c-Src kinase was activated when either murine NIH3T3 fibroblast cells or immunoprecipitated c-Src proteins were treated with nitric oxide generator, S-nitroso-N-acetyl penicillamine (SNAP) or sodium nitroprusside. Nitric oxide (NO) scavenger hemoglobin and N2O3 scavenger homocysteine abolished the SNAP-mediated c-Src kinase activation. Phosphoamino acid analysis and peptide mapping of in vivo labeled phospho-c-Src proteins revealed that SNAP promoted the autophosphorylation of tyrosine, which preferentially took place at Tyr-416. Peptide mapping of in vivo labeled c-Src kinase excluded the involvement of phospho-Tyr-527 dephosphorylation in the SNAP-mediated activation mechanism. Correspondingly, protein-tyrosine phosphatase inhibitor Na3VO4 did not abolish the SNAP-mediated activation of Src kinase, and the constitutively activated v-Src kinase was also further up-regulated in activity by SNAP. SNAP, however, failed to up-regulate the kinase activity of Phe-416 mutant v-Src. 2-Mercaptoethanol or dithiothreitol, which should disrupt N2O3-mediated S-nitrosylation and subsequent formation of the S-S bond, abolished the up-regulated catalytic activity, and the activity was regained after re-exposing the enzyme to SNAP. Exposure of Src kinase to SNAP promoted both autophosphorylation and S-S bond-mediated aggregation of the kinase molecules, demonstrating a linkage between the two events. These results suggest that the NO/N2O3-provoked S-nitrosylation/S-S bond formation destabilizes the Src structure for Tyr-416 autophosphorylation-associated activation bypassing the Tyr-527-linked regulation.

Nitric oxide (NO), which is synthesized enzymatically from L-arginine and molecular oxygen by nitric-oxide synthases (11) or NO-generating chemicals have been shown to affect a number of biological systems regulating various physiological and biochemical functions (12). Some of these include reduction of protein kinase C activity (13) and activation of p21WAF1 (14). Lander et al. (15) report that treatment of lymphoma cells with NO-generating agents increased the catalytic activity of p56lck kinase, possibly through potentiating the phosphotyrosine phosphatase activity. The present study, to our knowledge, provides the first time evidences that NO released from these chemicals activates Src kinase through a Tyr-527-independent and Tyr-416-linked mechanism, which involves S-nitrosylation/S-S bond-mediated modification of Src molecules.

**EXPERIMENTAL PROCEDURES**

**Cells and Chemicals**—The murine NIH3T3 fibroblast cell line over-expressing c-Src kinase was kindly provided by Dr. D. Shalloway of Pennsylvania State University. v-Src-transformed NIH3T3 cells were from our own stock. The cell lines were cultured in plastic plates with Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37 °C in a 5% CO2, 95% air incubator. After becoming confluent, cells were collected with 0.25% trypsin, 0.01% EDTA in phosphate-buffered saline and were split into 60-mm plastic plates with Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum for a further 20–24 h incubation. The cells were then rinsed with fresh modified Eagle’s medium twice and incubated in modified Eagle’s medium at 37 °C for 1 h before use. S-Nitroso-N-acetyl penicillamine (SNAP) was purchased from Research Biochemicals Int. (Natick, MA), and sodium nitroprusside (SNP), hemoglobin, and 6-homocysteine were from Sigma.

**Electrophoresis and Immunoblotting**—SDS-PAGE and immunoblots were performed as described elsewhere (16). Briefly, proteins obtained from lysed cells or immunoprecipitates (see below) were resolved on SDS-10% polyacrylamide gels and were then transferred to a polyvi...
nylride difluoride membrane. The membrane was incubated with anti-phosphotyrosine polyclonal antibody (Transduction Lab., Lexington, KY) or anti-Src monoclonal antibody (mAb327, donated by Dr. J. S. Brugge, State University of New York; Ref. 17) overnight at 4 °C and then with the appropriate second antibody for 2 h at room temperature. The blots were visualized by Western blot Chemiluminescence Reagent (NEN Life Science Products) as directed by the manufacturer. The molecular sizes of the developed proteins were estimated by comparison with prestained protein markers (New England Biolabs, Beverly, MA).

