Phosphorylation-dependent Conformational Changes Induce a Switch in the Actin-binding Function of MARCKS*

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Phosphorylation of myristoylated alanine-rich protein kinase C substrate (MARCKS) by protein kinase C eliminates actin filament cross-linking activity, but residual filament binding activity docks phosphorylated MARCKS on filamentous actin. The postulated actin-binding region of MARCKS, which includes a Ca\(^{2+}\)-calmodulin-binding site, has been portrayed with a helical structure, analogous to other calmodulin-binding domains. Previous speculation suggested that MARCKS may dimerize to form the two functional actin-binding sites requisite for cross-linking activity. Contrary to these hypotheses, we show that MARCKS peptide with actin-cross-linking activity has an extended structure in aqueous solution but assumes a more compact structure upon phosphorylation. We hypothesize that structural changes in the MARCKS peptide induced by phosphorylation create a dynamic structure that, on average, has only one actin-binding site. Moreover, independent of the state of phosphorylation, this peptide is monomeric rather than dimeric, implying that two distinct actin-binding sites are responsible for the actin-cross-linking activity of unphosphorylated MARCKS. These studies uniquely elucidate the mechanism by which phosphorylation of MARCKS induces structural changes and suggest how these structural changes determine biological activity.

Myristoylated alanine-rich protein kinase C substrate (MARCKS)\(^1\) is a primary and prominent protein substrate for protein kinase C (PKC) (1, 2) that binds calmodulin in a calcium-dependent manner (3), binds to phospholipid membrane (4), binds to and cross-links filamentous actin (5), and has been implicated in cellular processes associated with cytoskeletal restructuring, e.g. transmembrane signaling and neurotransmitter release (6–8). MARCKS is an acidic protein (pI 4.0) of 309 amino acids with an apparent molecular mass ranging from 65 to 85 kDa because of anomalous migration on SDS-polyacrylamide gels (9) and contains three highly conserved domains (6, 8). The first is a basic (pI 12.2) effector, phosphorylation site domain (PSD), which in rodents contains twelve lysines, one arginine, five phenylalanines, five serines, one glycine, and one leucine. Three of the serines (Ser\(^{152}\), Ser\(^{156}\), and Ser\(^{163}\)) are phosphorylated by PKC (10, 11). The second conserved domain of MARCKS is the myristoylated amino-terminal domain that, in conjunction with the PSD, helps anchor the protein to the inner surface of the plasma membrane (12). An important feature of the MARCKS protein is its ability to cycle between membrane and cytosolic fractions in a PKC phosphorylation-dependent manner (4, 13). In a nonphosphorylated state, MARCKS is tightly associated with the inner surface of the plasma membrane independently of a cytosolic face protein receptor (14) and translocates to the cytosol following PKC-mediated phosphorylation. The third conserved region is the MARCKS homology domain, which contains the splice junction for a single intron, and nothing is known regarding its function.

In addition to its role in associating MARCKS with the plasma membrane, the phosphorylation site domain of the MARCKS protein serves as an important binding site for both filamentous (F-actin) and calmodulin. MARCKS both binds to and cross-links F-actin (5, 15). Because the interaction between MARCKS and F-actin occurs in the phosphorylation site domain, PKC-mediated phosphorylation of MARCKS inhibits its capacity to cross-link F-actin while only diminishing its capacity to bind F-actin (5). By virtue of its action in cross-linking actin filaments at the plasma membrane, the MARCKS protein may play a significant role in translating extracellular signals to calcium/calmodulin-dependent and PKC-regulated intracellular events associated with actin plasticity and actin-plasma membrane interaction (16, 17).

All of the known interactions involving MARCKS, membrane, calcium/calmodulin, and F-actin, involve the 25-amino acid PSD and are mediated by reversible PKC phosphorylation. Although frequently depicted as an \(\alpha\)-helix (5, 6), several reports on MARCKS-PSD propose that it has little, if any, well-defined structure (9, 19–21). Many thermodynamic and kinetic studies of interactions between MARCKS and phospholipid membranes have led to the notion of a “myristoyl-electrostatic switch” (18), and electron paramagnetic resonance studies have suggested that MARCKS-PSD binds to membranes in an extended conformation (19). Studies using circular dichroism (20) and NMR (21) both concluded that MARCKS-PSD binds to calcium/calmodulin in a nonhelical structure, but electron paramagnetic resonance studies on the same system are consistent with MARCKS-PSD bound in an \(\alpha\)-helical conformation (22). Nothing is known about the conformational properties of MARCKS-PSD when bound to F-actin.

