The Tetratricopeptide Repeat Domain and a C-terminal Region Control the Activity of Ser/Thr Protein Phosphatase 5*

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Protein Ser/Thr phosphatase 5 is a 58-kDa protein containing a catalytic domain structurally related to the catalytic subunits of protein phosphatases 1, 2A, and 2B and an extended N-terminal domain with three tetratricopeptide repeats. The activity of this enzyme is stimulated 4–14-fold in vitro by polyunsaturated fatty acids and anionic phospholipids. The structural basis for lipid activation of protein phosphatase 5 was examined by limited proteolysis and site-directed mutagenesis. Trypsinolysis removed the tetratricopeptide repeat domain and increased activity to approximately half that of lipid-stimulated, full-length enzyme. Subtilisin removed the tetratricopeptide repeat domain and 10 residues from the C terminus, creating a catalytic fragment with activity that was equal to or greater than that of lipid-stimulated, full-length enzyme. Catalytic fragments generated by proteolysis were no longer stimulated by lipid, and degradation of the tetratricopeptide repeat domain was decreased by association with lipid. A truncated mutant missing 13 C-terminal residues was also insensitive to lipid and was as active as full-length, lipid-stimulated enzyme. These results suggest that the C-terminal and N-terminal domain act in a coordinated manner to suppress the activity of protein phosphatase 5 and mediate its activation by lipid. These regions may be targets for the regulation of protein phosphatase 5 activity in vivo.

Protein Ser/Thr phosphatases, together with protein Ser/Thr kinases, play an essential role in regulating a variety of cellular processes. The major structural family of protein Ser/Thr phosphatases, the PPP1 family, includes PP1, PP2A, and 2B, also known as calcineurin, as well as several newer family members about which little is known (1). A growing body of evidence indicates that PPP family members are subject to complex regulation. For example, the catalytic subunits of PP1 and PP2A are bound to regulatory subunits and docking proteins that control their activity, substrate specificity, and subcellular localization (2, 3). In addition to activation by calcium and calmodulin, calcineurin is also associated with proteins that control its intracellular location and activity (4, 5).

Protein phosphatase 5 (PP5) is a member of the PPP family with a C-terminal catalytic domain related to those of PP1, PP2A, and calcineurin, and an N-terminal domain consisting of three tetratricopeptide repeats or TPRs (6–8). Tetratricopeptide repeats occur in a variety of unrelated proteins and are thought to mediate protein-protein interactions (9–11). The TPR domain of PP5 interacts with a number of proteins in vitro, including the atrial natriuretic peptide receptor (7), Cdc16p and Cdc27p, which are two TPR-containing subunits of the anaphase-promoting complex (12), and hCRY2, a human homologue of the photolyase/blue light photoreceptor (13). Protein phosphatase 5 has also been shown to associate in vivo with heat shock protein 90 in complexes with glucocorticoid receptors (14, 15), and overexpression of the TPR domain of PP5 blocks glucocorticoid-induced gene transcription (14). These findings suggest that the TPR domain docks PP5 to other proteins. These and other observations (8, 16) also implicate PP5 in the regulation of cell growth and in signal transduction pathways controlled by atrial natriuretic peptide or steroid hormones. However, the role of PP5 in these processes and the mechanism(s) that control its activity are not known. We and others (17, 18) have shown that PP5 can be activated by polyunsaturated fatty acids and anionic phospholipids in vitro, suggesting that PP5 may be subject to regulation.

In the present study, we investigated the structural basis for the response of PP5 to lipid. Our results show that lipid activates PP5 by binding to its TPR domain and that proteolytic removal of the TPR domain partially activates the enzyme. In contrast, removal of 13 residues from the C terminus generates a fully activated form of PP5 that is rendered insensitive to lipid, indicating that without the C terminus the TPR domain alone cannot regulate activity. These results suggest that both the C terminus and the TPR domain act in concert to inhibit activity and mediate the activation of PP5 by lipid.

