Interaction between Actin and the Effector Peptide of MARCKS-related Protein

IDENTIFICATION OF FUNCTIONAL AMINO ACID SEGMENTS*

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It is widely assumed that the members of the MARCKS protein family, MARCKS (an acronym for myristoylated alanine-rich C kinase substrate) and MARCKS-related protein (MRP), interact with actin via their effector domain, a highly basic segment composed of 24–25 amino acid residues. To clarify the mechanisms by which this interaction takes place, we have examined the effect of a peptide corresponding to the effector domain of MRP, the so-called effector peptide, on both the dynamic and the structural properties of actin. We show that in the absence of cations the effector peptide polymerizes monomeric actin and causes the alignment of the formed filaments into bundle-like structures. Moreover, we document that binding of calmodulin or phosphorylation by protein kinase C both inhibit the actin polymerizing activity of the MRP effector peptide. Finally, several effector peptides were synthesized in which positively charged or hydrophobic segments were deleted or replaced by alanines. Our data suggest that a group of six positively charged amino acid residues at the N-terminus of the peptide is crucial for its interaction with actin. While its actin polymerizing activity critically depends on the presence of all three positively charged segments of the peptide, hydrophobic amino acid residues rather modulate the polymerization velocity.

Myristoylated alanine-rich C kinase substrate (MARCKS)1 and MARCKS-related protein (MRP) are the two members of the MARCKS protein family, a group of acidic rod-shaped proteins (1, 2). MARCKS is a ubiquitous 32-kDa protein, whereas MRP (also known as F52 or MacMARCKS) has a molecular mass of 20 kDa and is mainly expressed in brain and reproductive tissues (3, 4). A comparison of their primary structures reveals three conserved domains: 1) the N-terminal domain contains the recognition site for myristoylation (5); 2) the MARCKS homology 2 domain, a 15-amino acid residue-long stretch whose function is unknown, shows sequence identity with the cytoplasmic tail of the mannose 6-phosphate receptor (3); 3) an extremely basic domain, known as the phosphorylation site domain or effector domain that is 24–25 amino acid residues long, is responsible for most of the interactions of MARCKS proteins with their common ligands (see below). Both MARCKS and MRP bind to calmodulin (CaM) with nanomolar affinity (6, 7), are phosphorylated by protein kinase C (PKC) (8, 9), and bind to acidic lipid membranes (10, 11). Whereas binding of both MARCKS proteins to CaM is inhibited by phosphorylation in vitro (7, 12), phosphorylation-dependent inhibition of the binding to phospholipid vesicles has been reported for MARCKS (13), but not for MRP (14, 15). In cells, a connection between the subcellular localization of MARCKS and its phosphorylation state has been documented (16). Whereas both the N-terminal myristoyl chain and the effector domain are involved in membrane binding (5, 10, 14), binding to CaM and phosphorylation by PKC mainly occur at the effector domain (17, 18).

Only little information is available on the cellular functions of MARCKS and even less on MRP. Evidently, both proteins are essential for brain development (19–21). It is well documented that at the cellular level MARCKS proteins mediate cross-talk between CaM and PKC (reviewed in Refs. 22 and 23). Furthermore, these proteins colocalize with filamentous actin or actin-binding proteins in response to a number of cellular events during which a reorganization of the actin cytoskeleton is involved, such as cell spreading (24, 25), neurosecretion (26), or macrophage activation (27). In 1992, Hartwig et al. (28) found that MARCKS cross-links actin filaments in vitro. The findings that upon phosphorylation MARCKS is translocated from the membrane (16) and that binding of CaM inhibits its cross-linking of actin filaments (28) led to the model that the primary function of MARCKS is to regulate the structure of the actin cytoskeleton by cross-linking actin filaments to networks or bundles at the membrane (28). To this end, it is assumed that this protein–protein interaction is mediated to a large extent by the effector domain of MARCKS (3).

