The vasodilator-stimulated phosphoprotein promotes actin polymerisation through direct binding to monomeric actin

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Abstract The vasodilator-stimulated phosphoprotein (VASP) functions as a cellular regulator of actin dynamics. VASP may initialise actin polymerisation, suggesting a direct interaction with monomeric actin. The present study demonstrates that VASP directly binds to actin monomers and that complex formation depends on a conserved four amino acid motif in the EVH2 domain. Point mutations within this motif drastically weaken VASP/G-actin interactions, thereby abolishing any actin-nucleating activity of VASP. Additionally, actin nucleation was found to depend on VASP oligomerisation since VASP monomers fail to induce the formation of actin filaments. Phosphorylation negatively affects VASP/G-actin interactions preventing VASP-induced actin filament formation.

Key words: Actin dynamics; Ena/Mena/vasodilator-stimulated phosphoprotein; G-actin; Nucleation; Polymerisation

1. Introduction

The microfilament-associated vasodilator-stimulated phosphoprotein (VASP) is intimately involved in regulating actin dynamics. VASP is not only prominent in adhesive structures and stress fibres [1,2], but it also localises to the plasma membrane in protruding lamellipodia [3,4] and filopodial tips [5,6]. VASP belongs to the Ena/VASP proteins that are characterised by a tripartite domain structure (Fig. 1B): a central proline-rich domain is flanked by two Ena/VASP homology domains, EVH1 and EVH2. While the EVH1 domain targets VASP to cell adhesion sites [7–9], the EVH2 domain binds to and bundles actin filaments [10,11] and mediates VASP oligomerisation [11]. The central proline-rich domain interacts with profilin [12] thereby possibly recruiting profilin–actin complexes [13–15].

Recent evidence shows that lamellipodial protrusion and subcortical actin network organisation are regulated by Ena/VASP proteins [4] with the actin binding EVH2 domain playing a key role [16]. It has also been suggested that VASP may directly stimulate actin filament formation by direct binding to G-actin and stabilisation of actin nuclei [10,14,17,18]. Many G-actin binding proteins share short basic sequence motifs serving as the core of a recognition site for monomeric actin and the EVH2 domain harbours such a KLRK motif that is conserved among Ena/VASP family members (Fig. 1A). We thus tested for a direct interaction of VASP with monomeric actin. Our data reveal that VASP-induced actin polymerisation depends on the formation of VASP/G-actin complexes via the KLRK motif and is negatively affected by phosphorylation.

2. Materials and methods

2.1. Cloning of VASP constructs

Cloning of murine VASP was performed as described [10]. The deletion construct comprising amino acids 1–342 (VASP-ΔC) was generated by PCR using full-length VASP as a template. Amplification primers introduced *Eco*RI and *Xho*I restriction sites for further cloning into the bacterial expression vector pQE30 (Qiagen, Hilden, Germany). Site-directed mutagenesis was performed according to the manufacturer's instructions (Quick-change kit, Stratagene, Heidelberg, Germany) yielding VASP mutants with KLGE or KLEE instead of the KLRK motif. All PCR-generated constructs were sequenced prior to further cloning into bacterial expression vectors. Recombinant proteins were equipped with additional sequence tags for immunodetection: wild type VASP with the birch profilin (BiPro)-tag [19], the KLGE, KLEE and VASP-ΔC mutants with a FLAG-tag.

2.2. Protein expression, purification and analysis

Expression and purification of recombinant murine VASP constructs was as described [14]. Recombinant mouse profilin I and IIa were purified according to [20] with slight modifications [14]. Rabbit skeletal muscle actin was prepared as described [14,21].