EXPERIMENTAL PROCEDURES

Materials and Purified Proteins—All materials were purchased from Sigma unless otherwise noted. The catalytic subunit of cAMP-dependent protein kinase was purified from bovine heart (19) or purchased from New England Biolabs, Inc.

Cloning and Expression of PP5, PP5-(1–486), and PP5-(1–159)—The vector, pET-GST-PP5 (17), was used to express wild-type PP5. The PP5 C-terminal truncation mutant, PP5-(1–486), and the PP5 TPR domain construct, PP5-(1–159), were generated by using the polymerase chain reaction containing PP5-specific oligonucleotides and pET-GST-PP5 as templates. Polymerase chain reaction products were cloned downstream of the GST coding region of the bacterial expression vector pET-21a(+) GST (20) and sequenced. All constructs were transformed into Escherichia coli strain BL21 (DE3) (Novagen), and protein was expressed, purified by glutathione-agarose affinity chromatography, cleaved from

Received for publication, April 15, 1999, and in revised form, May 21, 1999.

* This work was supported in part by National Institutes of Health Grants NS31221 (to S. R.) and CA59935 (to H. C.) and American Heart Association Grant 3483670 (to S. R.). This is Journal Paper 10981 from Purdue University Agricultural Experimental Station. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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§ The abbreviations used are: PP, phosphoprotein phosphatase; AA, arachidonic acid; GST, glutathione S-transferase; ESI, electrospray ionization; HPLC, high performance liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MBP, myelin basic protein; MS, mass spectroscopy; pNPP, para-nitrophenyl phosphate; PP, protein phosphatase; TPR, tetratricopeptide repeat.

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Phosphatase activities were measured using an anion exchange chromatography (Mono Q HR 5/5, Amersham Pharmacia Biotech) at 25 °C. The column was eluted at a flow rate of 1 ml/min with a 50-ml gradient of 0–750 mM NaCl in 20 mM Tris-HCl, pH 7.6, 1 mM EGTA, and 4 mM MnCl₂. Chromatography buffer also contained either 1 mM phenylmethylsulfonyl fluoride or 0.1% 2-mercaptoethanol to prevent denaturation of the probease during digestion. Fractions containing active catalytic fragments, eluting at 140 mM NaCl for subtilisin digests or 220 mM NaCl for trypsin digests, were pooled, dialyzed overnight in 20 mM Tris, pH 7.6 (4 °C), 1 mM EGTA, 4 mM MnCl₂, 0.1% 2-mercaptoethanol, and 50% glycerol, and stored at −20 °C. Fractions containing the trypsin TP1 fragment eluting at 280 mM NaCl were dialyzed, and stored as described above.

Analysis of Catalytic Fragments by Mass Spectrometry—After anion exchange chromatography, the catalytic fragments were further purified by HPLC on a protein C-4 reverse phase column (250 × 4.6 mm, 5 mm, Vydac Hesperia, CA) using a gradient from 18 to 63% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. After lyophilization, samples were dissolved in 50% acetonitrile in 0.1% trifluoroacetic acid and directly analyzed by MALDI-MS or ESI-MS. The HPLC-puriﬁed tryptic catalytic fragment was further digested with 2 mg/ml trypsin in 50 mM ammonium bicarbonate, pH 8.0, for 4 h at 37 °C and analyzed by MALDI-MS. For analysis by ESI-MS, the peptide mixture was purified by using a POROS 50 R2 column (PerSeptive Biosystems, Framingham, MA) (25).

All MALDI analyses were performed using a Voyager RP time-of-flight spectrometer (PerSeptive Biosystems, Framingham, MA) in the linear mode using o-cyano-4-hydroxycinnamic acid as the matrix. The ESI analysis of the HPLC-puriﬁed subtilisin fragment was performed by using a Micromass Platform II (Altrincham, UK) single quadrupole mass spectrometer. The sample was infused into the mass spectrometer at 5 μl/min using a pressure injection vessel (26). A Micromass Q-Tof (Altrincham, UK) hybrid mass spectrometer, equipped with a quadrupole mass filter and an orthogonal acceleration time-of-flight mass spectrometer, was used for the analysis of the tryptic peptide mixture of the catalytic fragment after trypsinolysis. Analyses were performed on a 0-μl sample volume using a nanoelectrospray source.