Since the effector domains of MARCKS and MRP exhibit very high sequence homology (96% identity), a similar function has been assumed for MRP. To gain insight into the role of the effector domain during the presumed interaction of MRP with actin, we investigated the effect of a peptide corresponding to the effector domain of MRP, the so-called effector peptide, on rabbit skeletal muscle actin. The 24-amino acid residue-long

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1 The abbreviations used are: MARCKS, myristoylated alanine-rich C kinase substrate; MRP, MARCKS-related protein; CaM, calmodulin; F-actin, filamentous actin; G-actin, globular actin; group 1/2/3 peptide, effector peptide with modifications belonging to group 1/2/3; PKC, protein kinase C; PKM, catalytic subunit of PKC; wt, wt peptide, “wild type” MARCKS effector peptide.
MRP effector peptide is highly basic and has the following primary sequence: **KKKKKFSFKKPKLSGLSFKRNK**, with the 12 positively charged residues (i.e. 10 Lys and 2 Arg) typed in bold letters and hydrophobic residues in italics. It differs from the MARCKS effector domain mainly in the lack of one arginine after the five N-terminal lysine residues and in the proline residue, which is replaced by a serine in MARCKS (3). Thus, both peptides are highly polyctonic, but also possess hydrophobic regions which are probably involved in their binding to membranes (29). Experimental evidence has been obtained that the MARCKS effector peptide assumes a random coil structure both in solution (30) as well as when bound to CaM (31) or membranes (29), and similar properties can be assumed for the MRP effector peptide (31).

It has been shown that the structure of actin filament networks is strongly altered in the presence of very high concentrations of divalent cations (32) or by polycations (33). While high concentrations of divalent and polycationic cations lead to the formation of paracrystalline arrays (34), lower concentrations of polycations like polylysine or [Co(NH3)6]3+ align actin filaments laterally to form bundles (35). The effect of polycations can be explained by the condensation theory of Manning (36), which assumes that polycations are bound as counterions along the filaments and partially compensate the negative charges on actin, which normally prevent single filaments from forming bundles. In agreement with its polycationic nature, two studies have demonstrated that the effector domain of MARCKS or the corresponding effector peptide bundles actin filaments (28, 35). The interaction of this peptide with actin was, however, not investigated from a kinetic point of view. This latter aspect is addressed in particular in this article.

Moreover, the possibility for a regulatory function of CaM and PKC on the effector peptide activity is assessed by investigating their capability to affect the actin polymerization kinetics or the formation of higher actin filament structures induced by the MRP effector peptide.

Last but not least, to obtain some information on the role of the distinct sequence segments of the peptide during its interaction with actin, three groups of peptides were synthesized in which a particular amino acid segment was deleted or replaced by alanines: In group 1, some or all hydrophobic amino acid residues (i.e. phenylalanine and leucine) are replaced by alanines. In group 2, the N-terminal positively charged Lys residues are replaced by alanines or deleted, with or without replacement of the serine groups or the hydrophobic segments (for further details, see “Experimental Procedures”).

In group 3, the central and/or the C-terminal positively charged Lys and Arg residues are replaced by alanines.

These group 1 to group 3 “mutant” peptides are evaluated for their effect on both actin polymerization and/or actin filament reorganization into higher order structures, aimed at ultimately acquiring a more rational understanding of the interactions of the intact proteins MARCKS and MRP with actin.

**Experimental Procedures**

**Proteins and Peptides—**Actin was isolated and prepared from rabbit skeletal muscle according to the method of Spudich and Watt (37), as recently described by Steinmetz et al. (38), and stored at 4°C in G-buffer (2.5 mM imidazole, pH 7.4, 0.2 mM CaCl2, 0.2 mM ATP) containing 0.001% NaN3. For fluorescence measurements, actin was labeled at cysteine 374 with N-(1-pyrenyloiodoacetamide (Molecular Probes Europe B.V., Leiden, Netherlands) following the method of Kouyama and Mihashi (39). The extent of labeling was typically 80%. Actin concentration and its extent of pyrene labeling were determined as described by Cooper et al. (40). The absorption measurements were performed on a Kontron UVIKON spectrophotometer (Kontron Instruments, Zürich, Switzerland).

