Identification of Structural Elements Involved in the Interaction of Simian Virus 40 Small Tumor Antigen with Protein Phosphatase 2A*

(Received for publication, August 17, 1998)

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SV40 small tumor antigen (small-t) was used as a model to identify structural elements involved in the interactions between regulatory proteins and protein phosphatase 2A (PP2A). Using mutant proteins and synthetic peptides, we identified a small domain within small-t that is a major site for interaction with the dimeric form of PP2A. A series of small-t truncation mutants identified a region surrounding the first of two conserved cysteine clusters that was critical for interaction with PP2A. These mutants also identified additional regions of small-t that contribute to high affinity interaction. Deletion of residues 110–119, which encompass the first cysteine cluster, resulted in a protein that failed to bind to PP2A. Synthetic peptides that contained residues 105–122 of small-t blocked binding of small-t to PP2A. These peptides also inhibited the phosphatase activity of PP2A in a manner analogous to full-length small-t. The active small-t peptides adopt a β-strand structure that was essential for high affinity interaction with the PP2A dimer. Based on circular dichroism measurements, the same cysteine cluster-containing peptides that bind to PP2A also interact with zinc. Interaction with zinc required the conserved cysteines but was not required for interaction with PP2A.

A significant fraction of the serine/threonine phosphatase activity in most tissue and cell types is attributable to protein phosphatase 2A (1). The diverse functions of PP2A in cellular signaling are due to the presence of an assortment of holoenzymes. Each holoenzyme is made up of one of a diverse array of regulatory subunits associated with a conserved dimeric core (AC). The core complex is composed of a catalytic subunit (C) and a structural subunit (A). There are at least three unrelated families of PP2A regulatory subunits that have multiple isoforms and splice variants (2–5). A major function of the regulatory subunits is to differentially modulate the activity and specificity of the AC core enzyme (6–8). The small and middle tumor antigens of the polyomaviruses are a fourth set of proteins that can bind and regulate the activity of PP2A (9). Efficient transformation of quiescent cells requires the expression of small-t as well as large tumor antigen, the major transforming protein of SV40. Small-t appears to have a mitogenic role during transformation by SV40 (10, 11). Consistent with this idea is the observation that overexpression of SV40 small-t in mammalian cells activates growth factor-stimulated signaling pathways. These include the mitogen-activated protein kinase (12) and stress-activated protein kinase (13) pathways, as well as pathways that utilize nuclear factor-κB, protein kinase Cζ, and phosphatidylinositol-3 kinase (14). Activation of these pathways by small-t requires interaction with PP2A, indicating that this phosphatase plays an important and multifunctional role in cell signaling.

Relatively little is known about the molecular basis for the interaction of regulatory subunits and tumor antigens with the dimeric core of PP2A. None of the regulatory proteins form a stable complex directly with the catalytic subunit. A model for PP2A architecture suggests that the A subunit acts as an adapter protein that allows association of the C subunit with regulatory proteins (2, 4, 15). The regulatory subunits and tumor antigens bind in a mutually exclusive manner to a region of the A subunit that is distinct from the region that binds the C subunit (16). The diverse array of regulatory proteins that interact with PP2A bind to a common site within the A subunit despite a lack of any significant amino acid sequence similarity.

SV40 small-t is a 174-amino acid protein that contains an amino-terminal 82-amino acid common region that is shared with large tumor antigen and a 92-amino acid carboxyl-terminal domain that is unique to small-t (17). The small-t unique region contains the PP2A binding domain. Amino acid sequence comparisons have identified two cysteine cluster motifs (CXXCXC) that are absolutely conserved in the small-t proteins of the polyomavirus family (17). A region of the unique domain encompassing the first cysteine cluster is necessary for the binding of small-t to PP2A (12). Small-t associates with PP2A by displacing the cellular regulatory subunits, resulting in inhibition of phosphatase activity (18).

Because the regulatory subunits and tumor antigens bind to the same region of the A subunit of PP2A but share no significant sequence identity, we speculated that the different regulatory proteins share a common structural element. In this study, we have used SV40 small-t as a model regulatory protein to identify and define structural elements involved in interaction with PP2A. Using small-t mutants and synthetic peptides, we identified a small domain within SV40 small-t that is responsible for interaction with the A subunit, and we show that this domain can regulate phosphatase activity in a manner analogous to full-length small-t.