Immunoprecipitation and in Vitro Kinase Assay—Cells were lysed with 1 ml of ice-cold lysis buffer (100 mM Tris-HCl, pH 7.5, 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 0.5 mM Na3VO4, and 1 mM phenylmethylsulfonyl fluoride). The lysate was centrifuged at 15,000 × g for 30 min at 4 °C, and anti-Src monoclonal antibody (mAb327) was added to the supernatant. The immunoprecipitates were collected by incubating with protein A-Sepharose beads (Pierce) that had been conjugated with rabbit anti-mouse IgG antibody (MBL, Nagoya, Japan). The beads bearing the immunoprecipitates were washed three times with cold lysis buffer and used for either immunoblot or in vitro kinase assay.

In vitro kinase assay was done as described previously (18). Briefly, the immunoprecipitated Src proteins were washed 3 times with kinase buffer (10 mM Tris-HCl, pH 7.4, 5 mM MgCl2), suspended in the same buffer with 1.5 μg of acid-denatured enolase (Sigma) as an exogenous substrate, and radiolabeled [γ-32P]ATP (370 kBq) (NEN Life Science Products). This mixture was further added with or without chemicals as modulators for kinase assay. The kinase reaction was carried out for 20 min at 30 °C and was stopped by adding 30 μl of 2× SDS sample buffer with 2-mercaptoethanol (2-ME) unless otherwise noted. The immunoprecipitates were then heated in a boiling water bath for 3 min, and phosphoproteins were analyzed on 10% SDS-polyacrylamide gels. Gels were dried and exposed to x-ray film at ~80 °C for autoradiography.

Phosphoamino Acid Analysis—32P-Labeled Src protein bands were cut from the dried gel and rehydrated with 50 mM NH4HCO3. After rehydration, gel slices were cut into smaller pieces and incubated at 37 °C overnight with the addition of 50 μg/ml proteinase K. After centrifugation the supernatant was freeze-dried, dissolved in 6 N HCl, and incubated at 85 °C for 1 h. The HCl was removed completely by repeated freeze-drying. The hydrolysates thus obtained were mixed with a mixture of marker amino acids containing phosphoserine, phosphothreonine, and phosphotyrosine at a concentration of 1 mg/ml each. Samples were then analyzed in two dimensions on cellulose-coated glass plates (20 × 20 cm, Merck) by electrophoresis at pH 1.9 in the first dimension and pH 3.5 in the second dimension as described by Hunter and Sefton (19). After the second dimension electrophoresis, plates were dried and marker phosphoamino acids were detected by staining with ninhydrin, and the labeled phosphoamino acids were detected by autoradiography.

In Vivo Radiolabeling and Phosphopeptide Mapping of Src Proteins—NIH3T3 cells were labeled in vivo with phosphorus-32 (H3PO4, 10 mM/cm² NEN Life Science Products) in phosphate-free medium with 2% fetal calf serum at a final concentration of 1 mM/cm² for 8 h as described previously (7). Phosphorus-32-containing medium was discarded, and cells were washed three times with modified Eagle's medium. The cells were then lysed, and Src proteins were immunoprecipitated followed by analysis on 10% SDS-polyacrylamide gels. The in vitro and in vivo labeled Src proteins were excised from the dried gel and digested with 50 mg/ml cyanogen bromide (CNBr, Sigma) in 70% formic acid at room temperature for 1 h. Products were lyophilized four or five times with 1 ml of distilled water until the pH became neutral. The cleaved peptides were subjected to electrophoresis on a 24% acrylamide, 0.054% bisacrylamide gel (20, 21). Gels were dried and exposed to x-ray film as described above.

Construction of Phe-416 Mutant v-Src—A cDNA clone containing the entire coding sequence of v-Src gene, obtained from Schmidt-Ruppin strain of Rous sarcoma virus, was inserted between BglII and EcoRI sites of pBabe puro. Mutation for tyrosine 416 to phenylalanine was introduced by polymerase chain reaction (22, 23). In brief, primers containing the mutation were synthesized and used for amplification of v-Src sequences. The corresponding sequence of the v-Src gene was replaced with the mutated fragment. The mutated fragment was then sequenced to confirm that proper mutation was introduced.

RESULTS

NO Generator SNAP or SNP Activates c-Src Kinase Activity—When NIH3T3 cells overexpressing c-Src were incubated with 100–1000 μM SNAP or SNP, a number of cellular proteins were phosphorylated at the tyrosine residue (Fig. 1A), and the c-Src kinase activity was elevated compared with that in untreated (Nil) cells (Fig. 1B). The increase in catalytic activity of the kinase was shown for both autophosphorylation and phosphorylation of enolase, which was used as an exogenous substrate.