RESULTS

Proteolysis Increases PP5 Enzyme Activity—Protein phosphatase 5 can be activated by polyunsaturated fatty acids and anionic phospholipids in vitro (17, 18). To determine how PP5 is activated by lipid, we ﬁrst asked whether limited proteolysis could generate an active lipid-insensitive form of PP5 by removing a regulatory element. Treatment of PP5 with subtilisin led to a rapid and sustained 12–18-fold increase in activity using 10 μM casein as substrate (Fig. 1A). This activity was comparable to that of full-length PP5 assayed in the presence of a maximally activating concentration of AA, 250 μM. Similar changes in activity were observed regardless of whether PP5 was proteolyzed in the absence or presence of AA (Fig. 1A).

In the absence of AA, subtilisin treatment of full-length 58-kDa PP5 generated a 38-kDa fragment, which was converted over time to a stable 36-kDa fragment (Fig. 1B). Activity reached a maximum after digestion for 20 min, which corresponded with the loss of full-length enzyme, and was not further increased by the conversion of the 38-kDa fragment to the 36-kDa fragment (Fig. 1, A and B). Digestion in the presence of 250 μM AA appeared to generate the same 36- and 38-kDa fragments. In addition, a series of 14–18-kDa fragments appeared that were not observed in digests performed without AA (Fig. 1C). The largest of these 14–18-kDa fragments appeared at the earliest time point measured, 5 min. As digestion proceeded these fragments decreased in both size and yield and were no longer observed by 60 min. Endoproteinase Glu-C generated a similar pattern of proteolytic fragments and increased PP5 enzyme activity (data not shown).

Tryptic digestion of PP5 caused a 4–8-fold increase in phosphatase activity (Fig. 1D) representing 40–50% of the maximal activity of full-length, lipid-stimulated PP5. The increase in activity was observed regardless of whether AA was present or absent during digestion. In the absence of AA, trypsinolysis
generated a 39-kDa fragment that was converted over time to a 38-kDa fragment that remained stable up to 2 h, the longest time point measured, and a smaller fragment of 21-kDa that was degraded by 60 min (Fig. 1E). When proteolysis was performed in the presence of 250 μM AA, these same fragments were generated; however, the 21-kDa fragment was now slowly converted to a series of smaller fragments (Fig. 1F).

Proteolysis with subtilisin also increased activity toward MBP and histone by 15–20-fold, which was comparable to that of lipid-stimulated, full-length PP5, whereas trypsinolysis reduced carboxamidomethylated and maleyated lysozyme, and MBP was not altered by the addition of AA during proteolysis. However, when AA was included in phosphatase assays, the increase in phosphatase activity due to limited proteolysis was observed with multiple substrates. PP5 was subjected to a mock digest (■), subtilisin treatment (□), or trypsin treatment (△) in the absence of AA for 60 min, and then phosphatase activity was assayed using casein, MBP, or histone. All phosphatase reactions included the equivalent of 4 nM enzyme, and all but the control (■) were performed in the absence of AA. Data are presented as activity relative to control, defined as the activity of PP5 subjected to mock digestion, and then assayed without AA with each substrate (■). Control values for each substrate were as follows: casein, 75 ± 6 nmol of P_i released per min/mg; histone, 223 ± 32 nmol of P_i released per min/mg; MBP, 371 ± 96 nmol of P_i released per min/mg. Each time point represents the average ± S.D. of triplicate samples. Data are representative of two or more independent experiments performed under identical conditions.