Given the important role of phosphorylation in modulating the many interactions of MARCKS, we have examined the 25-amino acid MARCKS-PSD as a function of phosphorylation. NMR and molecular dynamics simulations both reveal signifi-
cant conformational changes between the phosphorlated and nonphosphorlated MARCKS peptides. Based on the conformational studies and actin cross-linking results, a new model is proposed in which MARCKS phosphorylation results in a large conformational change that, in turn, blocks one of two actin-binding sites and thus inhibits actin cross-linking.

**Experimental Procedures**

**NMR Spectroscopy**—NMR data were collected with a Varian Unity Plus 600 MHz at the University of Florida Center for Structural Biology and a Varian Unity Plus 720 MHz at the National High Magnetic Field Laboratory. Peptides for all experiments were synthesized by PEPTIDE Pharmaceuticals. Samples containing between 1 and 5 mM peptide were prepared with 90% H2O, 10% D2O, and 0.3 mM 3-trimethylsilyl (2,2,3,3-2H4) propionic acid for an internal chemical shift standard (0.0 ppm). Unless otherwise indicated, the pH (uncorrected for D2O) of each sample was adjusted to between 4.5 and 5.5 by using small volumes of concentrated HCl or ROH. Different experiments were collected with the salt concentration adjusted between 0 and 100 mM KCl. The temperature of the samples during data collection was either 15 or 20°C. NOE data with 300-ms mixing times were also collected at 4°C for both peptides; at that temperature we found similar patterns of cross-peaks but more spectral overlap than from data collected at higher temperature. The data were picked at 5-ps intervals and energy minimized using 1000 iterations of a5Å layer of explicit water molecules and energy minimized using 1000 iterations of steepest descents (to a convergence of less than 5 kcal/mol).

**Molecular Dynamics Simulations**—MARCKS peptide models were prepared with 90% H2O, 10% D2O, and 0.3 mM 3-trimethylsilyl (2,2,3,3-2H4) propionic acid for an internal chemical shift standard (0.0 ppm). Unless otherwise indicated, the pH (uncorrected for D2O) of each sample was adjusted to between 4.5 and 5.5 by using small volumes of concentrated HCl or ROH. Different experiments were collected with the salt concentration adjusted between 0 and 100 mM KCl. The temperature of the samples during data collection was either 15 or 20°C. NOESY data with 300-ms mixing times were also collected at 4°C for both peptides; at that temperature we found similar patterns of cross-peaks but more spectral overlap than from data collected at higher temperature. The data were picked at 5-ps intervals and energy minimized using 1000 iterations of steepest descents (to a convergence of less than 5 kcal/mol).

**Actin Filament Cross-linking Assays**—Low Speed Pelleting and Light Scattering of Actin Induced by MARCKS Peptides—Gel-filtered rabbit skeletal muscle actin was converted to Mg2+-actin, polymerized, and diluted to a final concentration of 7.8 mM in 2.0 mM MgCl2, 20 mM NaCl, 0.2 mM dithiothreitol, 0.1 mM ATP, 0.125 mM EGTA, 0.1 mM CaCl2 (buffer F). These actin filaments were incubated with varying concentrations of MARCKS peptides for 15 min and then pelleted at 18,000 × g for 15 min. Supernatants were run on SDS-polyacrylamide gels with quantification of theactin band by densitometry. The amount of pelleted actin was calculated based on a G-actin control for which it was assumed that no actin pelleted.

**Phosphorylated and Nonphosphorylated MARCKS PSD**—Yield Significantly Different Solution NMR Spectra—A helical wheel representation of MARCKS PSD suggests that it might form an α-helix (5, 6). Therefore, we used NMR to examine the solution conformations of both the phosphorylated and nonphosphorylated forms of the 25-amino acid MARCKS PSD.

**RESULTS**

**Phosphorylated and Nonphosphorylated MARCKS PSD Yield Significantly Different Solution NMR Spectra**—A helical wheel representation of MARCKS PSD suggests that it might form an α-helix (5, 6). Therefore, we used NMR to examine the solution conformations of both the phosphorylated and nonphosphorylated forms of the 25-amino acid MARCKS PSD.