* This work was supported by National Institute of Health Grant GM49505. 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 1734 solely to indicate this fact.
† This work is submitted in partial fulfillment for the degree of Doctor of Philosophy in the Cell Regulation Graduate Program at the University of Texas Southwestern Medical Center at Dallas.
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§ The abbreviations used are: PP2A, protein phosphatase 2A; GST, glutathione S-transferase; GST-A, fusion between GST and the A subunit of PP2A; CD, circular dichroism; small-t, SV40 small tumor antigen; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; MOPS, 4-morpholinepropanesulfonic acid.

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EXPERIMENTAL PROCEDURES

Purification of Proteins—Recombinant small-t was expressed in insect cells and purified as described previously (19). The dimeric AC form of PP2A (20) and the free catalytic form of PP2A (21) were purified from bovine cardiac muscle as described. The purification of glutathione S-transferase (GST) and the fusion between GST and the A subunit of PP2A (GST-A) were described previously (12). After purification, glutathione-agarose complexed to either GST or GST-A was washed three times with 3 bed volumes of PP2A storage buffer (25 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM DTT, and 50% glycerol) aliquoted and stored at −80 °C for later use. Recombinant baculoviruses expressing wild type small-t and mutant small-t proteins were generated using standard procedures. The expression of the mutant small-t proteins was verified by SDS-PAGE followed by Western blotting. Wild type small-t and mutants 1–110, 1–131, 1–150, 52–174, and 88–174 were expressed in Sf9 cells. Cells expressing the various proteins were harvested by centrifugation, washed with TBS (20 mM Tris-HCl, pH 7.0, 150 mM NaCl), and resuspended in 1:3 (w/v) lysis buffer (20 mM HEPES, pH 7.2, 500 mM NaCl, 10 mM DTT, 2 mM phenylmethylsulfonyl fluoride, 10% glycerol, and 1% Triton X-100). The suspension was incubated on ice for at least 30 min and then microcentrifuged for 15 min. The cleared supernatant was then applied onto a Superose-6 gel filtration column (Amersham Pharmacia Biotech) according to the manufacturer’s protocols. Virus stocks were produced and plaque-purified using standard procedures.

Preparation of 32P-Labeled Substrates and Protein Phosphatase Assays—Myosin light chain phosphorylation and phosphatase activity assays were determined as described previously (18). Briefly, the AC dimeric form of PP2A (final concentration, 0.5 nM) was combined with various proteins or peptide fragments in phosphatase assay buffer (20 mM MOPS, pH 7.0, 0.5 mg/ml bovine serum albumin, 10 mM DTT, and 1 mM MnCl2). The reaction was initiated by the addition of 32P-labeled myosin light chains (2 μM) and incubated for 5 min at 30 °C. The reaction was terminated by the addition of 25% trichloroacetic acid (final concentration, 10%) and 6 mg/ml bovine serum albumin (final concentration, 2.5 mg/ml). The samples were incubated on ice for 5 min, precipitated protein was pelleted by microcentrifugation, and the 32P-labeled peptide was purified with a Beckman LS 1801 liquid scintillation counter. The IC50 values for peptides 97–122, 105–122, and 105–122 (C-S) were calculated using the logistic equation for the determination of IC50 values as described by Bliss (29).

RESULTS

Inhibition of PP2A by Small-t Fragments—Wild type SV40 small-t antigen interacts with the AC dimeric core of PP2A and inhibits activity toward several phosphoprotein substrates (18). The binding of small-t is mediated primarily by interaction with the A subunit. To determine the contributions of different regions of small-t to interaction with the dimeric form of PP2A, we used recombinant fragments of small-t, the binding of which to the A subunit had been characterized (12). The recombinant fragments were expressed in Sf9 cells, partially purified, and quantitated. Their effects on phosphatase activity were determined by incubating varying amounts of the recombinant proteins with the AC dimeric form of PP2A. The activities of the resulting complexes were then determined using phosphorylated myosin light chain as substrate.

Fragments 52–174 and 88–174 (Fig. 1), containing truncations of the amino-terminal common region, and fragments 1–131 and 1–150, containing truncations of the carboxyl-terminal unique region, all inhibited the activity of the AC dimer (Fig. 2). The IC50 values for fragments 52–174 and 88–174 were 0.7 and 1.5 μM, whereas the IC50 values for 1–131 and 1–150 were 35 and 90 nM, respectively. In contrast, fragment 1–110, which cannot bind to the A subunit (12), had no effect on phosphatase activity. Although they inhibited activity to the same extent as wild type small-t, the recombinant fragments with truncations of the amino terminus had lower apparent affinity for the AC dimer than the wild type protein (IC50 = 30 nM). Sequences within the amino-terminal 88 amino acids were not required for inhibition of PP2A activity, but they were important for high affinity interaction of small-t with PP2A. Notably, removal of the first 51 amino-terminal residues of
small-t (fragment 52–174) resulted in a 140-fold increase in the IC$_{50}$.

