The Noncatalytic C-terminal Segment of the T Cell Protein Tyrosine Phosphatase Regulates Activity via an Intramolecular Mechanism

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Human T cell protein tyrosine phosphatase (TCPTP) is a nontransmembrane enzyme, the first of the protein tyrosine phosphatase family to be cloned. Alternative mRNA splicing results in variation in the sequence at the extreme C terminus of TCPTP and generates a 45-kDa form (TC45) that is targeted to the nucleus and a 48-kDa variant (TC48) associated with membranes of the endoplasmic reticulum. In this report, we assessed the role of the C-terminal, noncatalytic segment of TCPTP in regulating activity, concentrating primarily on the TC45 variant. We have demonstrated that limited tryptic proteolysis of TC45 releases first a 42-kDa fragment, then a 33-kDa catalytic domain. Using reduced carboxyamidomethylated and maleylated lysozyme as substrate (RCML), the catalytic domain displays 20–100-fold more activity than the full-length enzyme. Analysis of the time course of limited trypsinolysis revealed that proteolytic activation occurred following cleavage of a protease-sensitive region (residues 353–387) located at the C terminus of TC45. The activity of truncation mutants illustrated that removal of 20 C-terminal residues was sufficient to activate the enzyme fully. The 33-kDa catalytic domain, but not the full-length enzyme, was inhibited in a concentration-dependent manner by addition of the noncatalytic C-terminal segment of TC45. A monoclonal antibody to TCPTP, CF4, which recognizes an epitope located between residues 350 and 363, was capable of fully activating TC45. These data indicate that the noncatalytic segment of TC45 contains an autoregulatory site that modulates activity via a reversible intramolecular interaction with the catalytic domain. These studies suggest that the C-terminal noncatalytic segment of TC45, and possibly TC48, may not only direct the enzyme to different subcellular locations but may also modulate activity in response to the binding of regulatory proteins and/or posttranslational modification.

Protein tyrosine phosphatases (PTPs) are a diverse family of enzymes that act together with protein tyrosine kinases to govern the level of tyrosine phosphorylation and, therefore, are critical regulators of diverse physiological processes such as cell growth, gene expression, differentiation, cytoskeletal dynamics, and cell motility (1, 2). Enzymes of the PTP superfamily possess a conserved active site motif, HCX2R, and utilize a common mechanism of phosphate hydrolysis involving the formation of a phosphothiol intermediate with the invariant cysteine (1–4). The tyrosine-specific enzymes exist as both transmembrane receptor-like and intracellular nontransmembrane forms. Most intracellular PTPs contain a noncatalytic segment of variable size and sequence that is linked either to the N or C terminus of the catalytic domain. In certain PTPs, noncatalytic segments are involved in directing subcellular localization and in regulating activity (2).

The human T cell phosphatase (TCPTP) is an intracellular PTP that was originally cloned from a T cell cDNA library (5) but is now known to be expressed in many human tissues. Mouse and rat TCPTP homologs have also been cloned, expressed, and characterized (6, 7). TCPTP contains a conserved catalytic domain and a noncatalytic C-terminal domain that varies in its size, hydrophobicity, and function as a result of alternative mRNA splicing. Two splice variants of TCPTP differing only at their extreme C termini are expressed in humans and rodents (6, 8, 9). The 48-kDa splice variant of human TCPTP (TC48) contains a 34-residue hydrophobic tail, which is replaced by a hydrophilic six-residue sequence in the 45-kDa form (TC45), as shown in Fig. 1A. Alternative splicing provides a means to control the subcellular location of TCPTP since the TC48 variant is localized to the endoplasmic reticulum (ER), whereas TC45 is found in the nucleus (10–13). The hydrophobic tail as well as residues 346–358 are required for targeting TC48 to the ER (11). TC45 has two basic clusters (residues 350–358 and 377–381) that form a bipartite nuclear localization signal (NLS) (11–13). The hydrophobic C-terminal tail of TC45 must override the bipartite NLS to permit targeting to the ER (11).