Sedimentation equilibrium experiments were performed using absorption optics (collecting data at 258 nm) in a Beckman XL A centrifuge and analyzed as described previously (30). The 120-μl samples reached equilibrium in 48 h at 38,600 RPM at 20°C. Denaturants were determined by table or pycnometry. Partial specific volumes (ρ) were calculated (31) assuming that the amplitude from sedimentation of the sedimentation equilibrium gradient in 90% D2O and in H2O (32).

For sedimentation equilibrium studies including both actin and MARCKS, samples were prepared in 0.1 mM CaCl2, 0.1 mM ATP, 0.2 mM dithiothreitol, 0.01% NaN3, 5.0 mM Tris, pH 7.9, with 12 μM actin and 6.0 μM MARCKS peptide. Equilibrium was achieved at 14,400 RPM after 72 h at 4°C. The gradient was analyzed using a method of implicit boundary conditions as described previously (30). In brief, at 280 nm, absorption coefficients of MARCKS peptides were negligible relative to that of actin, because the MARCKS peptides have no aromatic residues other than phenylalanine. The concentration gradient measured at 290 nm could therefore be assumed to directly reflect the sum of the concentration of all detectable actin species. Curve fitting was based on the theoretical molecular weights of actin or of actin in complex with MARCKS peptides at a stoichiometry of 1:1 and 2:1 actin:MARCKS.

The species are assumed to be in chemical equilibrium at all radii, with dissociation constant Kd describing the site-specific interaction between actin and either of the two postulated binding sites on MARCKS, that is, the binding sites were considered to be independent and equal. Final curve fitting was constrained by the initial concentrations of both actin and MARCKS, and the fitting parameters included the concentrations of actin and MARCKS at a single radius and Kd.

**Actin Filament Cross-linking Assays**—Low Speed Pelleting and Light Scattering of Actin Induced by MARCKS Peptides—Gel-filtered rabbit skeletal muscle actin was converted to Mg2+-actin, polymerized, and diluted to a final concentration of 7.8 mM in 2.0 mM MgCl2, 20 mM NaCl, 0.2 mM dithiothreitol, 0.1 mM ATP, 0.125 mM EGTA, 0.1 mM CaCl2 (buffer F). These actin filaments were incubated with varying concentrations of MARCKS peptides for 15 min and then pelleted at 18,000 × g for 15 min. Supernatants were run on SDS-polyacrylamide gels with quantification of the actin band by densitometry. The amount of pelleted actin was calculated based on a G-actin control for which it was assumed that no actin pelleted.

90° light scattering assays were performed at 400 nm in quartz cuvettes at 22°C. A 7.8 μM Mg2+-F–actin stock with varying concentrations of MARCKS peptides was prepared in buffer F as for the pelleting assay. Light scattering intensity increased for the first 15 min after mixing actin with peptide and then stabilized. The maximum increment in intensity was approximately 8-fold. The experiments for the nonphosphorylated MARCKS peptide were repeated in buffer F in which the salt concentration was substituted with either 0 or 100 mM NaCl.
resolved in the phosphorylated peptide and significantly overlapped in the nonphosphorylated peptide, suggesting more structure in the phosphorylated than in the nonphosphorylated peptide around the glycine. Second, bulk water interacts to a much larger extent with backbone amides in the nonphosphorylated peptide than in the phosphorylated peptide. This difference in interaction is observed both in TOCSY and NOESY spectra collected without presaturation of water (23). The only difference in interaction is observed both in TOCSY and NOESY spectra collected with a 200-ms mixing time. The temperature for these experiments was 15 °C.

In addition to the phosphoserines, chemical shifts of several other amino acids are dependent upon pH. The largest of these shifts were observed in two of the three phenylalanines (Phe13 and Phe20) following the phosphoserines, the glycine (Gly17), and the amino terminus (Lys1). Several other residues showed small but significant pH-dependent shifts including the other phenylalanines (Phe7, Phe9, and Phe16), the nonphosphorylated serines (Ser16 and Ser23), and leucine (Leu15). To our surprise, all of the resonances in all of the lysine residues were constant with pH (except H1 of Lys1). The changes with pH are summarized in Fig. 2.

