AKAP79 Inhibits Calcineurin through a Site Distinct from the Immunophilin-binding Region*

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Targeting of protein kinases and phosphatases provides additional specificity to substrate selectivity in cellular signaling. In the case of the Ca2+/calmodulin-dependent protein phosphatase calcineurin, AKAP79 has been shown to bind calcineurin and inhibit its activity in vitro (Coghlan, V., Perrino, B. A., Howard, M., Langenberg, L. K., Hicks, J. B., Gallatin, W. M., and Scott, J. D. (1995) Science 267, 108–111). In the present study, we characterized the binding regions on calcineurin A (CnA) and AKAP79 that are important for this interaction. Residues 30–98 and 311–336 on CnA, and residues 108–280 on AKAP79 were found to be important for binding. The binding of CnA by AKAP79 does not require the calcineurin B subunit, and occurs in a region distinct from where the immunosuppressant-immunophilin complex binds. AKAP79 also bound to CnA in cells transfected with AKAP79 and CnA. To determine the function of AKAP79-calcineurin interaction in intact cells, we measured the dephosphorylation and subsequent activation of NFAT, a transcription factor that is a substrate of PKA, through AKAP79-calcineurin interaction in NFAT-mediated signaling.

Multiple hormones signal through common second messengers to activate the same protein kinase/phosphatase cascade yet elicit distinct cellular responses (1). One form of regulation for these diverse effects is the intracellular compartmentalization of the kinases and phosphatases involved (2–4). For example, although ubiquitously expressed, protein phosphatase 1 (PP1)1 is localized to specific subcellular compartments, including glycogen particles in liver, neuronal dendrites, and myofibrils of skeletal muscle and smooth muscle by its association with targeting subunits (2, 4). These targeting subunits confer in vivo substrate specificity not only by localizing PP1, but also by regulating the activity of PP1 toward different substrates (2, 4, 5).

Ca2+/calmodulin-dependent protein phosphatase 2B, or calcineurin (6), is a protein phosphatase that may be localized by targeting subunits. Calcineurin plays an essential role in many signaling pathways (6, 7). It consists of a catalytic A subunit (CnA), and a calcium-binding regulatory B subunit (CnB). In activated T cells, calcineurin dephosphorylates the cytoplasmic factor, NFAT, allowing it to translocate into the nucleus and activate interleukin-2 transcription (8, 9). Immunosuppressants, such as cyclosporin A (CsA) or FK506, when bound to their respective intracellular immunophilins, inhibit calcineurin phosphatase activity, preventing NFAT activation and subsequent cytokine gene induction (8, 9). Because of the diverse role calcineurin plays in cellular processes, it is likely that, similar to PP1, calcineurin targeting is an important regulatory mechanism.

Insight into calcineurin targeting may also be gained from consideration of the targeting of PKA. A group of proteins, referred to as AKAPs (A-kinase-anchoring proteins), target PKA to specific microenvironments by anchoring the regulatory subunits (RI and RII) of PKA (10–12). Anchoring of PKA by AKAPs has been shown to be important for regulation of PKA-mediated processes (12). One such AKAP, AKAP79, is enriched in neurons, but is also present in T cells. AKAP79 binds membrane vesicles through its NH2 terminus (13), and has been proposed to anchor PKA (14), calcineurin (15), and PKC (11). The interaction between AKAP79 and calcineurin (13, 15) has been partially characterized biochemically. When calcineurin was copurified with AKAP79 from rat brain extract, calcineurin was found to be inactive in the complex. Indeed, AKAP79 inhibits purified calcineurin in vitro (13, 15). However, the domains involved in the AKAP79 and calcineurin interaction are poorly defined and the functional consequence of this interaction is still unclear.

In the present study, we have further characterized the regions on CnA and AKAP79 that are required for their interaction by using yeast two-hybrid analysis as well as biochemical methods. Transfection studies with wild type and mutant versions of these proteins demonstrated that AKAP79 inhibited calcineurin-dependent dephosphorylation of NFAT, suggesting that AKAP79 plays a critical role in regulating calcineurin activity in vivo.