Identification of the Minimal Region of Small-t That Binds to the A Subunit—Based on the results described above and those reported previously (12), the region of small-t containing amino acids 111–130 appeared to contain a major PP2A binding site. To further define the regions necessary for interaction, small-t fragments containing residues 95–135 and 95–165 (Fig. 1) were generated and assayed for their ability to bind to the A subunit. 35S-Labeled small-t fragments were generated by in vitro transcription/translation and incubated with a glutathione-agarose resin complex of either GST-A or with GST alone. The agarose beads were washed and eluted with glutathione, and the eluted material analyzed by SDS-PAGE. Fig. 3A shows that both small-t fragments bound specifically to GST-A and not to GST. These results indicate that the region containing amino acids 95–135, which includes the putative binding domain, is able to bind to the A subunit. This 41 amino acid fragment of small-t therefore contains the residues that are sufficient for binding to the A subunit.

An amino acid sequence alignment of the putative PP2A binding domain from the small-t proteins of various polyomaviruses is shown in Fig. 4A. The alignment reveals several residues around the first cysteine cluster that are highly conserved. To investigate the importance of these residues, we employed a set of sequential carboxy-terminal truncation mutants to identify the carboxy-terminal boundary of the PP2A binding domain (Fig. 1). Various 35S-labeled small-t fragments were generated by in vitro transcription/translation and assayed for their ability to associate with GST-A. Truncations at or amino-terminal to residue 118 resulted in a complete loss of binding (Fig. 3B). However, fragments that were truncated at or carboxy-terminal to position 119 still bound to GST-A (Fig. 3B). These results indicate that amino acid 119 is the carboxy-terminal boundary of the PP2A binding domain. They also show that residues in addition to the conserved cysteines are required for binding to PP2A.

Because residue 119 of small-t is highly conserved (either Lys or Arg), we postulated that the positive charge at position 119 might be important for binding to PP2A. To test this hypothesis, the carboxy-terminal arginine at position 119 of the 1–119 fragment was mutated to alanine, lysine, or glutamic acid, and the mutant fragments were assayed for binding to the A subunit. Surprisingly, mutation of Arg$^{119}$ had no apparent effect. An equivalent amount of each truncation mutant bound to GST-A (data not shown). Therefore, the positive charge at amino acid 119 is not critical for interaction with GST-A. These results suggest that the amino acid at position 119, at least in the context of the 119-truncation fragment, makes important peptide backbone interactions that can be contributed by several different amino acids.

To test the idea that the conserved cysteine cluster is an integral component of the A subunit binding domain, a small-t deletion mutant lacking residues 110–119 was constructed and assayed for binding to PP2A. 35S-Labeled wild type small-t or small-t deletion mutant Δ110–119 (Fig. 1) were combined with 35S-labeled PP2A catalytic subunit and incubated with GST-A. Analysis of the bound material showed that removal of the first cysteine cluster resulted in a dramatic decrease in the ability of small-t to bind to the A subunit. The binding of the C subunit, added as a control for nonspecific effects, was not altered (Fig. 3C). In control experiments, neither wild type small-t, Δ110–119, nor the C subunit bound to GST alone (data not shown).

Peptide Analogs Block Binding of Small-t to the A Subunit—The effects of small-t truncations and deletions are consistent with the idea that the region surrounding the first cysteine cluster is a domain that interacts with the A subunit. However, an equally compelling argument is that deletion of residues 110–119 has a global effect on small-t structure that disrupts A
subunit binding. Consequently, a second approach utilizing small-t peptide analogs was used to corroborate the results from the mutagenesis studies. Synthetic peptides (Fig. 4B) spanning the putative PP2A binding domain of SV40 small-t (amino acids 91–131) were synthesized and assayed for their ability to block the association of 35S-labeled wild type small-t with GST-A. To ensure that the peptides had specific effects on small-t, the experiments were done in the presence of 35S-labeled C subunit. This strategy takes advantage of the non-overlapping binding sites for small-t and the catalytic subunit on the A subunit of PP2A (16).