Both the phosphorylated and nonphosphorylated MARCKS peptides have salt-dependent chemical shifts (data not shown). In both peptides, all the resonances shift upfield with increasing salt. Unlike the pH results, every amide resonance shifts with salt. The groups with the largest shifts are those that have more backbone intramolecular interactions in the phosphorylated than in the nonphosphorylated MARCKS peptide. Third, the phosphorylated peptide yields a large number of relatively strong adjacent amide to amide NOE peaks, whereas the nonphosphorylated MARCKS peptide has absolutely no peaks in the same region. No longer range amide-to-amide peaks are present in the phosphorylated MARCKS peptide spectrum, but there are several H1-H2+-H2+-H2 cross-peaks (Fig. 2). There are no medium or long range NOE cross-peaks in the nonphosphorylated MARCKS peptide NOESY spectra. The patterns of NOEs seen in the phosphorylated peptide do not correspond to any regular secondary structure and are most consistent with a series of partial turns or “nascent helix” (33). Fourth, the phosphorylated peptide NOESY spectrum shows many more interactions between phenylalanine aromatic rings and backbone amide protons than the nonphosphorylated peptide spectrum. This suggests that the rings are more compact and in closer proximity to the backbone in the phosphorylated than in the nonphosphorylated MARCKS peptide.

All of the NOESY and TOCSY data (Fig. 2) suggest a significant population of compact or “turn-like” conformations for the phosphorylated peptide and completely extended or “random” conformations for the nonphosphorylated MARCKS peptide. Although the two peptides have very different NMR spectra, neither shows convincing evidence for either an α (28) or 3_10 helix (28, 34).

**NMR Spectra for Phosphorylated MARCKS Demonstrate Large pH and Salt Dependence**—The nonphosphorylated MARCKS peptide has a net charge of +13. The phosphorylated MARCKS peptide with three phosphoserines has a pH-dependent net charge that varies between +13 (low pH) to +10 (intermediate pH) to +7 (high pH). Ionic interactions clearly play a major role in the biophysical properties of MARCKS PSD. Fig. 3 shows regions of TOCSY spectra for the phosphorylated MARCKS peptide at four different values of pH (4.61, 5.65, 6.31, and 6.88). Values of pH greater than 7 led to a severe reduction in amide intensities because of exchange. The groups in the phosphorylated peptide that titrate over this range of pH are the phosphoserines and the amino terminus. The largest pH-dependent shifts are the amide protons of the phosphoserines, which are all nearly 0.4 ppm downfield with increasing pH. Significantly smaller changes were recorded for the phosphoserine H1 or H1 resonances; these side chain protons move less than 0.1 ppm over the pH range investigated.

Computer Modeling Produces Compact Structures for Phosphorylated and Fully Extended Structures for Nonphosphorylated MARCKS—Molecular dynamics (MD) simulations on the phosphorylated and nonphosphorylated MARCKS peptides reveal dramatically different conformations. Fig. 4 shows families of energy minimized structures obtained at regular intervals from molecular dynamics trajectories. As described under
“Experimental Procedures,” all of the simulations were done with explicit water and charges on the peptides. The nonphosphorylated peptide has 13 positive charges that lead to significant ionic repulsion. Thus, the nonphosphorylated MARCKS peptide family of conformers are fully extended. In contrast, the presence of three phosphates on the phosphorylated MARCKS peptide adds either 3 or 6 negative charges, depending on the pH. Most of the simulations were done with singly charged phosphates to match the intermediate pH values used in the NMR experiments. The negatively charged phosphates placed along the phosphorylated peptide act to transiently attract different positively charged lysines or arginine. The dynamics simulations reveal multiple positively charged groups taking turns interacting with a given phosphate. The result of the transient ionic interactions is to create a compact “cup-like” backbone structure from a series of turns held together through “lever arm” actions of the side chains. Fig. 5 shows the end-to-end distances of the phosphorylated peptide with NMR restraints, the phosphorylated peptide without NMR restraints, and the nonphosphorylated MARCKS peptide as a function of time in the MD trajectories.

The MD simulations described above on the phosphorylated MARCKS peptide included NMR NOESY distance restraints as pseudo-potentials, so it is not surprising that the computed structures account for the NMR data. Therefore, we repeated the simulations on the phosphorylated peptide without any NMR restraints and found similar results to the simulations with restraints (structures not shown).