We next tested the direct effect of SNAP on the kinase activity of c-Src protein that had been isolated by immunoprecipitation from cell lysates of NIH3T3 cells. The results are shown in Fig. 1C. Compared with no SNAP addition control (Nil), 5 and 50 μM SNAP definitely promoted the c-Src kinase activity as shown by elevated autophosphorylation and phosphorylation of enolase. As low concentration as 0.5 μM SNAP was also minimally effective, whereas its high concentration, e.g., 500 μM, did not elevate the kinase activity over the control level (data not shown). A similar but less marked effect was observed with another NO generator SNP (data not shown).
A question arose whether the SNAP-mediated in vitro c-Src kinase activation was actually mediated by NO released from SNAP or its oxidized derivative N\(\text{O}_3\), which may be in equilibrium with NO (24). We showed that the SNAP-mediated c-Src kinase activation was prevented both by NO scavenger hemoglobin (25, 26) and by N\(\text{O}_3\) scavenger homocysteine (27) (Fig. 1D). This result supported the view that NO, or more specifically, its oxidized derivative N\(\text{O}_3\) was the ultimate effecter. Hemoglobin treatment caused even lower phosphorylation of c-Src and enolase than the basal control (Nil).

The concentration of SNAP needed for activation of Src kinase in cells (100–1000 \(\mu\)M; Fig. 1B) was much higher than that for activation of the isolated enzyme (5–50 \(\mu\)M; Fig. 1, C and D). Therefore, we tested whether the high concentration of SNAP-induced increase in enzyme activity in cells was really mediated by NO released from SNAP. As shown in Fig. 1E, NO scavenger hemoglobin completely inhibited the SNAP-induced activation of Src kinase. There might also be a possibility that the increase in Src kinase activity of the cells treated with SNAP (See Fig. 1B) was due to the NO action on Src molecules after cell lysis. To verify this we lysed the cells after stimulating with SNAP in the presence of hemoglobin to scavenge the possible action of NO after cell lysis. This treatment did not inhibit the in vivo SNAP effect to promote the enzyme activity (Fig. 1E), excluding the above mentioned possibility.

**Phosphoamino Acid and Phosphopeptide Mappings of in Vitro Radiolabeled c-Src Proteins**—We examined whether the promotion of c-Src kinase autophosphorylation by SNAP was at tyrosine residues. We performed phosphoamino acid analysis of c-Src kinase that had been excised from the dried gel after in vitro kinase assay. As shown in Fig. 2A, phosphorylation was only demonstrated at tyrosine but not at serine or threonine, and the phosphorylation at tyrosine was preferentially promoted by SNAP. This observation also indicated that the mechanism of the Src kinase activation mediated by SNAP does not involve an increase in phosphorylation at serine or threonine residues.

We next performed the CNBr cleavage mapping of in vitro activated autophosphorylated c-Src kinase to define whether the elevated tyrosine phosphorylation was linked to phosphorylation at Tyr-416 or Tyr-527. The result, as shown in Fig. 2B, indicated that only a 10-kDa peptide that should contain Tyr-416 (20) had been labeled with \(^{32}\)P, and SNAP treatment preferentially increased this labeling. From this result it is evident that SNAP activates the Src kinase in vitro linked to selective promotion of autophosphorylation at Tyr-416.

**Phosphopeptide Mapping of in Vivo Radiolabeled c-Src Proteins**—c-Src kinase remains inactivated in the resting cells due to phosphorylation of its carboxyl-terminal tyrosine 527 (3–5), and the enzyme becomes activated when phospho-Tyr-527 is dephosphorylated (2, 3). We then performed experiments to test whether phospho-Tyr-527 dephosphorylation would be involved in the mechanism of SNAP-mediated in vitro activation of Src kinase as shown in Fig. 1B. Cells were labeled with \(^{32}\)P in the presence or absence of SNAP followed by immunoprecipitation and SDS-PAGE. CNBr cleavage mapping was done with the \(^{32}\)P-labeled phosphorylated Src proteins that had been excised from the gel of SDS-PAGE. As shown in Fig. 3, in vivo labeling occurred mainly on 4- and 31-kDa peptides that contain Tyr-527 and Ser-17, respectively (7, 20). SNAP treatment did not reduce the overall labeling of the 4-kDa peptide, suggesting that dephosphorylation of phospho-Tyr-527 is not involved in the SNAP-mediated in vivo Src kinase activation. Interestingly, an additional labeling developed on 10-kDa peptide containing Tyr-416 upon treatment with SNAP. This result may suggest that SNAP-mediated in vivo activation of Src kinase also involves Tyr-416 phosphorylation.