In a control experiment, an excess of recombinant small-t inhibited the association of 35S-labeled wild type small-t with GST-A while having no effect on catalytic subunit binding (Fig. 5). The ability of different peptides to inhibit small-t binding to GST-A was determined by assaying for their ability to inhibit the association of wild type small-t with GST-A in the absence or the presence of 2.5 μM recombinant small-t (ST) or with peptides 91–110, 105–122, 97–122, or 117–128 as indicated (all at a final concentration of 25 μM). The bound material was analyzed by 12% SDS-PAGE followed by fluorography.

**Fig. 3.** Binding of small-t mutants to the A subunit of PP2A. 35S-Labeled wild type and mutant small-t proteins were translated in vitro, and combined with GST (−) or GST-A (+) complexed to glutathione agarose. The bound material was eluted with glutathione and analyzed by SDS-PAGE followed by fluorography. The binding of full-length small-t and small-t fragments 95–135 and 95–165 is shown in panel A. The binding of fragments 1–110, 1–116, 1–118, 1–119, 1–120, 1–125, and 1–131 is shown in panel B. In panel C, wild type small-t or the Δ110–119 mutant protein was combined with in vitro translated C subunit and assayed for binding to GST-A as described above.

**Fig. 4.** Summary of the activities of synthetic peptide. A, an alignment of the amino acid sequences of the putative PP2A binding domains of various polyomaviruses (residues 91–131 in SV40 small-t). The symbols above the sequence indicate absolutely conserved (*), highly conserved (#), and nonconserved (−) residues. B, the sequence of the synthetic peptides and their effects on PP2A. A summary of the GST-A binding and phosphatase assay results is shown at the right. ND, not determined.

**Fig. 5.** Synthetic peptides specifically inhibit the binding of small-t to PP2A. 35S-Labeled catalytic subunit and wild type small-t were combined with GST-A in the absence or the presence of 2.5 μM recombinant small-t (ST) or with peptides 91–110, 105–122, 97–122, or 117–128 as indicated (all at a final concentration of 25 μM). The bound material was analyzed by 12% SDS-PAGE followed by fluorography.

Peptide Analogs Inhibit PP2A Activity—Small-t mutants that were capable of binding to GST-A also inhibited the activity of the AC dimeric form of PP2A. To determine whether the small-t peptides could also affect activity, they were assayed for their ability to inhibit the phosphatase activity of the AC complex. The small-t peptides were incubated with either AC or the free C subunit and their effects on PP2A activity determined. None of the peptides had any effect on the phosphatase activity of the free catalytic subunit (Fig. 6A, open bars). However, the two peptides that blocked the association of small-t with GST-A caused a significant decrease in the activity of the AC complex (Fig. 6A, hatched bars). Because there was no effect on the free catalytic subunit, the results show that, like inhibition by wild type small-t, inhibition of PP2A by the pep-
The apparent affinities of peptides 97–122 and 105–122 were characterized by determining the IC\textsubscript{50} for inhibition of the AC complex. Increasing concentrations of peptides were combined with AC, and the phosphatase activity was determined using \textsuperscript{32}P-labeled myosin light chains as a substrate. Fig. 6 shows the dose-response curves for the various peptides. Peptides 91–110 and 117–128 showed no inhibition at any concentration tested, whereas peptides 97–122 and 105–122 inhibited AC activity with an IC\textsubscript{50} of 193 ± 19 and 223 ± 31 nM, respectively. Although these values are higher than the IC\textsubscript{50} of wild type small-t (5 nM), the peptides still interacted with relatively high apparent affinity.

In order to determine the role of the conserved cysteines in the activity of peptide 105–122, each of the cysteines was changed to a serine, and the mutant peptide was assayed for its ability to inhibit AC. When assayed over the concentration range in which wild type 105–122 peptide inhibited AC, peptide 105–122 (C-S) had no affect on activity (Fig. 6B). However, at higher concentrations, peptide 105–122 (C-S) was able to inhibit the core AC enzyme (IC\textsubscript{50} = 27 ± 3.6 μM). As observed with the other peptides, 105–122 (C-S) had no effect on catalytic subunit (data not shown). These results indicate that the cysteines are not required for inhibitory activity but are important for high affinity binding to AC.

The Association of Zinc with Small-t Peptides Requires the Conserved Cysteines—Mutation of the conserved cysteines, especially cysteine 116, results in small-t proteins that have reduced stability \textit{in vivo} (30) but are still able to bind to the A subunit (31). These observations suggest that mutation of the cysteines alters the structure of the protein while still permitting association with the A subunit. Our results with peptides 105–122 and 105–122 (C-S) are consistent with these observations. Both the wild type peptide and the mutant peptide were able to associate with AC, but the mutant peptide had significantly lower affinity.