A peptide corresponding to the effector domain of murine MRP (amino acid residues 86–109), the MRP effector peptide, was obtained from AMS Biotechnology (Lugano, Switzerland). The amino acid composition as well as the concentrations of the effector peptides were determined by quantitative amino acid analysis. CaM from bovine brain was purchased from Sigma (Division of Fluka Chemie AG, Buchs, Switzerland), and the catalytic subunit of rat brain protein kinase C (PKM) was obtained from Calbiochem. “Wild type” MARCKS effector peptide (wt peptide) and the 13 mutant peptides, which were a kind gift of Prof. Felix Althaus from the Tierspital, University of Zurich, Switzerland, were synthesized by Alexis Corp. (Läuffelfingen, Switzerland). All other chemicals were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland) in the highest purity grade available.

The following peptides were used (“mutations” are underlined).

| Peptide | Sequence |
|---------|----------|
| wt peptide | KKKKKFSFKKPKLSGLSFKRNK |
| Group 1 | |
| M-13: | KKKKKPAASKASAGSAKKNNK |
| M-14: | KKKKKPSASKLSGFSKRNK |
| M-16: | KKKKKPSASKLSGFSKRNK |
| Group 2 | |
| M-1: | AAAAAFSFKKPKLSGFSKRNK |
| M-8: | FFSFKKPKLSGFSKRNK |
| M-9: | FFKKPKLSGFSKRNK |
| M-10: | AASKASAGSAKKNNK |
| M-11: | AAAAAFAFKKPKLSGFSKRNK |
| M-12: | AAAAAAKSAGSAKKNNK |
| M-15: | AAAAAFAFSFKKPKLSGFSKRNK |
| Group 3 | |
| M-2: | KKKKKPSASKLSGFSKRNK |
| M-3: | KKKKKFSFKKLVSFLSGFSKRNK |
| M-4: | KKKKKFSFKKLVSFLSGFSKRNK |

**Electron Microscopy—**Actin polymerization was monitored by following the fluorescence increase of the pyrenated actin moiety, according to Koyama and Mihashi (39). Unless indicated otherwise, pyrene-labeled actin was mixed with unlabeled actin to yield a final extent of 5%. 400 µl of actin solution was put into a 5 × 5-mm quartz glass cuvette (Hellma Suprasil, Hellma, Müllheim, Germany), and the fluorescence was recorded at an angle of 90°. The sample was excited at 365 nm with a bandwidth of 1.5 nm, and emission was measured at 407 nm with a bandwidth of 5 nm. To minimize scattering effects, a cut-off filter at 395 nm was used for the emitted light. The measurements were performed on a Jasco Spectrofluorometer FP-777 (Japan Spectroscopic Co., Ltd., Tokyo, Japan). In all experiments, the temperature was kept constant at (20 ± 1) °C. The data were evaluated with EasyPlot 3.0 (by Spiral Software, Brekline, MA and Massachusetts Institute of Technology, Cambridge, MA). Unless indicated otherwise, polymerization of actin was induced by adding MgCl2 to 2 mM and KCl to 50 mM to G-buffer (G-buffer). All polymerization experiments were performed with at least three different actin preparations to ensure that the observed results were not due to variations in the quality of a particular actin preparation.