EXPERIMENTAL PROCEDURES

Materials—Vectors and cDNA library for the two-hybrid work were obtained from Dr. Steven Elledge, Baylor College of Medicine. The y153ΔCnB mutant yeast strain was a gift from Dr. Crabtree, Stanford University. CnA and CnB constructs were a gift of Dr. Brian Perrino and Dr. Thomas Soderling at Vollum Institute; pGL3-NFAT1 was a gift of Dr. Anjana Rao, Harvard University. pGL3-NFATc1erase consists

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1 The abbreviations used are: PP1, protein phosphatase 1; CnA, calcineurin A; CnB, calcineurin B; CsA, cyclosporin A; AKAPs, A kinase anchoring proteins; PKA, cAMP-dependent protein kinase A; RI, type I regulatory subunit of PKA; RII, type II regulatory subunit of PKA; PKC, protein kinase C; HER293, human embryonic kidney 293 cells; GFF, green fluorescent protein; PCR, polymerase chain reaction; GL-GFP, green lantern green fluorescent protein; PMA, phorbol 12-myristate 13-acetate; FKBP, FK506-binding protein; PBS, phosphate-buffered saline; HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis; Pipes, 1,4-piperazine-diyethanesulfonic acid.

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AKAP79 Binds and Inhibits Calcineurin in Cells

AKAP79 binds to calcineurin, a T-7 epitope tag (5' TACACAAAAATTTTATGCTAGCATGACTGGTGGACAG

CACAATGGGGT) was added to the NH2 terminus of full-length, 1-335 amino acids (N-335) or 1-420 amino acids (V-420) of rat brain CaN (22) by PCR. Each PCR reaction used a common 5' primer encoding a specific restriction site, followed by a Kozak consensus sequence, a T-7 epitope, and the codons for amino acids 2-7 of the NH2 terminus of rat brain CaN. The products were digested with the appropriate restriction enzymes, and cloned into the episomal mammalian expression vector pcCPE4 (Invitrogen). A Flag-epitope (5' -GATATTTTGGCTGAATGGGAGATGACGATGA-

cAACATGCAAGAGATGGTTGAGCAACACAG-3') was added to the NH2 terminus of AKAP79 (full-length), AKAP79/1-108, and AKAP79/108-427 by PCR. The 5' primer used encoded a specific restriction site followed by a Kozak consensus sequence, a T-7 epitope, and the codons for residues of the NH2 terminus of human AKAP79 (17). The PCR products were subcloned into pcCPE4 and pcDNA3.1 (Invitrogen). GFP or GL-GFP (Life Technologies, Inc.) was also attached to the COOH terminus of AKAP79 and subcloned into pcDNA3.1 (Invitrogen).

Biotinylated AKAP79/108-247 or AKAP79/247-427 were expressed in a modified Pinpoint system (Promega) using the arabinose promoter (23, 24). The plasmids were transformed into E. coli E104E (Xoma Corp.), grown at 30 °C in the presence of 4 mM biotin to an OD600 of 0.7. Biotinylated AKAP79/1-108, AKAP79/108-247, and AKAP79/247-427 were expressed using T7-CnA or its various mutants into COS cells using Superfect (Qiagen). Sixteen hours following transfection, the cells were washed with CMF-PBS, lysed, scraped in 1.2 ml of CB buffer (10 mM Pipes, pH 6.8, 100 mM NaCl, 3 mM MgCl2, 1 mM EDTA, 300 mM sucrose, 0.5% Triton X-100, 10 μg/ml leupeptin and aprotinin, 2 μg/ml pepstatin-A) and transferred to microcentrifuge tubes. Lysates were clarified by centrifugation at 500 × g for 3 min and incubated on ice for 1 h with 10 μg of anti-T7 monoclonal antibody (Novagen), or 10 μg of anti-Flag M2 monoclonal antibody (Eastman-Kodak). The lysates were rotated for 30 min at 4 °C with 40 μl of CB buffer-washed Protein-G-Sepharose (Pharmacia). The immunoprecipitates were washed 3 × 300 μl of CB buffer, 1 × 300 μl of 100 mM NaCl, 10 mM Pipes, pH 7.2, 20 μg/ml aprotinin. The immunoprecipitates were resuspended in 50 μl of 60 mM NaCl, 10 mM Pipes, 2% SDS, 24% glycerol, 0.5 mg/ml bromphenol blue. One-fifth of the immunoprecipitates were subjected to immunoblot analysis as described below.

For characterizing the effect of AKAP79 on endogenous calcineurin activity, HEK293 cells were transfected with control vector or GFP-AKAP79, in the presence of an NFAT responsive promoter controlling luciferase expression to measure NFAT activation. After 16 h, the cells were washed twice with PBS. Fresh medium was added and the cells were returned to the incubator. Assays for NF-AT activation were performed 24 h later.