Circular dichroism was used to determine whether mutating the conserved cysteines in peptide 105–122 altered its structure. Fig. 7A shows the CD spectra for peptides 97–122, 105–122, 117–128, and peptide 105–122 (C-S). Peptide 117–128 exhibited a minimum peak at 200 nm indicating that this peptide had an unordered structure in solution (32). Both peptides 97–122 and 105–122 (C-S) are consistent with these observations. Both the wild type peptide and the mutant peptide were able to associate with AC, but the mutant peptide had significantly lower affinity.

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tide (Fig. 7A). This result suggests that the reduced affinity of peptide 105–122 (C-S) for the AC complex may be due to the loss of secondary structure.

SV40 small-t has been shown to bind to 2 mol of zinc (19), and the conserved cysteines are likely to be involved in coordination of the associated zinc atoms (33). To determine whether the cysteines in the synthetic peptides were able to coordinate zinc, the CD spectra for peptides 117–128, 97–122, 105–122, and 105–122 (C-S) were determined in the presence and absence of zinc (Fig. 8). The addition of zinc to peptides 97–122 and 105–122 caused the minima to shift to the right and also caused a decrease in their intensity (Fig. 8, B and C). These spectral changes indicate that zinc associates with these peptides resulting in a change in their structure. Subsequent treatment with EDTA caused the CD spectra of peptides 97–122 and 105–122 to return to the zinc free state (data not shown). Conversely, addition of zinc to peptides 117–128 or 105–122 (C-S) did not cause a change in structure (Fig. 8, A and D). The lack of effect of zinc on the structure of peptide 105–122 (C-S) indicates that the cysteines in the conserved cluster are involved in the coordination of zinc.

DISCUSSION

Previous work detailing the interaction of small and middle tumor antigens with PP2A has shown that there are multiple sites of interaction between the two proteins. The A subunit of PP2A is composed of 15 repeats of a loosely conserved 40–45-amino acid sequence that has been defined as an armadillo (arm) repeat. Arm repeats are present in a variety of proteins and are thought to be involved in protein-protein interactions (34). A model for the A subunit has been proposed (15) that is consistent with the three-dimensional structure of the arm repeat region of β-catenin, the ortholog of the Drosophila armadillo protein (35). Tumor antigens and cellular regulatory subunits interact with multiple regions within repeats 1–10, whereas the catalytic subunit binds to carboxyl-terminal repeats 11–15 of the A subunit (16). A requirement for multiple arm repeats has also been demonstrated for the interaction of β-catenin with its accessory proteins (35).

Consistent with the involvement of multiple sites on the A subunit, multiple regions of small-t are also involved in the association with PP2A. In addition to the region encompassing the first cysteine cluster (12), a conserved region encompassing residues 97–103 also plays an important role in interaction (31). Mutational analysis has shown that the equivalent region is also important for interaction of polyoma small and middle tumor antigens with PP2A (36, 37). Although it is clear that the 97–103 region is important for high affinity binding to PP2A, it is not crucial. Mutations in this region reduce the apparent affinity of interaction but do not completely inhibit the interaction with PP2A in vitro (31).

Mutation of additional regions of polyoma small and middle tumor antigens affects interaction with PP2A. Mutations within residues 142–145, which lie between the first and second cysteine clusters, inhibited co-immunoprecipitation of polyoma small-t with PP2A (37). Although the sequence of SV40 small-t in this region is not highly related to polyoma small-t, it may also play a role. The amino-terminal common domain of SV40 small-t is not required for interaction with PP2A (12, 31) but it may play an accessory role. Deletions and point mutations of polyoma middle-t antigen showed that the
first 25 residues are important for interaction with PP2A in co-immunoprecipitation assays (36). Our data are consistent with a role for the amino-terminal region of SV40 small-t also being involved in association with PP2A. Removal of the first 51 amino acids caused a 140-fold reduction in apparent affinity for the AC complex (2). The amino-terminal domain of small-t shares sequence similarity with the J-domains of DnaJ proteins (38). The amino-terminal common domain of small-t can functionally replace the J-domain of the Escherichia coli DnaJ molecular chaperone (39, 40). Results from mutational analysis of polyoma and SV40 small-t suggest that the J-domain of small-t could be involved in interactions with PP2A.