**SNAP-mediated in Vitro Src Kinase Activation Is Phospho-Tyr-527 Dephosphorylation-independent**—There is a possibility that the potentially co-immunoprecipitated phosphotyrosine phosphatase, which dephosphorylates phospho-Tyr-527, could be involved in the mechanism of SNAP-mediated in vitro c-Src kinase activation. This is, however, not very likely, because c-Src proteins were prepared for in vitro kinase assay from the cell lysates in the lysis buffer containing Na\(_3\)VO\(_4\), a potent phosphotyrosine phosphatase inhibitor. To further rule out this possibility we treated the immunoprecipitated c-Src protein again with Na\(_3\)VO\(_4\) immediately before in vitro kinase
SNAP-mediated activation of Src kinase is independent of phosphotyrosine phosphatase/Tyr-527-linked control. A, NIH3T3 cells were lysed, and c-Src proteins were immunoprecipitated. Then 5 μM SNAP or 500 μM Na$_2$O$_3$ followed by 5 μM SNAP at a 20-min interval was added to the reaction mixture of immunoprecipitated Src proteins for the kinase assay. Left lane, no addition (Nil), control. B, NIH3T3 cells that overexpress c-Src (left panel) or v-Src (right panel) were lysed, and c-Src and v-Src proteins were immunoprecipitated and treated with 5–50 μM SNAP for the kinase assay. Positions of molecular mass markers are shown on the left, and those of the Src protein and enolase (E) are shown on the right. A representative of three experiments with consistent results is shown.

SNAP-mediated in Vitro Src Kinase Activation Is Tyr-416-linked—To examine whether Tyr-416 autophosphorylation is the major event in SNAP-mediated in vitro activation of Src kinase, we prepared v-Src with a mutation at tyrosine 416, replacing with phenylalanine (Phe-416 mutant v-Src), and tested the mutated enzyme with SNAP for in vitro kinase assay. The background enzyme activity of the Phe-416 mutant v-Src to phosphorylate substrate was lower than that of the wild type v-Src (left panel). These results suggested that the dephosphorylation of phospho-Tyr-527 is not a prerequisite for the Src kinase activation mediated by SNAP.

SNAP-mediated Src Kinase Activation Results from a Sulfhydryl (SH) Group Modification-mediated Reversible Mechanism—The possible role of SH group(s) modification in the mechanism of SNAP-mediated activation of Src kinase was then investigated. As expected from the earlier reports (28–30) on chemical properties of NO and related chemical compounds, we thought that NO released from SNAP or its oxidized derivative N$_2$O$_3$ would react with thiol group(s) of Src molecules to form protein-S-NO, which might eventually form disulfides (S-S) with vicinal thiols by oxidation-reduction processes. This change might underlie the mechanism of SNAP-induced Src kinase activation. If this is the case, disruption of S-S bond(s) originally formed by NO/N$_2$O$_3$ through S-nitrosylation would abolish the SNAP-mediated increase in the kinase activity. As shown in Figs. 6A (top) and 6B, SNAP-activated c-Src kinase (lane 2) lost its high catalytic activity when treated with reducing agents such as 2-ME and dithiothreitol (lane 3), which should interrupt the NO/N$_2$O$_3$-mediated SH group modification forming S-S bond. It is noted that the treatment of the kinase with 2-ME also diminished the spontaneous increase in the catalytic activity of the kinase in vitro (compare lane 3 and lane 5 with lane 1 as control in Fig. 6A). Surprisingly, however, by re-exposing the 2-ME-treated kinase after washing to SNAP, the catalytic activity of the enzyme was re-established (lane 4). During washing, after treating with 2-ME of the Src kinase, some of the proteins might have been lost. To verify this possibility we performed an immunoblot experiment using anti-Src antibody with individual samples. As shown in Fig. 6A...
Nitric Oxide Controls Src Kinase Activity

Fig. 7. Linkage between SNAP-mediated activation and aggregation of Src molecules. c-Src proteins isolated by immunoprecipitation were incubated with or without 5 μM SNAP in duplicate in kinase buffer at 30 °C for 20 min together with [γ-32P]ATP. The reaction was stopped by adding 2-ME (+) (left panel) and 2-ME (−) (right panel) sample buffer, and the Src proteins were then subjected to SDS-PAGE. Positions of molecular mass markers are shown on the left, and those of the monomeric and aggregated Src proteins are shown on the right. A representative of three experiments with consistent results is shown.