Cell Stimulation and Luciferase Assay—Cells co-transfected with the NFAT luciferase gene (1 μg) and AKAP79 or gravin constructs (1 μg) were washed with 1 × 106 cells per well plated with 1 ml of minimal essential medium containing 1 mM CaCl2, and supplemented with 3 μM CsA (vehicle control), or PMA (40 ng/ml) + ionomycin (2 μM), or PMA (40 ng/ml) + ionomycin (2 μM) + CsA (1 μM) for 6 h at 37 °C. Cells were harvested, lysed in 200 μl of reporter lysis buffer (Promega), and centrifuged at 15,000 × g in a 4 °C microcentrifuge. 20 µl of the supernatants were assayed for luciferase activity using the luciferase substrate (Promega) as per the manufacturer’s instructions. Assays were read on a Dynatech ML3000 Microtiter luminometer.

Western Blot Analysis—Immunoprecipitates from transfection studies were also analyzed by Western blot. Proteins were subjected to 10% SDS-PAGE (Novex), transferred to Immobilon (Millipore), and blocked in Blotto (5% non-fat dry milk/TBS). Three different sets of antibodies were used for the immunoblot: anti-T7/HRP-conjugated monoclonal antibody (Novagen, 1:5000); anti-AKAP79 monoclonal antibody (183E, 1:2000) followed by goat anti-mouse/HRP (Boehringer, 1:7500); or anti-Flag M2 monoclonal antibody (10 μg/ml) followed by goat anti-mouse HRP. Immunoblots were washed with TBS, 0.2% Tween 20 between incubations and immunoreactivity detected by chemiluminescence (NECN Life Science Products). In NFAT dephosphorylation experiments the cells and the codons for culture medium containing Me2SO, PMA + ionomycin, or CsA + PMA + ionomycin for 1 h at 37 °C. Cells were harvested and resuspended in 30 μl of 40 mM Tris, pH 8.0, 60 mM sodium pyrophosphate, 10 mM EDTA. After addition of 30 μl of 10% SDS, the sample was subjected to three rounds of vigorous vortexing, followed by boiling for 10 min, then analyzed by SDS-PAGE. Western blot analysis was performed using anti-Flag antibody, anti-AKAP79 antibody 183E, or anti-AKAP79 antibody.

RESULTS

AKAP79 Binding Region on Calcineurin.—To determine the AKAP79-binding site on CaN, COOH-terminal and NH2-terminal CaN truncations were examined in a two-hybrid yeast strain expressing AKAP79. For the COOH-terminal region, deletion of the last 151 residues of CaN (C336), containing the regions that bind CnB (28), calmodulin (29), and the autoinhibitory region (30), did not affect AKAP79 binding. Similar results were obtained in a two-hybrid yeast strain devoid of
Two-hybrid analyses were performed using AKAP79 region(s) on CnA. Binding was assayed by and CnA deletions containing the GAL4 activation domain. Binding to FKBP12 in the presence of FK506 was also measured. The CnA catalytic domain, mutants and (Fig. 1A), we examined CnA point mutations surrounding residues 335 and 337. AKAP79 binding was not affected by Cys335 → Ala, Ser336 → Ala, Pro337 → Ala, or Pro339 → Ala substitutions (Fig. 1B). Consecutive triplicate alanine substitutions spanning residues 311–334 were used to further delineate the residues important for the AKAP79-CnA interaction. Alanine mutations spanning residues 311–316 did not affect AKAP79 binding. In contrast, AKAP79 binding was reduced or abolished in CnA alanine mutants spanning residues 317–334 (Fig. 1B). Similar results were found when the AKAP79 expressing yeast strain lacking CnB was used. To show that the mutants are functional structurally, one of the triplicate alanine mutations, N329IR → AAA was chosen for characterization of functional binding to FKBP12. This CnA/NIR mutant interacted with FKBP12 in the presence of FK506 in yeast two-hybrid analysis, as well as in vitro binding studies (data not shown). When the purified CnA/NIR mutant was reconstituted with CnB, it showed similar protein phosphatase activity as wild type calcineurin (data not shown), indicating that the mutants did not affect calcineurin structure or activity.