Limited proteolysis converts TC48 to a 33-kDa fragment and results in a 30-fold increase in activity toward RCML and a 2-fold decrease with myelin basic protein (14, 15). A 37-kDa truncated form of TC48 (residues 1–317) lacking most of the C-terminal noncatalytic segment had similar enzymatic prop-

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The abbreviations used are: PTP, protein tyrosine phosphatase; TCPTP, T cell protein tyrosine phosphatase; TC45, 45-kDa TCPTP splice variant; TC48, 48-kDa TCPTP splice variant; RCML, reduced, carboxyamidomethylated, and maleylated lysozyme; pNPP, p-nitrophenyl phosphate; PMSF, phenylmethylsulfonyl fluoride; GST, glutathione S-transferase; PCR, polymerase chain reaction; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; MALDI-TOF, matrix-assisted laser desorption-time of flight; NLS, nuclear localization signal; ER, endoplasmic reticulum; Mes, 4-morpholineethanesulfonic acid; Tricine, N-tris(hydroxymethyl)methylglycine.

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A common feature shared by most autoinhibited enzymes is their ability to be activated by limited proteolysis, which destroys or releases their autoinhibitory domains. The response of TCPTP to limited proteolysis and to truncation suggests that its C-terminal domain might regulate activity via a similar intramolecular mechanism.

Despite being one of the first PTPs identified, the function(s) of TCPTP is unknown. Identification of the regulatory mechanisms controlling the activity of TCPTPs will be required to fully understand its physiologic function. Toward this end, we have examined the role of the C-terminal domain in regulating the activity of TC45. Limited proteolysis and truncation mutagenesis were used to define the domain substructure of TC45 and to identify regions within the noncatalytic C-terminal segment that are involved in controlling activity. Additional analyses provided direct evidence that the C-terminal segment of TC45 can regulate activity through an intramolecular interaction with the catalytic domain.

EXPERIMENTAL PROCEDURES

Construction of Plasmids for Expression of TC45 and TC45 Mutants—PCR was used to prepare a DNA fragment encoding the full-length TC45 splice variant using a TC45 cDNA (5) as template. The following primers were used: forward, 5′-CGTGGATCCCATGGGGGTTGGGACTT-3′; reverse, 5′-CCCGGTATTACCTATCGTTTGT-3′. PCR products were ligated into the BamHI-EcoRI-di- gestion site in pET-TC45. The authenticity of all mutants was confirmed by nucleotide sequencing.

Expression of GST Fusion Proteins of the C-terminal Segments from TC45 and PTP1B—The noncatalytic C-terminal segments of TC45 and PTP1B (24) were expressed in E. coli as thrombin-cleavable GST fusion proteins. The expression of the fusion proteins in E. coli was controlled by the tac promoter.

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protein concentration of about 4 mM. Phosphatase Assays—RCML (Life Technologies, Inc.) and casein (Sigma) were phosphorylated using GST-Lyn kinase with 4 mM [γ-32P]ATP (400–800 cpm/pmol) as described (26). Protein substrates were assayed at 30 °C for 10 min in reaction buffer containing 50 mM imidazole, pH 7.2, 0.1% 2-mercaptoethanol, 1 mg/ml bovine serum albumin as described previously (26). Assays using p-nitrophenyl phospho-
te (pNPP) as substrate were performed as described (26) in buffer containing 25 mM imidazole, pH 6.5, 0.1% 2-mercaptoethanol, 25 mM pNPP at 30 °C for 10 min.

Limited Proteolysis—Limited tryptic cleavage was performed at 30 °C in 1.0 ml of buffer containing 200 mM Tris-HCl, pH 8.0, 2 mM CaCl2, 50 μg of TC45, and various weight ratios of L-1-phenylethyl chloromethyl ketone-treated trypsin (Sigma) to TC45 as indicated. For SDS-PAGE analysis, reactions were terminated at varying times by trichloroacetic acid precipitation. A 100-μl aliquot of the reaction was mixed with 185 μl of 20% (w/v) trichloroacetic acid and centrifuged. Precipitates were washed three times with acetone, dissolved in SDS-PAGE loading buffer, and electrophoresed on SDS gels containing 12% (w/v) polyacrylamide. For zero time points, samples were withdrawn prior to adding trypsin. To collect samples for both PTP assays and SDS-PAGE, a 99-μl aliquot of the reaction was withdrawn at each time point and added to a tube containing 1 μl of 100 mg/ml PMSF. A 50-μl aliquot of the PMSF-treated sample was transferred to an HPLC column containing 10 μl of trypsin inhibitor (6 mg/ml) and diluted for measuring activity. The remainder of the PMSF-treated sample was precipitated by addition of 93 μl of 20% (w/v) trichloroacetic acid and analyzed using reverse-phase HPLC on a C18 column. Two partially re-