2.3. Actin polymerisation assay

Actin polymerisation in the presence of VASP, profilin I or IIa was monitored by fluorimetry with 10% pyrene-labelled actin added to unlabelled actin. Polymerisation assays with untreated actin were as described [14]. 1 μM actin was pre-incubated in the absence or presence of 1 μM profilin I or IIa, respectively, at 25°C for 30 min in G-actin buffer (25 mM HEPES, 0.2 mM $CaCl_2$, 0.5 mM dithioerythritol, 1 mM ATP, pH 7.0). Polymerisation was initiated under F-actin conditions by adjusting the solution to 25 mM NaCl, 15 mM KCl, 2 mM $MgCl_2$ and adding 0.25 μM VASP protein (wild type or mutants). In polymerisation experiments with *Escherichia coli* protease-treated (ECP)-actin (see below) 2 μM ECP-actin was pre-incubated in G-actin buffer. 2 μM VASP, 25 mM NaCl, 15 mM KCl were added as

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Abbreviations: VASP, vasodilator-stimulated phosphoprotein; EVH, Ena/VASP homology domain; BiPro, birch profilin; ECP, *Escherichia coli* protease

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described above in the presence of either 0.2 mM $CaCl_2$ or 2 mM $MgCl_2$. Fluorescence was monitored for 60 min as described [14].

2.4. Preparation of ECP-actin

Proteolytically cleaved actin (ECP-actin) was prepared by incubating purified rabbit skeletal muscle actin (3 mg/ml) with partially purified *E. coli* A2 protease ECP32 at an enzyme to actin mass ratio of 1:100 for 2 h at room temperature as described previously [22]. Because ECP-actin is resistant to further degradation by this protease, and the enzyme displays an extremely limited substrate specificity [23] no protease inhibitor was added to stop the reaction. Cleaved actin was lyophilised in the presence of 2 mM sucrose and stored in aliquots at −80°C.

2.5. VASP phosphorylation, chemical crosslinking and Western blot analysis

Phosphorylation of wild type VASP by PKA was performed for 60 min as described [14]. All crosslinks were carried out in crosslinking buffer (10 mM sodium phosphate buffer, pH 7.0; 20 mM NaCl; 50 mM KCl; 0.2 mM $CaCl_2$; 0.2 mM ATP, 0.1 mM dithiothreitol (DTT); 20 U/ml aprotinin) with 1 μM VASP proteins and/or 2 μM ECP-actin (final concentrations) in the absence of magnesium ions. Samples were incubated at 30°C for 25 min prior to chemical crosslinking using final concentrations of 7 mg/ml *N*-hydroxysulfosuccinimide and 1.5 mg/ml 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide as described [2]. Reactions were terminated after 5 or 15 min by addition of SDS sample buffer. Samples were analysed by SDS–PAGE and subsequent Western blotting. Actin was detected using a polyclonal anti-actin antibody (Sigma) while wild type BiPro-VASP and FLAG-tagged VASP mutants were detected with a monoclonal BiPro-tag antibody (4A6) [19] or the anti-FLAG antibody M2 (Sigma), respectively.

2.6. Solid phase binding assay

The interaction between VASP and G-actin was monitored in an ELISA essentially as described [14]. 50 pmol G-actin per well were coated. Incubation with 0.1–100 pmol BiPro-tagged dephosphorylated VASP or PKA-phosphorylated VASP was performed under physiological conditions (phosphate-buffered saline: 136 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2 with 0.05% Tween 20 and 0.5 mM DTT). Bound VASP was detected with the 4A6 BiPro-tag antibody as described [14].

3. Results

3.1. VASP mutants generated to analyse G-actin binding

VASP harbours a basic sequence motif within a highly conserved region in the EVH2 domain that is reminiscent of the core sequence of other G-actin binding proteins (Fig. 1A). In thymosin β4, mutations of lysine residues in this motif significantly reduced its binding to G-actin [24]. Hence, VASP constructs were generated that yielded two VASP mutants, KLGE and KLEE, respectively (Fig. 1B). In addition, we also created a deletion construct (VASP-ΔC) lacking the C-terminal oligomerisation domain [11]. All proteins were expressed as his-tag proteins in *E. coli* bearing additional sequence tags for immunodetection (see Section 2).