Calcineurin A Binds to AKAP79 in Vivo—To determine whether CnA interacts with AKAP79 in cells other than yeast, T7-CnA was co-transfected with full-length Flag-AKAP79 in COS cells. T7-CnA was detected in anti-Flag AKAP79 immunoprecipitates (Fig. 2A, lane 4). In reciprocal experiments, Flag-AKAP79 was detected in anti-T7 CnA immunoprecipitates (Fig. 2B, lane 3). T7-CnA (Fig. 2A, lanes 1, 3, and 4) or Flag-AKAP79 (Fig. 2B, lanes 1, 3, and 5) was expressed only in cells transfected with the respective plasmids. Expression levels of each were not affected by co-expression of the other protein (for T7-CnA, compare Fig. 2A, lanes 1 and 3; for Flag-AKAP79, compare Fig. 2B, lanes 1 and 5).

AKAP79 Binding Requires Residues Around 335 on Calcineurin A—To further characterize the specificity of the interaction between AKAP79 and CnA, constructs encoding AKAP79 and CnA truncations were co-transfected into COS cells. Two COOH-terminal T-7 CnA truncations terminating at residue 335 (CnA/N335) and 420 (CnA/N420) were tested for their ability to co-immunoprecipitate with AKAP79. The expression levels of all T7-CnA constructs were similar, as indicated by T-7 immunoprecipitates (Fig. 3A), AKAP79 co-immunoprecipitated with CnA/N420 (Fig. 3B, lane 6), but not with CnA/N335 (Fig. 3B, lane 5). These results are consistent with the yeast two-hybrid analysis (Fig. 1), indicating that the region around residue 335 of CnA is important for AKAP79 binding.

Calcineurin A Binding Involves Residues 108–280 of AKAP79—Reciprocal experiments were performed to characterize the region on AKAP79 that is important for CnA binding. A series of AKAP79 deletion mutants were prepared and tested by two-hybrid analysis. CnA associated with AKAP79/280 (residues 1–280), but not AKAP79/236 (residues 1–236; Fig. 4A), suggesting that residues around 236–280 on AKAP79 are important for CnA binding. As a control, these AKAP79 mutants were also tested for their ability to bind RII in two-hybrid analysis (Fig. 4A), To characterize whether the NH2-terminal region of AKAP79 is also important for the interaction with CnA, biochemical experiments were carried out using NH2-terminal truncations of AKAP79. Biotinylated AKAP79/108–427 bound to CnA in a specific and saturable manner, with an affinity of 191 ± 113 nM (Average ± S.E., n = 3; Fig. 4B). This is similar to the affinity (243 ± 22 nM; n = 12) obtained for the binding of biotinylated CnA to full-length AKAP79 (data not shown). However, binding was not observed when biotinylated AKAP79/247–427 was used as the ligand (Fig. 4B, n = 3), suggesting that residues between 108 and 247 on AKAP79 are necessary for binding to CnA. As expected, both biotinylated AKAP79/108–427 and AKAP79/247–427 bound RII (data not shown). Taken together, the two-hybrid analysis and in vitro binding studies indicate that residues including 236–280 on AKAP79 are critical for CnA binding.

In order to determine whether the regions important for binding are also necessary for inhibition of calcineurin activity,
we tested to see if AKAP79/108–427 or AKAP79/247–427 could inhibit calcineurin activity toward a peptide substrate in vitro. AKAP79/108–427 was found to inhibit calcineurin activity with a predicted IC50 of 272 \( \pm 22 \) nM (Fig. 4C, \( n = 3 \)), similar to the IC50 of 430 nM obtained in a previous study (13). Consistent with the inability of AKAP79/247–427 to bind calcineurin, this fragment did not inhibit calcineurin activity (Fig. 4C, \( n = 3 \)). Likewise, a previous study has shown that AKAP79/1–108 does not bind or inhibit calcineurin (13). The results confirm that residues 108–427 of AKAP79 are necessary for both binding to CnA and inhibiting calcineurin activity.

To determine whether region(s) identified on AKAP79 in vitro are also important for CnA binding in vivo, we transfected Flag-AKAP79/1–108 or AKAP79/108–427 along with T-7 CnA into COS cells. Flag-AKAP79/1–108 was detected in anti-T7 CnA immunoprecipitates (Fig. 5, lane 3) but AKAP79/1–108 was not (Fig. 5, lane 2). Both forms of AKAP79 were expressed at comparable levels, as demonstrated in the anti-Flag-AKAP79 control immunoprecipitates (Fig. 5, lanes 4 and 5). Thus, consistent with the in vitro results described here and in Ref. 13, the first 108 residues of AKAP79 are not required for binding of CnA inside cells.