**Fig. 1.** Limited proteolysis of PP5 increases enzyme activity. Recombinant PP5 was digested with either subtilisin (A) or trypsin (D) in the absence (○) or presence (□) of 250 μM AA, and at various times an aliquot from the digest was terminated and assayed for phosphatase activity toward casein. Reactions contained the equivalent of 4 nM PP5. Data are presented as activity relative to control, which is the activity of full-length PP5 subjected to mock digest and assayed without AA. Each time point represents the average ± S.D. of triplicate samples. For each time point from subtilisin digests performed in the absence (B) or presence (Ε) of AA and trypsin digests performed in the absence (D) or presence (F) of AA, matched samples were resolved by electrophoresis on 15% SDS-polyacrylamide gels and visualized by Coomassie staining. The time of digest (min) is noted across the top of each gel, and the migration of molecular weight standards is indicated to the left of each gel. Results are representative of three or more independent experiments performed under identical conditions.

**Fig. 2.** Subtilisin generates a more active proteolytic fragment than does trypsin. Recombinant PP5 was digested using subtilisin (■) or trypsin (△) in the presence of 250 μM AA or was subjected to a mock digestion (○) without addition of protease. The mock-digested enzyme and active proteolytic fragments were then purified, and casein phosphatase activity was assayed in the absence or presence of 250 μM AA. Data are the average ± S.D. of at least five independent experiments performed in triplicate. Mock digested, full-length PP5 assayed in the absence of AA released 56 ± 16 nmol of P_i per min/mg.

**Fig. 3.** Limited Associates with the TPR Domain of PP5 in Vitro—N-terminal sequence analysis was performed to determine the identity of proteolytic fragments. The recombinant enzyme contains four residues, Gly-Ser-Gly-Ser, preceding the initial methionine residue of native PP5. The 18–21-kDa fragments generated during trypsinolysis in the absence or presence of AA had identical N termini, Gly-Ser-Gly-Ser-Met, and thus were all derived from the TPR domain (Table I). Subtilisin generated 14–18-kDa fragments with N termini beginning at Ala⁴ or Gly⁶ in the presence of AA, or Glu¹⁰ in the absence of AA, indicating that these fragments were also all derived from the TPR domain (Fig. 4 and Table I). Since the TPR domain-derived fragments were more resistant to degradation in the presence of AA than in its absence (Fig. 1, B, C, E, and F), the effect of lipid on TPR domain degradation was explored further. Protection of the cleaved TPR domain by AA was also observed with other proteases, including endoproteinase Glu-C and chymotrypsin (data not shown). We previously showed that only unsaturated
TABLE I
Characterization of TPR-containing proteolytic fragments

| Protease | Time digested | 250 μM AA | N terminus | Proteolytic fragment |
|----------|---------------|-----------|------------|---------------------|
| Subtilisin | 15 min | + | Ala¹-Glu-Gly-Glu-Arg- Glu¹²-Glu-Arg-Thr-Glu- Glu¹⁵-Cys-Ala-Glu-Pro | 18 |
| Subtilisin | 15 min | – | | |
| Subtilisin | 20 min | + | Gly¹⁵-Cys-Ala-Glu-Pro | 16 |
| Trypsin 5 | +/– | Gly-Ser-Gly-Ser-Met¹ | 21 |
| Trypsin 10 | – | Gly-Ser-Gly-Ser-Met¹ | 20 |
| Trypsin 15 | + | Gly-Ser-Gly-Ser-Met¹ | 20 |
| Trypsin 60 | + | Gly-Ser-Gly-Ser-Met¹ | 19 |

* Estimated from migration of fragments by SDS-polyacrylamide gel electrophoresis analysis.

**Fig. 4. Sequence analysis of PP5 proteolytic fragments.** The N-terminal sequences of proteolytic fragments from subtilisin or trypsin digests were determined as described under “Materials and Methods.” The black arrowheads indicate points of cleavage for subtilisin within the N terminus. The length of digest (min) is denoted. Cleavage points marked +/AA or –/–AA were only observed for fragments generated in the presence or absence of AA, respectively; all other cleavage points occurred in both the absence and presence of AA. Recombinant PP5 has an additional four amino acids (Gly-Ser-Gly-Ser) at the N terminus not considered in the numbering of residues at various cleavage points. The approximate locations of TPRs (■) and the catalytic domain [ Families of PP5 are shown.