(bottom), however, the amount of Src protein remained basically the same among the samples. These results suggested that the Src kinase was subjected to a reversible SH group modification for either activation or inactivation. A question arises here whether some oxidants would show similar effects or not. To verify this point, we added hydrogen peroxide to the isolated Src kinase for analyzing activity of the enzyme. As shown in Fig. 6C, hydrogen peroxide also activated Src kinase in a concentration-dependent manner. This result suggested that a redox mechanism possibly following S-nitrosylation with N2O3 for S-S bond formation would play an important role in the chemical pathway of Src kinase activation.

SNAP-mediated Src Kinase Activation Accompanies Promotion of S-S Bond-mediated Aggregation of the Kinase Molecules—We next conducted an experiment to ask if the Src proteins are actually subjected to modification due to S-S bond formation as a manifestation of the NO/N2O3-provoked mechanism. After incubation of c-Src proteins with or without SNAP together with [γ-32P]ATP for the kinase assay, the reaction was stopped by adding sample buffer plus or minus 2-ME. As shown in Fig. 7, for both SNAP-untreated and -treated assays, considerable amounts of autophosphorylated Src proteins were detected at the upper portion of the separating gel under unreducing conditions (right panel), which were dissociated under reducing condition (left panel). The ratio of aggregated to non-aggregated autophosphorylated Src proteins from the SNAP-treated assay, determined using appropriate computer software, was 1.61, which was higher than 0.94, the ratio of untreated control. This result evidenced the involvement of the S-S bond-mediated modification of the kinase molecules in the mechanism of the SNAP-induced kinase activation.

DISCUSSION

Here for the first time we report evidences of an NO/N2O3-provoked redox-based chemical reaction-mediated, Tyr-416 autophosphorylation-linked mechanism for Src kinase activation, which bypasses the known phospho-Tyr-527 dephosphorylation (2, 3) or amino-terminal serine/threonine phosphorylation (31, 32) linked control. This evidence includes 1) activation of c-Src kinase either in vivo (in cells) or in vitro by treatment with NO-releasing SNAP or SNP (Fig. 1, B and C) and inhibition by treatment with NO-scavenging hemoglobin and by N2O3-scavenging homocysteine (Fig. 1D); 2) selective promotion by SNAP of phosphorylation at Tyr-416 of the Src kinase (Fig. 2); 3) no dephosphorylation of Tyr-527 after in vivo treatment of cells with SNAP (Fig. 3); 4) failure in prevention of the SNAP-mediated Src kinase activation with Na2VO4 as a phosphotyrosine phosphatase inhibitor (Fig. 4A) and further activation by SNAP of constitutively activated Tyr-527-defective v-Src kinase (Fig. 4B); 5) failure in promotion by SNAP of the kinase activity of Phe-416 mutant v-Src (Fig. 5); 6) mutually counteracting reversible controls of Src kinase activity by SNAP and reducing agents such as 2-ME (Fig. 6A) and dithiothreitol (Fig. 6B); 7) association of the SNAP-mediated Src kinase activation with promotion of S-S bond-mediated aggregation of the Src molecules (Fig. 7).