**Phosphorylation—**Phosphorylation of the MRP effector peptide at its Ser residues was achieved as described recently (7). Briefly, 5.4 µM MRP effector peptide was phosphorylated for 9 h at 30 °C in a total volume of 130 µl containing 6 mM MgCl2, 0.1 mM ATP, 0.001% Triton X-100, 2.5 mM imidazole, pH 7.4, and 20 mM PKM. Positive controls included the same reaction mixture except that PKM was substituted by the PKM storage buffer (Calbiochem data sheet). For negative controls, PKM was substituted by the PKM storage buffer, and the effector peptide was substituted by an equal amount of G-buffer. The stoichiometry of phosphorylation was determined by parallel incubation of aliquots with [γ-32P]ATP and by quantification of the incorporated radioactivity as described (7).
The effector domain of MRP is involved in most of the interactions with its reaction partners, including CaM, membranes, and PKC (5, 17, 18). Moreover, it has been proposed that the effector domain of MARCKS, which is almost identical to the effector domain of MRP, is essential for its actin cross-linking activity. Hence, we investigated the possibility that a peptide representing the effector domain of MRP might mediate an interaction of MRP with actin. In particular, with respect to the dynamic properties of the actin cytoskeleton, we have investigated these interactions from a kinetic point of view.

The Effector Peptide Induces Actin Polymerization and Filament Bundling—The 24-amino acid-long effector domain of MRP contains 12 positively charged residues (e.g., 10 Lys and 2 Arg), so it is conceivable that its strong polycationic nature might influence actin polymerization. To assess this question, a peptide consisting of these 24 amino acids, the MRP effector peptide, was added to actin in G-buffer at different stoichiometries.

Immediately upon addition, the effector peptide causes actin to polymerize in a concentration-dependent fashion (Fig. 1a, curves 3 and 4). Upon reaching steady state, 2 mM MgCl2 and 50 mM KCl were added to polymerize the remaining actin (arrows). Comparison of the steady-state values in the absence and presence of Mg2+/K+ clearly documents that the fraction of polymerized actin correlates with the concentration of effector peptide. This concentration dependence suggests the formation of a stoichiometric complex between actin and the effector peptide.

These results can best be explained by assuming that the effector peptide binds to actin monomers and saturates the low affinity cation binding sites, which are normally occupied by mono- and divalent cations (41–43). In this way, the effector peptide could “mimic” the cations that lead to filament formation under physiological salt concentrations. Hence, it might be argued that the observed polymerization reaction is an unspecified event that could be initiated by many different polycations (34, 44). To test this hypothesis, polymerization of G-actin was induced by addition of polylysine at an equimolar actin:Lys18 ratio. As can be gathered from curve 1 in Fig. 1a, polylysine is indeed capable of inducing actin polymerization in the absence of any other mono- or multivalent cations, but the kinetics is about 2 orders of magnitude slower than when using MRP effector peptide. A comparably fast polymerization kinetics was only observed with polylysines of a very high degree of polymerization (for example, Lys82; data not shown). Evidently, the effector peptide interacts with actin monomers in a specific manner. We propose that the effector peptide has a distinct structure (e.g., amino acid sequence, secondary structure, aggregation state, or charge distribution), which, together with electrostatic interactions, leads to very fast association of the peptide with actin monomers in the absence of any other cations.

Since the experiments described so far were performed in a rather unphysiological environment, it is necessary to also evaluate how the MRP effector peptide affects actin filament formation when polymerization is induced by addition of monovalent and divalent cationic salts; upon increasing the ionic strength J by a factor of 100–200 by adding 2 mM MgCl2, 50 mM KCl, the Debye-length $\lambda_D$ will decrease approximately by a factor of 10 (since $\lambda_D=1/\sqrt{J}$) (45). Therefore, the interaction between actin monomers and the MRP peptide should be attenuated. Fig. 1b displays the fluorescence signal upon addition of an equimolar concentration of MRP effector peptide to 4 mM pyrene-labeled G-actin in the presence of 2 mM MgCl2 and 50 mM KCl. As expected, the actin polymerization velocity is still markedly increased (curve 2), however, at a considerably slower rate than at low salt concentration. The relatively long lag phase indicates that elongation rather than nucleation is enhanced by the peptide.

It should be noted that more effector peptide is required to enhance actin polymerization when increasing the KCl concentration from 50 to 100 mM (data not shown). This suggests that the binding of the effector peptide to actin is, at least to some extent, governed by electrostatic interactions. However, the mechanism of the interaction is independent of the salt concentration. Only the effective concentration of effector peptide complexed with actin is lowered.