Phosphorylated and Nonphosphorylated MARCKS PSD Are Monomeric—The equilibrium distribution of the nonphosphorylated MARCKS peptide in an analytical ultracentrifuge corresponded to that expected for a 3054-Da monomer with a partial specific volume ($\bar{\delta}$) of 0.77 ml/g as calculated from the amino acid sequence (Fig. 6). The steeper gradient seen with the phosphorylated MARCKS peptide cannot be accounted for by its slightly greater molecular mass (3292 Da) but could indicate some extent of oligomerization or a difference in $\bar{\delta}$. A comparison of the sedimentation gradient of the phosphorylated MARCKS peptide in H$_2$O and D$_2$O solutions suggested that $\bar{\delta}$ was 0.74 ml/g (Fig. 6, inset), with an identical result when $\bar{\delta}$ was calculated from the peptide sequence after adjustment for phosphorylated amino acids. Using this value for $\bar{\delta}$, the equilibrium gradient for the phosphorylated MARCKS peptide was also seen to be consistent with monomer. Results were similar, with no evidence for dimer or other oligomers, at both pH 6.1 (Fig. 6) and pH 7.0 (data not shown).

In the presence of the phosphorylated MARCKS peptide, G-actin sediments in the analytical ultracentrifuge precisely as expected for a monomeric protein of 42 kDa. In the presence of the nonphosphorylated MARCKS peptide, the data no longer fit the theoretical curve for an actin monomer (Fig. 7, upper panel) but instead show evidence of aggregation. Repetition of the experiment with a second preparation of actin gave identical results. One explanation compatible with the observed degree of aggregation would be for the nonphosphorylated MARCKS peptide to have two or more actin-binding sites on which it can cross-link actin. A model for the nonphosphorylated MARCKS peptide with two independent and equal actin-binding sites can be shown to adequately fit the data when the site-specific dissociation constant equals 17 $\mu$M. Other more complicated models are tenable but cannot be justified as superior to the fit achieved for this simple model of interaction.

Both Phosphorylated and Nonphosphorylated MARCKS Peptides Cross-link Actin but at Significantly Different Concentra-
In a pelleting assay in 20 mM NaCl, the nonphosphorylated MARCKS peptide was able to induce pelleting of 7.8 μM F-actin at low concentrations, with 50% of the actin pelleted when the peptide concentration was 1.0 μM (Fig. 8A). The phosphorylated MARCKS peptide also induces F-actin pelleting, but only at much higher concentrations, with nearly 50% of the F-actin pelleted at a peptide concentration of 7.0 μM. Under these conditions in the absence of MARCKS, less than 5% of the F-actin pelleted. These observations are consistent with the hypothesis that the nonphosphorylated MARCKS peptide efficiently cross-links actin filaments by binding to separate filaments with each of its two actin-binding sites. Pelleting by the phosphorylated MARCKS peptide implies that it can also cross-link actin filaments but with much lower affinity than the nonphosphorylated peptide. Cross-linking was not inhibited at very high concentrations of the nonphosphorylated MARCKS peptide, a result that could be explained if the dose response of the pelleting assay is not linear with respect to the extent of cross-linking, so that only a small fraction of residual cross-link was sufficient to pellet nearly all of the F-actin. Alternatively, saturation by ligand of both of two cross-linked filaments may not lead to loss of cross-linking if the peptide is able to bind to two distinct sites on actin.

Data from light scattering were qualitatively similar to data from the pelleting assay. The nonphosphorylated MARCKS peptide much more efficiently increased scattering intensity than the phosphorylated peptide both in maximal extent of increase and increase at comparable concentrations of peptides (Fig. 8B). In separate assays, neither MARCKS peptide had any effect on either the elongation rate or steady-state F-actin.
levels for pyrene-labeled actin (data not shown). These results suggest that the scattering intensity is a measure of the extent of actin-filament cross-linking induced by MARCKS. Although the nonphosphorylated MARCKS peptide cross-links F-actin in 100 mM NaCl, the results showed a large dependence on salt concentration, with significantly decreased cross-linking at higher salt concentrations.