Our data define amino acids 105–122 of SV40 small-t as a site that interacts with PP2A. Deletion of residues 110–119 resulted in a protein that was unable to bind to the A subunit of PP2A. Additionally, peptides, which contained residues 105–122, blocked the association of wild type small-t with GST-A and inhibited PP2A activity. These results suggest that this region contains a primary interaction site, whereas other regions of small-t contribute to high affinity binding to PP2A. Although this region encompasses the first cysteine cluster of small-t, the conserved cysteines are not required for interaction with PP2A. A peptide in which each of the cysteines was mutated to serine still interacted with the AC dimer, although with lower affinity (Fig. 6). These results are consistent with previous studies showing that mutation of one or two cysteines within the first cluster had no dramatic effect on interaction with PP2A (31).

Mutation of the highly conserved cysteines at amino acids 111, 113, and 116 affected the structure of the 105–122 peptide. Mutating the cysteines to serines caused the structure of the peptide to change from a β-strand to a random conformation and decreased apparent affinity for the AC dimer by 2 orders of magnitude. These results indicate that the cysteines contribute directly to the structural integrity of the peptide and presumably play a similar role in full-length small-t. Accordingly, mutation of these residues in full-length small-t causes decreases in stability that are consistent with alteration in protein structure (30, 31, 33). Collectively, these results argue that the conserved cysteines play an important role in forming the structure of this region of small-t and that the presence of a β-strand secondary structure is critical for high affinity binding to PP2A.

Small-t is a metalloprotein that binds to 2 mol of zinc (19), and association of zinc is important for the stability and solubility of the protein (30, 33). Mutation of conserved cysteines in the first cluster also causes a reduction in zinc binding (33). The reduced stability of the cysteine mutants suggests that binding of zinc is important for the structural stability of small-t. Although it has been proposed that the conserved cysteines are involved in the coordination of zinc, the actual metal binding ligands in small-t have not been identified. The fact that mutation of the cysteines within peptide 105–122 to serines abolished its zinc-induced conformational change strongly supports the proposal that the cysteines are involved in the direct coordination of zinc.

It has been suggested that coordination of zinc by small-t involves an arrangement of the cysteine clusters that is similar to the binuclear thiolate zinc cluster of the GAL4 transcription factor (19). However, the cysteine at position 103 and the histidine at position 122 (Fig. 4A) are also conserved throughout the polyomavirus family (17). Furthermore, mutation of cysteine 103 and histidine 122 decrease the stability of small-t, indicating that these residues could also be involved in the coordination of zinc (31, 33). The combination of cysteine 103, histidine 122, and the conserved cysteine clusters of small-t generates a putative zinc binding domain with the sequence (CX_CXXX_CX_HX_HX_HX_HX_C, CXXX, C), which could serve as a zinc binding center. This sequence does not readily conform to any of the known zinc binding domains (42) and may represent a novel motif.

It has been pointed out previously that small-t, as well as the cellular regulatory subunits, binds to overlapping regions of the A subunit of PP2A. A puzzling aspect has been the fact that none of these regulatory subunit families share significant amino acid similarity with each other or with small-t. This lack of significant sequence homology is also seen in the proteins that associate with the arm region of β-catenin (35). This lack of a shared sequence motif contrasts with the presence of conserved sequence motif in proteins that interact with protein phosphatase 1 (43). The lack of a conserved sequence suggests that a conserved structural element is responsible for interaction with PP2A. The CD data provided here represent the initial information regarding the nature of such a structural element within a PP2A regulatory protein. The secondary structure of the region of small-t encompassed by residues 105–122 is predicted to be a β-strand. The CD spectra of peptides that inhibit the phosphatase activity of the AC dimer and block small-t binding to GST-A indicate that they have the predicted β-strand structure. There are several examples in protein structure data bases in which proteins lacking any amino acid identity contain structurally related domains. Structural stabilization, induced by coordination of metals, could be duplicated by appropriate side-chain interactions. It has been postulated that the structure of metal binding motifs may not be unique to metalloproteins, but may represent more general folding motifs (41). Therefore, despite the lack of amino acid sequence similarity and the absence of cysteine clusters, the PP2A regulatory subunits may share a common structural element with small-t antigen. Our data suggest that this common element may be a region of β-strand secondary structure.

Acknowledgment—We thank Dr. Jose Rizo-Rey for assistance with circular dichroism measurements.

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J. Biol. Chem. 1998, 273:35339-35346.
doi: 10.1074/jbc.273.52.35339

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