AKAP79 Inhibits Calcineurin-dependent NFAT Induction—To extend the in vitro results on the AKAP79 inhibition of calcineurin phosphatase activity, we tested the functional consequence of AKAP79 overexpression on calcineurin activity. Calcineurin has been shown to be essential for NFAT activation (16). Consequently, we examined the induction of NFAT using a transcription reporter (Figs. 6A and Fig. 7). HEK293T cells were used as the activation of NFAT in these cells is calcium-dependent and sensitive to CsA (Fig. 6A) (32–35), indicating that the response is calcineurin dependent. In cells that co-expressed AKAP79, PMA/ionomycin-induced NFAT activity was inhibited 95% compared with cells co-transfected with vector alone (Fig. 6A). The AKAP79-mediated inhibition of NFAT luciferase activity was specific, as co-expression of gravin, an AKAP79-related protein which also binds PKA and PKC, but not calcineurin (36), resulted in only 20% inhibition of NFAT luciferase activity (Fig. 6A). Western blot analysis verified that AKAP79 and gravin were expressed in transfected cells (Fig. 6B). These results suggest that AKAP79 can inhibit calcineurin function in vivo.

To test if the AKAP79 truncations that were found to be important for interacting with CnA in vitro are also important for regulating calcineurin function in intact cells, NH2-terminal AKAP79 truncations were co-transfected into HEK293T...
cells along with the NFAT luciferase plasmid to measure calcineurin activity (Fig. 7). Overexpression of the AKAP79/108–427 in HEK 293T cells inhibited NFAT luciferase activity (80%), as effectively as full-length AKAP79 (Fig. 7). In contrast, AKAP79/1–108 caused only a ~30% decrease in NFAT luciferase activity (Fig. 7). Western blot analysis using anti-Flag antibody showed that both the 1–108 as well as the 108–427 fragments were expressed at levels comparable to that of full-length AKAP79 in transfected HEK293T cells (data not shown). Since NFAT transactivation requires the calcineurin-dependent activation of cytosolic NFAT as well as PKC-mediated activation of the nuclear AP1 component (33, 37, 38), inhibition of PKC by the 1–108 fragment (39) could account for the partial decrease of NFAT-driven luciferase. Likewise, the binding of PKC by gravin could also account for its ~10–20% inhibition of NFAT luciferase activity (Fig. 6). The inhibition of NFAT luciferase by the 108–427 fragment was not due to binding of PKA, as a Val400→Pro point mutation, which disrupts RII-AKAP79 interaction (17), still effectively inhibited NFAT luciferase activity (Fig. 7). Consistent with in vitro binding and inhibition data, these results suggest that AKAP79/108–427 inhibits calcineurin-dependent NFAT activation by decreasing calcineurin phosphatase activity.

Inhibition of Calcineurin Dephosphorylation of NFATp by AKAP79—Since signaling via PKC and other pathways contributes to the induction of NFAT luciferase, we assessed calcineurin phosphatase activity in HEK293 cells stably expressing AKAP79 by examining the dephosphorylation of NFATp. NFATp has been shown to be a direct substrate of calcineurin in vitro (40–42). The calcineurin-mediated dephosphorylation of NFATp results in a distinct shift to a lower apparent molecular weight on SDS-PAGE. Upon stimulation with ionomycin alone, NFATp shifted from an apparent molecular mass of ~130 kDa to an apparent molecular mass of ~120 kDa. This shift of NFATp on SDS-PAGE was blocked in cells treated with CsA during ionomycin stimulation, indicating that NFATp dephosphorylation is dependent on calcium signaling and calcineurin, as previously reported (40–42) (Fig. 8, lane 4). In HEK293 cells stably expressing a GFP-AKAP79 fusion protein, however, no change in apparent molecular weight of NFATp was observed upon stimulation with ionomycin (Fig. 8, lane 7). Interestingly, a small amount of the NFATp shifted to the lower apparent molecular weight in cells treated with Me2SO vehicle control (Fig. 8, lane 2). This effect is likely due to a
AKAP79 binds to CnA and inhibits calcineurin activity in vivo. The regions that are critical for binding were identified by two-hybrid analysis, by in vitro biochemical studies, and through co-immunoprecipitation experiments with transfected cells. AKAP79 was found to bind to calcineurin at sites distinct from that of the immunosuppressant-immunophilin complex, suggesting that AKAP79 and the immunosuppressants inhibit calcineurin activity by different mechanisms. The inhibition of calcineurin activity by AKAP79 was also shown in intact cells. Overexpression of AKAP79 inhibited the dephosphorylation of NFAT by endogenous calcineurin, resulting in a decrease in NFAT activation. Since NFAT activation is a key component of interleukin-2 transcriptional regulation, the inhibition of NFAT activation by AKAP79 will decrease T cell signaling.

