce cell proliferation signal through activation of receptor tyrosine kinases that phosphorylate downstream enzymes and/or adaptor proteins leading to phosphorylation and activation of mitogen-activated protein kinase. Recent data provide evidence that mitogen peptides acting on G protein-coupled receptors can also promote phosphorylation and activation of mitogen-activated protein kinase via activation of tyrosine kinase (48). Conversely, one could be expected that growth inhibitory peptides acting via G protein-coupled receptors may induce growth inhibition through activation of PTPase resulting in tyrosine dephosphorylation of mitogen-induced tyrosine phosphorylation of signaling molecules. Such a stimulation of PTPase by growth inhibitory peptides acting via G protein-coupled receptors has been previously reported for angiotensin II AT2 receptors (49). The effector molecules that act downstream of sst2-SHP-1 complexes are not known. However, it has been shown that somatostatin dephosphorylates tyrosine phosphorylated EGF receptors (9), suggesting that growth factor receptors could be one of the substrates of SHP-1. sst1 and sst3 somatostatin receptor subtypes expressed in heterologous cells have been also demonstrated to mediate somatostatin stimulation of PTPase activity (14, 50, 51). Whether SHP-1 binds to other somatostatin receptor subtypes has yet to be investigated. If this was the case, then activation of SHP-1 could be an early signal pathway shared by the somatostatin receptor family.

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SHP-1 Associates with sst2