Sequence Analysis of Limited Proteolytic Fragments—For protein sequence analysis, limited proteolytic fragments were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes as described by Matsuda 999 (27). After staining with Coomassie Blue, 120A on-line PTH analyzer or an Applied Biosystems model 491 gas phase sequencer.

Mass Spectrometry—Samples for mass spectrometric analysis were further purified and exchanged into compatible buffers by reverse-phase HPLC using either a C4 column (Vydac, 2.1 × 250 mm) for wild type and mutant TC45 or a C18 column (Vydac, 2.1 × 250 mm) for proteolytic fragments. Columns were eluted with a linear 0.1% trifluoroacetic acid/acetoni trile at a flow rate of 150 μl/min. All automated gas-phase sequence analyses were performed using either an Applied Biosystems model 470A sequencer equipped with a model 120A on-line PTH analyzer or an Applied Biosystems model 491 gas phase sequencer.

RESULTS

Expression and Purification of Recombinant Human TC45—The TC48 and TC45 splice variants of human TCPTP were expressed in E. coli under control of the T7 promoter. Most of the TC48 variant was insoluble, whereas TC45 composed about 20–25% of the soluble protein. Therefore, the TC45 splice vari-

Limited Tryptic Proteolysis of TC45—Previous studies (14, 15) have shown that limited trypsinolysis of human TC45 pro-
duces an activated 33-kDa fragment, but the precise positions of the tryptic cleavage site(s) have not been determined. To analyze the role of the noncatalytic C-terminal domain in controlling the activity of TCPTP and to identify potential regulatory sequences, limited trypsinolysis was used to map tryptic
cleavage sites and probe the domain organization of TC45.

Digestion with a 1:20 ratio of trypsin (1:20 weight ratio of trypsin:TC45) for 30 min resulted in nearly quantitative con-
version of the native enzyme to a protease-resistant fragment of 33-kDa (Fig. 3A). Use of less trypsin (1:50 weight ratio) showed that the appearance of the 33-kDa fragment was pre-
ceded by the formation of a transient 42-kDa intermediate (Fig. 3B). Under these conditions, multiple fragments with sizes between 42 and 45 kDa were observed at early time points (Fig. 3B). Three separate samples of fragments migrating at positions between full-length TC45 and the 42-kDa band were subjected to amino acid sequence analysis. Each sample yielded a single sequence (data not shown) that was identical to the N terminus of the uncleaved protein, suggesting that most of these fragments are being converted to the 42-kDa interme-
diate by the removal of C-terminal residues.

The presence of Coomassie-stained material at or near the dye front of SDS gels (Fig. 3) suggested that polypeptides of 14 kDa or less were formed during limited tryptic proteolysis. To isolate and identify these small fragments, aliquots of a digest (1:50 trypsin) were withdrawn at several times and separated by reverse-phase HPLC on a C18 column. Two partially re-

Electrospray mass spectrum of these fragments are being converted to the 42-kDa interme-
diate by the removal of C-terminal residues.

To map their position within the TC45 sequence, proteolytic fragments were subjected to N-terminal sequencing and mass spectrometric analyses. The 33- and 42-kDa fragments were derived from the N terminus of the protein since their se-
quences were identical to that of recombinant TC45 (Table I). The electrospray mass spectrum of the 33- and 42-kDa fragments each showed one major species with a mass that closely matched that predicted for residues 2–288 and 2–352, respectively (Table I).

Samples from the HPLC fraction containing the low molecular mass fragments were separated on Tricine gels. The N-
terminalsequence of the 12- and 7-kDa fragments obtained from this gel began with Trp-289, suggesting that they were both generated by cleavage after Arg-288. Despite the heterogeneity observed upon electrophoresis, samples taken directly from the HPLC fraction yielded a single sequence that also began with the cognate amino acid at position 289.
The column was eluted at a flow rate of 150 μl/min with a 63-min linear gradient from 0 to 30% acetonitrile in 0.1% trifluoroacetic acid, followed by a 2-min gradient from 30 to 60% acetonitrile. The position of molecular size standards are shown on the left.