A rather puzzling result is found when the amount of peptide is lowered to substoichiometric concentrations at a given salt concentration; compared with control experiments with actin, polymerized in the absence of the effector peptide, the polymerization rate is decreased (data not shown). We have recently developed a model approach that can qualitatively account for this interesting phenomenon (49).
Interaction between Actin and the MRP Effector Peptide

The MARCKS effector peptide instead of the MRP effector peptide (data not shown), indicating that the proline residue in the middle of the effector domain of MRP does not significantly affect its interaction with actin.

**CaM and PKC Regulate the Interaction of the Effector Peptide with Actin**—Next, we investigated whether the interaction of the effector peptide with actin can be modulated by CaM, a well-documented binding partner of MRP. Incubation of actin with an MRP effector peptide preincubated with CaM completely abolishes spontaneous actin polymerization (Fig. 1c, curve 2). Nevertheless, under these conditions actin still retains its ability to polymerize, since addition of salts (2 mM MgCl₂, 50 mM KCl) induces the formation of filaments (second part of curve 2). Note that no difference in the polymerization kinetics can be observed relative to the control experiment in which actin is polymerized by salts in the absence of effector peptide and CaM (Fig. 1c, curve 4).

Remarkably, the polymerizing activity of the MRP effector peptide can even be reversed by CaM. Fig. 1c, curve 3, documents that addition of CaM to an F-actin solution, polymerized by the MRP effector peptide in the absence of salts, causes a decrease of the fluorescence signal to almost the signal of pure G-actin. Subsequent addition of mono- and divalent cations (i.e. 2 mM MgCl₂, 50 mM KCl) induces polymerization of all actin in solution.

To better understand the effect of CaM on the properties of the MRP effector peptide, aliquots were retrieved at the time points indicated in curve 3 of Fig. 1c (i.e. a–c) and prepared for electron microscopy. As expected, up to time point a actin bundles are depicted, looking almost identical to the structures described in Fig. 2, b and c (data not shown). Upon addition of CaM (Fig. 2d), however, the filament bundles are completely dissolved, and most of the filaments are depolymerized. Yet, some structures seem to indicate that the actin depolymerization into monomers is not complete, since occasionally irregularly shaped aggregates are observed. Finally, upon addition of 2 mM MgCl₂, 50 mM KCl (shown in Fig. 2e), a filament network is formed as one would expect for pure actin polymerized by salt only.

Two explanations can account for the effects of CaM on the interaction of the effector peptide with actin: 1) the complexes of actin subunits and effector peptide molecules are dissociated by CaM, possibly due to a higher affinity of CaM for the effector peptide; 2) alternatively, an actin-peptide-CaM complex might form in which the effector peptide loses its polymerizing activity. In this respect, the structures that are found after CaM-mediated depolymerization of the actin filaments (Fig. 2d) may indeed represent actin-effector peptide-CaM ternary complexes, which, in turn, may nucleate salt-induced repolymerization into filaments, so that no lag phase is observed (see Fig. 1c, curve 3).

According to the model that has been proposed for the regulation of MARCKS by CaM and PKC (3, 4, 22), it may be speculated that also phosphorylation of the MRP effector peptide affects its kinetic activity with actin. To evaluate this possibility, the MRP effector peptide was phosphorylated as described under "Experimental Procedures" (stoichiometric ratio: 1.6 mol of phosphate incorporated per mol of peptide). Then 2 µM actin was polymerized by addition of 1.8 µM phosphorylated MRP effector peptide. Since the enzymatic activity of PKC requires Mg²⁺, all experiments were performed in the presence of 2 mM MgCl₂.