**Fig. 5. Recombinant TPR domain is protected from proteolysis by lipid.** Recombinant PP5-(1–159) consisting of the TPR domain was digested using trypsin for varying amounts of time in the absence (A) or presence (B) of 250 μM AA. Samples were resolved by electrophoresis on 15% SDS-polyacrylamide gels and visualized by Coomassie staining. The length of digest (min) is noted across the top of each gel, and the migration of molecular weight standards is indicated to the left of each gel.

| Lipid additive | Slowed TPR digestion | Stimulated enzyme activity |
|----------------|----------------------|---------------------------|
| Arachidonic acid | + | + |
| Arachidonyl alcohol | – | – |
| Arachidic acid | – | – |
| Arachidonic acid methyl ester | – | – |
| Linoleic acid | + | + |
| Oleic acid | – | – |
| Stearic acid | – | – |
| Palmitoleic acid | + | + |
| 1,3-Diolein | – | – |
| 1-Oleoyl 2-acetyl sn-glycerol | – | – |
| Caproic acid methyl ester | – | – |

* Lipid was added to a final concentration of 250 μM.
* The sustained presence of multiple proteolytic fragments in the size range of 14–20-kDa after 10 min of either trypsin or subtilisin digestion when compared to the control digest without lipid were scored positive. Similar results were observed in at least two independent experiments.
* Lipids demonstrating at least an 8-fold increase in activity toward casein were scored positive. Similar results were observed in at least two independent experiments.
significantly higher activity than the tryptic fragment with an intact C terminus raised the possibility that residues 490–499 might be involved in regulating activity.

C-terminal Truncation of PP5 Increases Enzyme Activity—A truncation mutant, PP5-(1–486), was generated to assess the role of the C terminus in controlling PP5 activity. PP5-(1–486) exhibited specific activities toward casein and MBP that were comparable to those of full-length, lipid-stimulated PP5 (Fig. 6). Similar results were observed with histone, phosphorylase a, and pNPP. Arachidonic acid did not increase the activity of PP5-(1–486) toward casein and increased activity toward MBP only 2-fold (Fig. 6). Thus, removal of 13 residues from the C terminus alone was sufficient to activate PP5 and diminish its sensitivity to lipid.

Trypsinolysis of PP5-(1–486) revealed that the truncation mutant was more rapidly converted into catalytic and N-terminal fragments than the full-length enzyme (Fig. 7, A and B). However, the rates at which the released TPR-derived fragments were degraded and the sites of cleavage for the catalytic fragments were the same as those of the full-length enzyme (data not shown). This suggests that removal of the C-terminal 13-residue segment promotes a conformation that is more susceptible to trypsinolysis.

Attempts to Inhibit PP5 with the TPR Domain or with a Peptide Representing the C-terminal Segment—For some enzymes, such as calcineurin, a discrete region of the protein acts as an autoinhibitory domain (27, 28). Studies were performed to determine whether the TPR domain or the C-terminal segment of PP5 function in this manner. The activity of catalytic fragments purified from subtilisin or trypsin digests was assayed in the presence of recombinant PP5-(1–486), representing the TPR domain. Up to 100 μM TPR domain, which represented a 15,000-fold molar excess, had no effect on the catalytic fragments. The purified 21-kDa trypsic fragment, which encompasses the TPR domain, also failed to inhibit the catalytic fragments up to a 20-fold molar excess. Likewise, synthetic peptides corresponding to residues 481–499 or 487–499 were unable to inhibit specifically full-length PP5 or PP5-(1–486). Thus, neither the TPR domain nor the C terminus appear to function as direct autoinhibitory segments. However, these polypeptides may not assume the conformation of the corresponding segments in native protein and therefore may not accurately reflect their functional properties.