The effective concentration of SNAP that increased the catalytic activity of Src kinase was 100–1000 μM for cells (Fig. 1B, Fig. 3) and 5–50 μM for isolated Src proteins (Fig. 1C and others). We showed that activation of Src kinase by exposing the cells to 1000 μM SNAP (Fig. 1E) and by exposing isolated Src proteins to 50 μM SNAP (Fig. 1C) was inhibited by NO scavenger hemoglobin. Activation of Src kinase by the latter was also prevented by N2O3 scavenger homocysteine. These results proved the involvement of NO and N2O3, a closely related oxidized derivative of NO, in the activation mechanism of both Src kinase in cells and isolated Src proteins. Earlier studies examined the release of NO from SNAP and SNP, showing that millimolar concentrations of these NO donors release free NO at concentrations in the physiological range (33–36) and demonstrated that 50–1000 μM NO donors are needed for NO-mediated regulation of activities of protein kinase C (13), matrix metalloproteinase (37, 38), prostaglandin H synthase (34), N-methyl-D-aspartate receptor-channel complex (28), and iron-responsive element-binding protein (39). In our experimental system only a small portion of NO released from SNAP that was added to the culture of cells probably entered the cell to affect the intracellular Src kinase, and low concentrations (0.5–5 μM) of SNAP were shown to be effective in activating isolated Src kinase. Taken together, we consider that the effective concentration of NO released from SNAP in the present study can be physiologically relevant.

It has been reported earlier that NO/N2O3 can aid S-S bond formation (28–30) through reaction of protein-SH with NO/N2O3 to generate protein-S-NO, which then oxidizes with another protein SH (28). This NO/N2O3-induced intermolecular S-S bond formation must underlie the SNAP-mediated Src protein aggregation that accompanied increased autophosphorylation, although NO/N2O3 might also induce Src protein modification by S-nitrosylation and subsequent intramolecular S-S bond formation. There are nine cysteines in c-Src and v-Src, some of which could be located in a position to be potentially modifiable externally. Our data suggest that reaction of NO/N2O3 with these cysteine residues facilitates formation of the S-S bond between Src molecules, thereby inducing their aggregation. The demonstrated aggregation of Src proteins due to intermolecular S-S bonds might mimic intracellular accumulation of those kinase proteins following ligand-mediated cross-linkage of cell surface receptors with which the kinase proteins should associate and that are thereby crucial for Src kinase activation.

Recently defined tertiary structures of the Src and Src family kinases (8–10) demonstrate that associations between the tail phospho-Tyr-527 (in case of c-Src) and SH2 domain and between the SH3 domain and kinase N-lobe domain connected by the SH2 kinase linker sequence stabilize the whole molecular structure of the kinase. This might preclude the Tyr-416 (in the case of c-Src) phosphorylation-linked local switch in the kinase domain (40). Dephosphorylation of phospho-Tyr-527 or binding of the SH3 domain to some proline-rich sequences destabilizes the structure to turn the local switch on for activation (8–10).
Our present results suggest an alternative pathway to destabilize the kinase structure for activation through compelling individual Src molecules to interact with each other by S-S bond. We do not, however, exclude the possibility that some modification through S-nitrosylation and redox reaction for intramolecular S-S bond formation, which could occur in addition to intermolecular S-S bond formation, might also be involved in the mechanism of Src kinase activation. In any case, the newly demonstrated mechanism for Src kinase activation did not work all on the F416 mutant Src kinase. It should therefore be that both, formerly known Tyr-527-dependent and new Tyr-527-independent, mechanisms put on the Tyr-416 phosphorylation-linked local switch for Src kinase activation.

c-Src in resting cells is basically in an inactive form (3–5) but is partially activated spontaneously due to a yet unexplained reason when isolated in vitro. Because treatment of the Src kinase with reducing agents in vitro reversibly diminished the catalytic activity and because the environment inside the cell is normally reducing, the reducing condition may be physiologically crucial for maintaining the kinase inactive in the resting cells. The demonstrated SH group modification or redox-linked mechanism could work when cells bearing Src kinase are put in the pathological microenvironment where NO/\textsubscript{2}NO\textsubscript{3} or superoxide is produced by inflammatory cells in response to microorganism invasion. The same mechanism might also play a role in the yet unidentified physiological pathway of Src kinase activation, possibly providing the second messenger of the putative cell surface receptor-mediated signal.

In short, the present results support the following summarized view. The Src molecule is normally stabilized under reducing conditions through intradomain association between SH2 and tail phospho-Tyr-527 and among SH3, SH2-kinase linker, and kinase N-lobe, which puts off the local switch for the kinase activation. At least two different mechanisms could destabilize the kinase structure to put on the Tyr-416 phosphorylation-linked local switch for increased catalytic activity. One is through dissociation of the tail from SH2 by phospho-Tyr-527 dephosphorylation, and another is through SH group or redox reaction-mediated modification of Src proteins including intermolecular S-S bond formation for their aggregation.

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