Addition of unphosphorylated MRP effector peptide in the presence of 2 mM MgCl₂ causes strong acceleration of actin polymerization compared with polymerization of actin alone (Fig. 1d, curves 1 and 2). In contrast, when actin polymeriza-
Absence of Cations—Introduction). The peptides were categorized into three groups (see polymerization and/or bundling. Based on these experiments, peptides were tested for their ability to cause actin filament segments were deleted or replaced by alanines. These mutant peptides were synthesized in which characteristic amino acid mine which amino acid residues are involved in the interaction of the MARCKS and MRP effector peptides with actin, several pho-effector peptide were analyzed by electron microscopy. Fig. 2f clearly documents that upon phosphorylation the MRP effector peptide has almost completely lost its actin bundling activity. This is in good agreement with the assumption that the negative charges of the phosphate groups compensate the polycationic character of the MRP effector peptide.

Identification of Functional Amino Acid Segments—To determine which amino acid residues are involved in the interaction of the MARCKS and MRP effector peptides with actin, several peptides were synthesized in which characteristic amino acid segments were deleted or replaced by alanines. These mutant peptides were tested for their ability to cause actin filament polymerization and/or bundling. Based on these experiments, the peptides were categorized into three groups (see Introduction).

Group 1 Peptides: Actin Polymerization Is Induced in the Absence of Cations—Each group 1 peptide (with some or all hydrophobic amino acid residues replaced by alanines; for details see "Experimental Procedures") was incubated with 4 μM pyrene-labeled G-actin at a 1:1 peptide:actin molar ratio, and the fluorescence signal was monitored. After 1 h, 2 mM MgCl2 and 50 mM KCl were added to complete actin polymerization.

Very similar to the wt MARCKS effector peptide, the group 1 peptides strongly enhance the actin polymerization kinetics (Fig. 3a). Thus, the hydrophobic amino acid residues are not required for the induction of actin polymerization in the absence of salts. However, in contrast to experiments involving the wt peptide, a significant lag phase is observed with all group 1 peptides, indicating that nucleation takes place at a slower rate than with the wt peptide. Also, the polymerization kinetics for the mutant peptides is slower by a factor of 2.

For all peptides, aliquots were taken from the solutions after each polymerization step (i.e. before and after addition of mono- and divalent cations) and inspected by electron microscopy. Figs. 4, a and b, display electron micrographs taken from F-actin solutions after incubation with group 1 peptides (a) and after addition of salt (b) to complete the polymerization/association process. In each assay, the polycationic peptides caused formation of actin bundles consisting of up to 100 actin filaments aligned side-by-side. No significant difference in the appearance of the actin bundles was observed between samples containing wt or group 1 peptides.

Group 2 Peptides: Interaction with Actin Is Abolished—4 μM pyrene-labeled actin was incubated with equimolar concentrations of each of the group 2 peptides (with the N-terminal five Lys-residues replaced by alanines; for details see "Experimental Procedures"), and the fluorescence signals were recorded. Contrary to the wt peptide and group 1 peptides, these mutant peptides fail to induce actin polymerization in the absence of salts (data not shown). The small increase in pyrene fluorescence (~1%) might be due to the formation of very few filaments, induced by a weak interaction with the cationic peptides (note the small differences among the intrinsic signals at the time of addition of Mg2+ and K+; Fig. 3b). After 1 h, 2 mM MgCl2 and 50 mM KCl were added to the sample (indicated by the arrow in Fig. 3b), yielding an actin polymerization kinetics and steady-state level very similar to the control reaction where 4 μM actin was polymerized by the addition of 2 mM MgCl2,50 mM KCl only. Although the kinetic trace is not identical to the control curve for every group 2 peptide, the difference in polymerization velocity is always less than 15% (estimated from the slope at the point of inflection). This behavior suggests that, if at all, only a very weak interaction occurs between actin monomers and group 2 peptides.

Aliquots from actin and the group 2 peptides in G-buffer were inspected by electron microscopy. In the absence of salts,
only few short filament-like structures were depicted on the electron microscopy grid (Fig. 4c). This is consistent with the observation that the fluorescence signal increased by only ~1% upon incubation of actin and peptide. When mono- and divalent cations were added to induce complete actin polymerization, an actin network was observed (Fig. 4d), which both in filament density as well as in filament structure is comparable with the loose filament networks obtained in the control (Fig. 2a). Hence, in contrast to group 1 peptides, group 2 peptides do not cause any significant F-actin bundle formation. The complete loss of actin polymerizing or filament bundling activity suggests that in fact one actin binding site of MARCKS is located within this lysine-rich segment of the effector peptide (48).