**FIG. 3. SDS-PAGE analysis of the time course of limited tryptic proteolysis.** TC45 was digested at 30 °C with two different ratios of trypsin: A, 1:20 weight ratio of trypsin:TC45; B, 1:50 weight ratio of trypsin:TC45. At the indicated times, aliquots corresponding to 5 μg of TC45 were removed from the digest, precipitated with trichloroacetic acid, and separated on 12% SDS-polyacrylamide gels as described under “Experimental Procedures.” Samples for the zero time point were taken before addition of trypsin. Gels were visualized by Coomassie Blue staining. The position of molecular size standards are shown on the left.

**FIG. 4. Analysis of fragments recovered from reverse-phase HPLC of limited tryptic digests.** TC45 was treated with a 1:50 weight ratio of trypsin at 30 °C, and samples were removed at various times and separated on a Vydac C18 column (2.1 × 250 mm) in 0.1% (v/v) trifluoroacetic acid. The column was eluted at a flow rate of 150 μl/min with a 63-min linear gradient from 0 to 30% acetonitrile in 0.1% trifluoroacetic acid followed by a 2-min gradient from 30 to 60% acetonitrile in 0.1% trifluoroacetic acid. Left panel, a sample removed from the digest at 15 min was separated by HPLC and each of two partially resolved peaks eluting at 26% acetonitrile were collected, concentrated, and separated on a 10% Tricine gel that was visualized by silver staining. Lane 1, first peak eluted; lane 2, second peak eluted. Molecular size markers are shown on the left. Right panel, lane 3, the peak eluting at 60% acetonitrile was concentrated and resolved on a 12% SDS-polyacrylamide gel that was stained with Coomassie Blue. Full-length TC45 (FL) was loaded in the indicated lane. Protein molecular size standards are shown on the right.

from 7 to 12 kDa originated from cleavage after Arg-288 and differ only in their C termini. Although the signal for the two 7-kDa fragments dominated, analyses of this HPLC fraction using MALDI-TOF mass spectrometry detected several additional species of higher mass (data not shown), which account for the larger species observed in the gels. Two 7-kDa peptides with masses that match calculated values for residues 289–350 and 289–352 were detected by electrospray mass spectrometric analysis of the HPLC fraction (Table 1).

These data indicate that the C-terminal, noncatalytic segment of TC45 contains two distinct regions (see Fig. 1B). The C-terminal end (residues 353–387) is exposed and rapidly degraded by trypsin, whereas the remainder of the residues are contained within the 7-kDa fragment (residues 289–350/352) and are protected from tryptic proteolysis. A significant portion of TC45 appears to be cleaved first at sites within this C-terminal, trypsin-sensitive region to give the 42-kDa intermediate, which then undergoes cleavage at Arg-288 to release the active 33-kDa fragment (residues 2–288) containing the catalytic domain of TCPTP and the 7-kDa fragment. Alternatively, TC45 may be cleaved first at Arg-288 to yield 33 and 12-kDa fragments. The 12-kDa fragment is then further degraded at its C terminus to give the 7-kDa fragment. Both the 42-kDa and 12-kDa transient intermediates predicted by these two routes of cleavage were detected in our digests (Figs. 3 and 4).

**Activation of TC45 during Limited Proteolysis—**The specific activity of trypsinized TC45 toward RCML (20 μmol/min/mg) was nearly identical to that reported for proteoloyzed TC48 (14) and was 20–100-fold higher than that of the full-length enzyme. Variation in the fold activation can be attributed to differences in the basal activity of our TC45 preparations. Activity toward the small compound pNPP was not affected by tryptic proteolysis.

To follow the time course of proteolytic activation, 1:100 trypsin was used to slow the rate of cleavage, which proceeded primarily by removal of the C-terminal protease-sensitive region to form the 42-kDa fragment (Fig. 5B). Maximum activation (~50-fold) was achieved by 25–30 min (Fig. 5A) when most of TC45 was cleaved to species of slightly lower molecular mass, but less than half was converted to the 42-kDa fragment. Thus, activation occurred early in the cleavage reaction as the C-terminal protease-sensitive region was being degraded but prior to formation of the 42-kDa form of the enzyme, suggesting that partial removal of the C-terminal protease-sensitive region might be sufficient to activate TC45.