It was previously postulated, based on sequence similarity to FKB12 and peptide inhibition studies, that residues 88–102 of AKAP79 were responsible for binding calcineurin (15). How-
ing subunits and protein phosphatases, we compared the three-dimensional structure of calcineurin to PP1 (Fig. 9). A peptide based on a consensus binding sequence found in many PP1 targeting subunits has been co-crystallized with PP1 (5). This peptide binds to a hydrophobic channel that is remote from the catalytic site. Interestingly, the COOH-terminal β13/β14 sheets in PP1 have been shown to be important contact areas for the binding of this peptide to PP1 (5). This hydrophobic channel is on the opposite side of the β13/β14 sheets where the corresponding AKAP79-binding region on calcineurin is. While the exact residues in the β13/β14 sheets are not conserved between CnA and PP1, the overall tertiary structure in the core region is similar (Fig. 9). Based on the deletion analysis and structural similarity, it is attractive to postulate that AKAP79 may also bind to a similar region on CnA. Co-crystallization of AKAP79 with CnA will clarify whether AKAP79 and the PP1 targeting subunits bind to similar functional domains on calcineurin and PP1, respectively.

The binding of AKAP79 to calcineurin diminished calcineurin-dependent signaling in intact cells (Figs. 6–8). Deletion of the membrane-targeting NH2-terminal 108 residues of AKAP79 did not affect its ability to inhibit calcineurin activity (Fig. 7), suggesting that AKAP79 inhibition of NFAT activation was unlikely to be due solely to AKAP79 sequestering calcineurin at the membrane, away from NFAT. This inhibitory effect of AKAP79 on calcineurin activation of NFAT also differs from that on PKA signaling to the nucleus, since anchoring of PKA by AKAP79 enhances nuclear phosphorylation of CREB (46). The association of calcineurin with AKAP79 may represent an inactive “pool,” or the association may provide a mechanism by which calcineurin-dependent signaling is regulated. Whether and how the association between AKAP79 and calcineurin is regulated remains to be determined.

In addition to regulating T cell signaling, AKAP79 could also modulate calcineurin activity in other cells by targeting it to specific areas where its substrates are localized, or coordinate signaling between calcineurin and other effector molecules that interact with calcineurin (42, 47, 48). In brain, AKAP79 (49) and calcineurin (50) are found in dendritic spines in the hippocampal pyramidal neurons where excitatory glutamate receptors are localized. Considering that calcineurin plays important roles in immune and neuronal functions, understanding the role of calcineurin targeting will provide not only a better understanding of cell-specific mechanisms of calcineurin regulation, but also a potential novel target for designing cell-specific calcineurin inhibitors.

Fig. 8. Inhibition of NFAT dephosphorylation in HEK 293 cells stably expressing AKAP79. HEK293 or HEK293 cells stably expressing GFP-tagged AKAP79 (HEK-AKAP79GFP) were transfected with vector alone (vector) or a construct encoding NFATp (NFAT). The cells were treated with Me2SO (D), ionomycin (1 μM, I), or ionomycin (1 μM) + CsA (1 μM) (CsA/I) for 30 min at 37 °C. SDS whole cell extracts were made and analyzed by 6% SDS-PAGE followed by Western blotting with anti-NFATp antibody. Lanes 9 and 10 show immunoblot using anti-AKAP79 antibodies, demonstrating the presence of AKAP79 in HEK/AKAP79GFP.

Fig. 9. Three-dimensional structure of calcineurin and protein phosphatase 1. A, the AKAP79-binding regions on calcineurin is highlighted in the three-dimensional structure for CnA using the axis provided by Ref. 45. The purple regions highlights the β13/β14 region of CnA (residues 311–340 of bovine CnA) which was shown in the present study to be important for AKAP79 binding (see Fig. 1B), with the violet area showing residues NIR. The NH2-terminal region, residues 31–97 of bovine CnA (45) corresponding to residues 30–96 in mouse CnA that are important for AKAP79 binding, is shown in cyan color. B, three-dimensional structure of PPI (51) with the β13/β14 sheets, also highlighted in purple. Arrow shows the hydrophobic groove that has been shown to bind a peptide encoding a consensus sequence important for PP1 targeting (5).
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