**DISCUSSION**

Our NMR, sedimentation, cross-linking, and molecular dynamics data support the following conclusions. 1) The phosphorylated MARCKS peptide forms a more compact structure than the nonphosphorylated MARCKS peptide. The phosphorylated peptide differs from the nonphosphorylated peptide by the addition of phosphates to three serines. NMR data and molecular dynamics simulations both demonstrate that there are large conformational changes resulting from the phosphates. All data support fully extended structures for the nonphosphorylated peptide and a compact structure for the phosphorylated MARCKS peptide. The compactness is a result of transient interactions between the negatively charged phosphates and several different positively charged lysines or arginine. Although this “compactness” might be considered a “nascent helix” (33), we see no NOE interactions that would be fully consistent with an α-helix. 2) The nonphosphorylated MARCKS peptide has two actin-binding sites. The observation that nonphosphorylated MARCKS peptide cross-links actin (5) led to the conclusion that either it had one actin-binding site and forms dimers or that it has two actin-binding sites. Our sedimentation results conclusively show that the nonphosphorylated MARCKS peptide is monomeric in solution. This finding is not surprising considering the +13 charge on the peptide at neutral pH. We cannot rule out the possibility that the nonphosphorylated peptide dimerizes in the presence of actin filaments, but it seems unlikely considering the relatively uniform distribution of positive charges across the molecule. Aggregation of G-actin by the nonphosphorylated MARCKS peptide confirms the 2:1 actin:nonphosphorylated MARCKS peptide binding stoichiometry. The apparent binding constant of 17 μM is consistent with the extent of dimerization but may have no bearing on actual affinity of MARCKS for F-actin, where the binding site may depend on conformational changes or inter-subunit contacts that are specific to F-actin. All available evidence (based on co-localization) currently suggests that both the phosphorylated and nonphosphorylated MARCKS peptides...
have selective affinity for F-actin (6). 3) The nonphosphorylated MARCKS peptide cross-links actin at much lower concentrations than the phosphorylated MARCKS peptide. Previous reports demonstrated that both the phosphorylated and nonphosphorylated MARCKS peptides bound to actin filaments but that only the nonphosphorylated peptide cross-linked actin (5). Our actin cross-linking assay has shown that the phosphorylated MARCKS peptide does cross-link actin but only at 7-fold greater concentrations than the nonphosphorylated peptide. Moreover, our sedimentation results suggest little or no nonphosphorylated MARCKS peptide dimer in solution.

At least two models for the modulation of actin cross-linking emerge from these studies. In both models, the phosphorylated and nonphosphorylated MARCKS peptides contain two actin-binding sites. In the absence of phosphates (the nonphosphorylated MARCKS peptide), both actin-binding sites interact with actin filaments with relatively high affinity and thus cross-link two different filaments. In the first model, the addition of three phosphates leads to a molecule (the phosphorylated MARCKS peptide) that has one high affinity actin-binding site and a second site with roughly 7-fold lower affinity for actin because of ionic or steric interactions. In this model, the observed conformational changes are simply coincident to and not directly controlling the actin cross-linking. In the second model, the conformational changes in the phosphorylated MARCKS peptide, which arise from the addition of the three phosphates, mask one of the two actin-binding sites. This conformational masking is the direct result of the compactness of the molecule. Based in part on our observations that there appears to be no dominant phosphoserine-lysine salt bridge, the compact structures are transient and would expose the second actin-binding site roughly one-seventh of the time. In this model, the conformational changes provide the direct control of actin cross-linking.

Why would it be important to regulate the cross-linking activity of MARCKS by phosphorylation? The presence of two-binding sites allows for F-actin to be cross-linked into a variety of specific structures. Neither the low speed pelleting nor light scattering assays specify whether nonphosphorylated MARCKS peptide cross-links F-actin into parallel bundles, orthogonal networks, or random aggregates of filaments. Previous studies using electron microscopic techniques have also not been conclusive, with published images showing examples of each of these types of structures (5). Because nonphosphorylated MARCKS localizes to the plasma membrane, perhaps specifically to focal adhesions, it is reasonable to speculate that MARCKS regulates cytoskeletal-membrane interactions, either controlling membrane stability or providing a potential communication link between extracellular matrix and cytoskeleton.

Several lines of evidence suggest that MARCKS may play an important role in neuropsychiatric events during brain development, in the adult animal during learning, and in the long term response to psychotropic drugs used in the treatment of manic-depressive illness (35–38). In fact over the course of brain maturation, MARCKS expression decreases in the majority of brain regions but remains elevated in areas associated with a high level of plasticity including hippocampus and olfactory bulb (39, 40). The PKC-dependent interaction of the PSD region of MARCKS with the plasma membrane, calmodulin, and F-actin indicates that MARCKS is well positioned to modulate regional actin-membrane plasticity in response to specific patterns of PKC and calcium-calmodulin signaling during long term events requiring cytoskeletal remodeling in both the developing and adult brain. A better understanding of the biophysical properties of MARCKS in actin cross-linking will offer novel strategies for elucidating the mechanisms of signal-dependent plastic changes in cell systems.

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