**Removal of 20 C-terminal Residues Fully Activates TC45—** Constructs for expression of a series of six truncation mutants, from which 8–35 C-terminal residues were removed, two internal deletion mutants (Δ350–358 and Δ353–371), and a construct encoding the 33-kDa catalytic domain (residues 1–288) were prepared by in vitro mutagenesis. Mutant enzymes were expressed in E. coli and purified essentially as described for TC45. SDS-PAGE analysis (data not shown) confirmed that each mutant had the expected mobility and exhibited greater than 95% homogeneity.

Using RCML as substrate, the activity of the truncation mutant TCPTP-(1–288), which corresponds to the 33-kDa tryptic fragment, was comparable to the maximum value achieved during limited tryptic digestion of TC45 (see above) and was 121-fold more active than full-length enzyme (Fig. 6). TCPTP-(1–288) had a K_m of 48 nM and a V_max of 23 μmol/min/mg, which are close to the kinetic parameters of the 37-kDa form of TCPTP-(1–317) reported by Zander et al. (14) also using RCML as substrate. The truncation mutant TCPTP-(1–352), which corresponds to the 42-kDa tryptic fragment, displayed a specific activity similar to that of TCPTP-(1–288) and was 130-fold more active than the full-length enzyme (Fig. 6).

We observed a gradual increase in activity as increasing numbers of residues were deleted from the C terminus (Fig. 6). TCPTP-(1–352) and TCPTP-(1–359) exhibited the highest activities and were about 130-fold more active than the full-length enzyme. The TCPTP-(1–367) and TCPTP-(1–363) mutants, lacking 20 and 24 C-terminal residues, respectively, had...
lower activity but were greater than 83% fully activated. In contrast, TCPTP-(1–379) and TCPTP-(1–371), in which 8 and 16 residues were deleted from the C terminus, were not fully activated. Similar results were obtained with casein as substrate, but the maximal activation (9-fold) was less (data not shown). Internal deletions of residues 350–358 and 353–371 were partially activated by 29- and 36-fold, respectively, suggesting that simply reducing the length of the C-terminal tail was not sufficient to achieve full activation (Fig. 6). These truncation mutants demonstrate that removal of 20 residues from the C-terminal protease-sensitive segment is sufficient to achieve near-maximal stimulation of TC45. These data are consistent with the time course of proteolytic activation, showing that TC45 was activated early in the digest as the C-terminal protease-sensitive segment was being degraded but prior to formation of the 42-kDa fragment.

Removal of the C-terminal Domain Alters the Response of TC45 to Increasing Ionic Strength—The activity of full-length TC45 and the 33-kDa catalytic domain, TCPTP-(1–288), toward RCML responded differently to increasing KCl concentration. Increasing KCl to 100 mM stimulated the activity of TC45 about 76-fold, whereas KCl concentrations above 100 mM decreased activity (Fig. 7). In contrast, 10 mM KCl resulted in a slight enhancement (~1.2-fold) in activity of the 33-kDa catalytic domain, but KCl concentrations above 100 mM decreased activity with 50% inhibition occurring at about 150 mM KCl (Fig. 7). At KCl concentrations greater than 300 mM, the activity of the 33-kDa catalytic domain and full-length TC45 were similar. These effects were independent of the cation and resulted from increasing the ionic strength. With both full-length and truncated forms of TC45, activity toward pNPP was relatively insensitive to salt concentration with a small reduction in activity (<10%) at 100 mM KCl (data not shown). Differences in the response of the full-length and 33-kDa forms of the enzyme to ionic strength indicate that the C-terminal domain of TC45 influences the activity of the catalytic domain toward polypeptide substrates.

Addition of the Isolated C-terminal Segment of TC45 to the 33-kDa Catalytic Domain Inhibits Activity in a Concentration-dependent Manner—Activation of TC45 by truncation or proteolysis suggests that the C-terminal noncatalytic segment contains a regulatory site that interacts with the catalytic domain.