**Group 3 Peptides: Actin Polymerization Is Drastically Retarded**—Each group 3 peptide (with the central and/or C-terminal Lys/Arg segments replaced by alanines; for details see “Experimental Procedures”) was incubated with 4 µM G-actin at equimolar concentration, and the fluorescence signal was recorded. As for group 2 peptides, no fast actin polymerization is observed in the absence of salts (not shown). Fig. 3c reveals that incubation of the group 3 peptides with G-actin samples only leads to a small fluorescence increase (~5–10%), a behavior similar to that of the group 2 peptides. After 1 h, 2 mM MgCl₂ and 50 mM KCl were added to induce or complete the actin polymerization process (Fig. 3c, arrow). Interestingly, and contrary to polymerization in the presence of group 2 peptides, addition of Mg²⁺ and K⁺ ions induces actin polymerization at a much slower rate than found in control experiments (see Fig. 3c). These results indicate that group 3 peptides strongly interact with actin monomers. Note that incubating large amounts of group 3 peptides (12 µM) with 4 µM actin results in a very slow spontaneous polymerization of actin and leads, in the presence of salts, to a less pronounced inhibition of actin polymerization (data not shown). Thus, we suggest that the group 3 peptides bind to actin monomers, thereby rendering them less accessible to mono- and divalent cations. This results in a slow actin polymerization, which is comparable with the polymerization kinetics observed with polylysine (Lys₁₅₀, Fig. 1a).

Aliquots from the actin solutions containing group 3 peptides were inspected by electron microscopy. Incubation of G-actin with group 3 peptides yields a mixed population consisting of a few actin filaments and bundles, probably due to the polycationic nature of the bound peptides (Fig. 4e). Upon addition of MgCl₂ and KCl, the remaining actin monomers polymerize and form predominantly filament bundles (Fig. 4f).

Last but not least, it should be noted that dividing the mutant peptides into three subgroups certainly is an oversimplification, since incubation of G-actin with some of the group 2 peptides still leads to a small, but visible, increase in the fluorescence signal (~2% of the final signal), indicating a weak remaining interaction, possibly caused by the polycationic nature of the remaining lysine residues. In particular, one peptide in which all but the C-terminal lysines were replaced by alanines (named “M-6”) does not fit into the presented scheme because, contrary to the other group 2 peptides that were tried, it accelerates actin polymerization in the presence of salts (data not shown).

**Conclusions and Outlook**—Based on the results presented in this report we propose that the MRP effector peptide assumes a distinct structure which, together with electrostatic interactions, leads to very fast association of the peptide with actin monomers. Evidently, the N-terminal lysine residues are required for this interaction, thus confirming Aderem’s assumption that they harbor one actin binding site (3), whereas the seven lysine residues within the central and C-terminal basic residue clusters are necessary to induce fast, spontaneous polymerization. The large hydrophobic residues only moderately influence the lag phase, which suggests a role for them in the overall peptide structure rather than a direct interaction with actin.

Both CaM and PKC modulate the interaction of the MRP effector peptide with actin, possibly by competitive binding to or phosphorylation of the effector peptide. Hence, it will be of interest to investigate and compare a possible regulation of MRP protein-actin complexes by CaM and PKC.

Last but not least, it is intriguing to speculate on whether some of the peptides investigated in this work could be used as “tools” for producing specific actin filament aggregates. In fact, such a scenario might be of particular interest if we think of a way to obtain nuclei-like actin oligomers, consisting of a small number of subunits (e.g., Figs. 2d and 4c). Distinct oligomers with, for example, significant nucleating properties might be employed as drugs for regulating the spatial architecture and mechanical properties of the actin cytoskeleton.

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