**DISCUSSION**

The TPR Domain and the C-terminal Region Control PP5 Activity—This study confirms that full-length PP5 has latent phosphatase activity that is revealed by limited proteolysis or lipid treatment. We demonstrate herein that both the C-terminal region (residues 490 to 499) and the TPR domain suppress the activity of PP5. Both regions are required for lipid stimulation, and removal of either region alone resulted in increased activity and a loss of lipid activation. Removal of the TPR domain alone partially activated PP5, whereas C-terminal truncation resulted in full activation. Proteolytic removal of both the TPR domain and the C-terminal region with subtilisin resulted in a catalytic fragment with activity equal to or exceeding that of full-length, lipid-stimulated enzyme. These observations suggest that the two regions may act in concert to suppress enzyme activity. Although both the C terminus and the TPR domain are required for lipid stimulation, the TPR

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**TABLE III**

| Protease   | Time digested | Proteolytic fragment | N-terminal sequence | Observed mass | Predicted mass | Residue number |
|------------|---------------|----------------------|--------------------|---------------|---------------|---------------|
| Subtilisin | 5             | 38                   | Ile-Ala-Gly-Asp-Glu-His- | 38,623 ± 35    | 38,551        | 152–489       |
| Subtilisin | 60            | 36                   | Thr-Ile-Glu-Asp-Glu-Tyr-  | 36,445 ± 5      | 36,440        | 171–489       |
| Trypsin   | 60            | 38                   | Ser-Val-Val-Asp-Ser-Leu-  | 38,695 ± 150   | 38,690        | 160–499       |

*Estimated from migration of fragments by SDS-polyacrylamide gel electrophoresis analysis.

**FIG. 6**. Comparison of PP5 and PP5 C-terminal truncation mutant activity. Phosphatase activity of full-length PP5 (■) or the C-terminal truncation mutant, PP5-(1–486) (□), was assayed in the absence or presence of 250 μM AA using casein or MBP as substrate. Each value represents the average ± S.D. of triplicate samples and is representative of at least three independent experiments performed under similar conditions. Full-length PP5 assayed in the absence of AA released 62 ± 17 nmol of Pi per min/mg using 10 μM casein and 32 ± 3 nmol of Pi per min/mg using 10 μM MBP.

**FIG. 7**. Truncated PP5 is rapidly proteolysed. Recombinant PP5-(1–486) (A) or full-length PP5 (B) was digested with trypsin for varying amounts of time in the absence or presence of 250 μM AA. For each time point, material from trypsin digests was resolved by electrophoresis on SDS-polyacrylamide gels and visualized by Coomassie staining as described under “Experimental Procedures”. The size of digest (min) is noted across the top of each gel, and the migration of molecular weight standards is indicated to the left of each gel. Results are representative of at least three independent experiments.
domain appears to be the site at which lipid binds to activate PP5. This conclusion is supported by the observation that, once released from the catalytic domain, the TPR domain was proteolyzed at a rate that is lipid-dependent. In addition, the effect of lipid on the proteolytic destruction of the TPR domain was specific for polyunsaturated fatty acids that stimulate enzyme activity.

The findings that lipid binds to the TPR domain and that removal of the TPR domain activates PP5 are in agreement with results reported by Chen and Cohen (18). However, in their study trypsin fully activated PP5, and no role for the C terminus was observed. The discrepancy in findings with trypsinolysis may be due to differences in assay conditions, the properties of the recombinant proteins used, or the boundaries of the fragments generated. Nevertheless, our results demonstrate that the tryptic fragment generated in the present study was not fully active because it retained its C terminus.

Limited proteolysis of PP5 also indicated that the catalytic domain is folded into a compact structure that is connected by a solvent-exposed, protease-sensitive linker region to the TPR domain. This finding is consistent with the three-dimensional structure of the TPR domain in which the linker domain beyond the third TPR forms an extended α-helix (29). It is not clear whether the C-terminal segment is susceptible to proteolysis prior to release of the TPR domain.