![Figure 6](image-url)
to modulate activity. To test this possibility, we expressed the noncatalytic C-terminal segment of TC45 (residues 289–387) in E. coli (Fig. 8B) and determined whether it could inhibit the 33-kDa catalytic domain. As a control, residues 231–300 from the C-terminal segment of PTP1B, a closely related nonreceptor PTP, were also produced in bacteria using similar methods (Fig. 8B). TC45-(289–387) inhibited the activity of the 33-kDa catalytic domain in a concentration-dependent manner with half-maximal inhibition at 40 μM (Fig. 8A). In contrast, equivalent concentrations of PTP1B-(231–300) had a slight inhibitory effect; at the maximum concentration tested (4 mM), only 17% inhibition was observed. The lack of a significant effect with the fragment derived from PTP1B shows that inhibition is a specific property of the TC45 C-terminal segment. At concentrations up to 4 mM, the activity of the full-length TC45 was unaffected by addition of the C-terminal segments from either TC45 (Fig. 8A) or PTP1B (data not shown). This demonstrates that inhibition is not substrate-directed and suggests that inhibition requires a binding site that is inaccessible in the full-length enzyme, presumably because it is occupied by its own intrinsic C-terminal segment.

Monoclonal Antibody CF4 Activates TC45—The monoclonal antibody CF4 recognizes a previously unidentified epitope within the C-terminal segment of TC45 and TC48 (11). Purified CF4 antibody stimulated TC45 activity in a concentration-dependent manner, whereas DH8, an antibody that recognizes PTP1B but not TCPTP, failed to stimulate TC45 (Fig. 9). Addition of equivalent concentrations of bovine serum albumin also had no significant effect on TC45 activity (data not shown). At the highest concentration of CF4 antibody available (1.2 mg/ml), TC45 activity with RCML was stimulated 8-fold to a specific activity of 25 μmol/min/mg, which is comparable to the value obtained by either limited proteolysis or C-terminal truncation. Thus, binding of CF4 converts TC45 to a fully activated state. The 8-fold increase in activity observed with antibody binding is about twice the 3–4-fold activation we obtained with proteolyzed or truncated enzyme in the high ionic strength phosphate buffer used in these experiments. Immunoblotting of our truncation, deletion, and point mutants was used to map the epitope of CF4 to a position between residues 350 and 363 within the C-terminal segment. The location of the CF4 epitope is consistent with results of limited proteolysis, which show that this region of the molecule is solvent-exposed and protease-sensitive.

DISCUSSION

The role of the noncatalytic, C-terminal segment in directing the intracellular localization of the two TCPTP splice variants is well documented (10–13). These studies provide evidence that TC45 utilizes a C-terminal autoregulatory site to modulate activity through an intramolecular interaction with the catalytic domain. These studies have focused on the TC45 splice variant, but it is possible that such autoregulation may also occur with the TC48 splice variant since these enzymes differ only at their extreme C termini and respond similarly to proteolysis and truncation. However, we cannot exclude the possibility that the hydrophobic tail of TC48 could either prevent or alter intramolecular interactions between the C-terminal and catalytic domains.

Analysis by limited proteolysis indicated that TC45 contains a protease-sensitive region near Arg-288 that links the 33-kDa catalytic domain (residues 1–288) to the C-terminal noncatalytic segment (see Fig. 1B). The pattern of limited proteolytic cleavage revealed the presence of two distinct regions within this noncatalytic segment. The extreme C terminus of the noncatalytic segment (residues 353–387) is protease-sensitive, exposed to solvent, and readily accessible to interacting pro-
teins. In contrast, residues 289–350 contained within the 7-kDa fragment encompass several potential tryptic cleavage sites that are protected from proteolysis, suggesting that this region of the C-terminal segment is folded into a more compact structure, despite its relatively small size.