Mechanisms for the Regulation of PP5 Activity by the TPR Domain and the C-terminal Region—Several mechanisms could account for the regulation of activity by the TPR domain and the C terminus. The TPR domain and C-terminal segment may physically interact to induce a conformation of PP5 with reduced activity or to block access of substrates to the catalytic site. Alternatively, each of these inhibitory segments alone may bind directly to the catalytic domain, blocking substrate access or promoting an inactive conformation. The three-dimensional structure of the TPR domain of PP5 consists of six antiparallel amphipathic helices (29), which may mediate hydrophobic interactions with the catalytic domain and/or the hydrophobic C terminus. Lipid binding to the TPR domain may disrupt these hydrophobic interactions.

Our results are consistent with a model in which the TPR domain and the C terminus act in a coordinated manner to inhibit activity and argue against a model in which the TPR domain or the C-terminal region alone blocks the catalytic site or promotes an inactive conformation of the catalytic domain. In the absence of the C terminus, the TPR domain is more rapidly released from the catalytic domain by proteolysis, indicating that the linker region between the TPR and catalytic domains is more accessible to protease. The observation that the C-terminal truncation mutant is highly active although it contains the TPR domain also supports the notion that inhibition requires both regions. Finally, attempts to inhibit catalytic domain fragments with recombinant PP5-(1–159) containing the TPR domain were unsuccessful, as were attempts to inhibit full-length or truncated enzyme with synthetic peptides representing the C terminus. However, further studies will be required to rule out an independent inhibitory role for each region and to determine how inhibition is achieved.

How Is PP5 Regulated in Vivo?—Since TPR domains typically mediate interactions with other proteins (9), the binding of proteins to the TPR domain could potentially regulate PP5 activity in a manner similar to that observed with lipids. Although several proteins have been shown to bind PP5 through its TPR domain (7, 12–14), only hCRY2, a human homologue of the blue light receptor, has been reported to affect PP5 by inhibiting its activity (13). It will be important to assess the effect of other interacting proteins on PP5 activity. In addition to the TPR domain, this study reveals the potential of the C terminus as a target for regulation in vivo.

Does the C Terminal Play a Common Regulatory Role for PP1, PP2A, and PP5?—The C-terminal regions of other members of the PPP family have been shown to regulate phosphatase activity. Calcineurin has an extensive C-terminal region of more than 150 residues beyond its catalytic domain containing a B subunit-binding site, a calmodulin-binding site, and an autoinhibitory domain (4). Protein phosphatases 1 and 2A, like PP5, have 20–40-residue C-terminal segments that extend beyond the conserved catalytic domain (1). In the case of PP1, phosphorylation of a threonine residue in the C terminus of the catalytic subunit inhibits its activity in vitro (30, 31). In vitro studies of PP2A indicate that tyrosine phosphorylation near the C terminus inhibits activity (32), and methylation of the C terminus increases activity (33). Unlike PP5, the catalytic subunits of PP1 and PP2A lack extensive N-terminal domains and instead are associated with one or more regulatory subunits in cells (1, 2). These regulatory subunits are not required to observe the effects of post-translational modification in vitro, suggesting that the C terminus directly affects the catalytic domain of PP1 and PP2A (30, 32). However, a modulatory role for regulatory subunits cannot be excluded, since both PP1 and PP2A have been shown to be phosphorylated on their C termini in vivo under conditions in which the catalytic subunit is associated with regulatory subunits (31, 32, 34, 35). In the crystal structure of PP1 however, the C terminus is unstructured (36, 37) so it is unclear how this region influences activity. Additional structural studies will be required to learn whether the C terminus of PP5 interacts with the catalytic domain, TPR domain, or both and to determine whether the C termini of PP1, PP2A, and PP5 modulate activity by a similar mechanism.

Acknowledgments—We thank Dr. Alan Mahrenholz and Mary Bower (Purdue University) for providing assistance and advice with amino acid sequencing and peptide synthesis. Work performed in the Purdue Laboratory for Macromolecular Structure (peptide sequencing and oligonucleotide synthesis) was supported in part by National Institutes of Health Grants DK20542 and CA25168. Work performed in the DNA sequencing Facility of the Purdue University Cancer Center (DNA sequencing) was supported by National Institutes of Health Grant CA25168. The Purdue Cancer Center was supported by an American Cancer Society institutional grant.

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