Several lines of evidence indicate that the C-terminal noncatalytic segment of TC45 contains an autoregulatory site that is capable of controlling activity through an intramolecular interaction with the catalytic domain. Using RCML as substrate, the activities of the 33-kDa catalytic domain lacking the entire noncatalytic segment and a 42-kDa fragment lacking the C-terminal protease-sensitive region (residues 353–387) are more than 100-fold higher than that of the full-length enzyme. Increasing ionic strength activates the full-length enzyme but inhibits the 33-kDa catalytic domain, indicating that the C-terminal segment affects the enzymatic properties of the catalytic domain. Addition of a polypeptide containing residues 289–387 from the noncatalytic C-terminal segment of TC45 inhibits the activity of the 33-kDa catalytic domain in a concentration-dependent manner, providing evidence that the C-terminal segment contains a site that binds directly to the catalytic domain. In the full-length enzyme, this interaction must be intramolecular since TC45 is monomeric. The lack of an effect of exogenous C-terminal segment on full-length TC45 is consistent with such an intramolecular interaction since the binding site on the catalytic domain of the full-length enzyme would be occupied by its own endogenous C terminus. The activation of full-length TC45 by increasing ionic strength suggests that electrostatic interactions may mediate the binding of the autoregulatory region and the catalytic domain.

The epitope of the CF4 monoclonal antibody is located between residues 350 and 363 within the noncatalytic C-terminal segment. The activation of TC45 by purified CF4 antibody provides further evidence in support of an autoregulatory mechanism. The effect of CF4 antibody is most easily explained by its ability to bind the noncatalytic domain and induce the dissociation of the autoregulatory motif from its docking site on the catalytic domain. Protein kinase C, which is known to be regulated by an autoinhibitory mechanism, has also been shown to be activated completely by an antibody that recognizes an autoinhibitory sequence (28). In this case, the antibody is thought to mimic physiologic activators of the kinase.

The activation of TC45 by removal of 20 C-terminal residues and by an antibody directed to an epitope located just upstream from this region, suggests that the last 20–35 residues of the C terminus might comprise an autoregulatory site that binds the catalytic domain. Although this is an attractive hypothesis, we cannot exclude the possibility that proteolytic removal of this region or antibody binding could lead to activation through an indirect mechanism in which the conformation of a distal regulatory site is altered so that it cannot bind the catalytic domain. The existence of a binding site on the catalytic domain that recognizes and responds to autoregulatory site(s) might be exploited to develop reagents that could be used to modulate TCPTP activity selectively in vivo.

The precise mechanism by which the C-terminal domain regulates activity has not been defined. Hence, we have used the term autoregulatory rather than autoinhibitory to characterize the function of the C-terminal segment. With the exception of the basic substrate myelin basic protein, removal of the noncatalytic segment increases activity with all peptide/protein substrates tested to date, albeit to differing degrees (14–16). However, studies employing artificial substrates may not accurately reflect the behavior of TC45 with its physiologic substrates. We have found that truncation of TC45 has no effect on activity with pNPP, and Zhao et al. (29) observed that the kinetic parameters for phosphotyrosine were unchanged by truncation of the TC48 variant. These observations suggest that the C-terminal regulatory domain only affects the activity of the enzyme toward large substrates such as peptides or proteins. The absence of an effect of the C-terminal domain on small substrates argues against a mechanism where the active site is occluded due to its occupancy by an autoinhibitory site. Instead, the autoregulatory region of TC45 may act in part by influencing the accessibility of protein substrates to the active site.

Most kinases and phosphatases that are autoinhibited can be activated by an allosteric regulator such as Ca\(^{2+}\)/calmodulin, which binds at or near the autoinhibitory domain to disable it and prevent its interaction with the catalytic domain. To date, no such regulatory compound or macromolecule has been identified for TCPTPs. However, the CF4 antibody clearly demonstrates the potential for a protein to regulate the activity of TC45 by binding to exposed regions near the C terminus of the protein. We have recently reported that the nuclear import factor p97 (30) and a novel p116 protein can associate with the basic clusters of the TCPTP bipartite NLS (13); however, the identity of p116 remains unknown, and therefore we are unable to characterize fully the role of these proteins in activating TCPTP. In addition, TC45 is phosphorylated on Ser/Thr when overexpressed in COS1 cells and phosphorylation may indeed provide another means of modulating the autoregulatory region of TCPTP. It will be important to identify both the proteins that interact with the noncatalytic C-terminal domain of TCPTP as well as to evaluate the potential role of subcellular targeting, phosphorylation, and proteolysis in controlling TCPTP activity.

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