3.2. VASP-stimulated actin polymerisation requires an intact KLRK motif and VASP oligomerisation

VASP-induced actin polymerisation can be monitored by fluorescence spectroscopy using pyrenyl-labelled actin [10,14]. When tested in this assay (Fig. 2A) only wild type VASP induced actin polymerisation, whereas the curves for both KLRK mutants and the VASP-ΔC construct were comparable to that of the actin control. This loss in actin polymerisation activity was not due to improper folding of the mutants, since all proteins bound to F-actin in co-sedimentation assays (data not shown). Western blot analysis of all

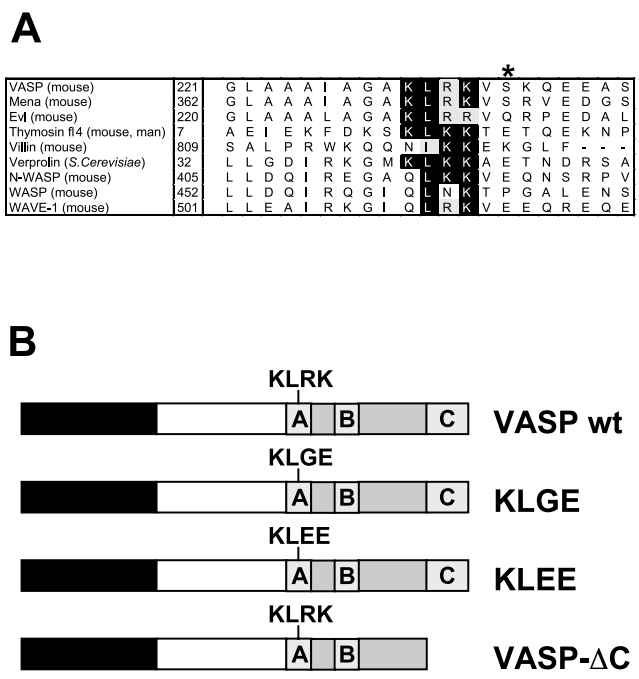

Fig. 1. VASP harbours a putative G-actin binding motif in the EVH2 domain. A: Sequence comparison of recognised and suspected G-actin binding motifs in several actin-associated proteins. The KLKK motif crucial for G-actin binding in thymosin β4 [24] also occurs in Ena/VASP proteins (black boxes). Homologous amino acids are shaded. Asterisk indicates the in vivo phosphorylation site S235 of murine VASP. B: Bar diagrams of murine VASP constructs used in this study. The EVH1 domains (black), the proline-rich regions (white) and the EVH2 domains (grey) are indicated. A = putative G-actin binding region; B = F-actin binding region; C = oligomerisation domain.

VASP proteins subjected to chemical crosslinking in the absence of actin revealed that the VASP-ΔC construct remained monomeric as predicted while the KLGE and KLEE mutants were indistinguishable from the wild type. VASP dimers were already prominent after 5 min of crosslinking, higher VASP oligomers were observed after 15 min (Fig. 2B). These data reveal that efficient stimulation of actin polymerisation by VASP depends on VASP oligomerisation as well as an intact KLRK motif.

3.3. Direct binding of VASP to G-actin involves the KLRK motif

Under physiological conditions, actin filament formation from G-actin is rapidly initiated and in the presence of recombinant murine VASP large aggregates of VASP and F-actin are formed. Hence, to test for direct binding of VASP to monomeric actin, we used actin cleaved by ECP32 which splits actin between glycine 42 and valine 43. The resulting fragments remain in a non-convalent complex with the native actin conformation [25]. ECP-actin does not polymerise under calcium conditions and only to a very reduced level under magnesium conditions [22,25]. These properties were confirmed in actin polymerisation assays (Fig. 3A and data not shown). In the presence of 0.2 mM $CaCl_2$, even the addition of wild type VASP at equimolar concentrations did not promote the formation of actin filaments, while some polymerisation was observed for Mg^{2+}-actin under similar conditions. Subsequently, chemical crosslinks were performed in the absence of magnesium ions and analysed by Western blotting.

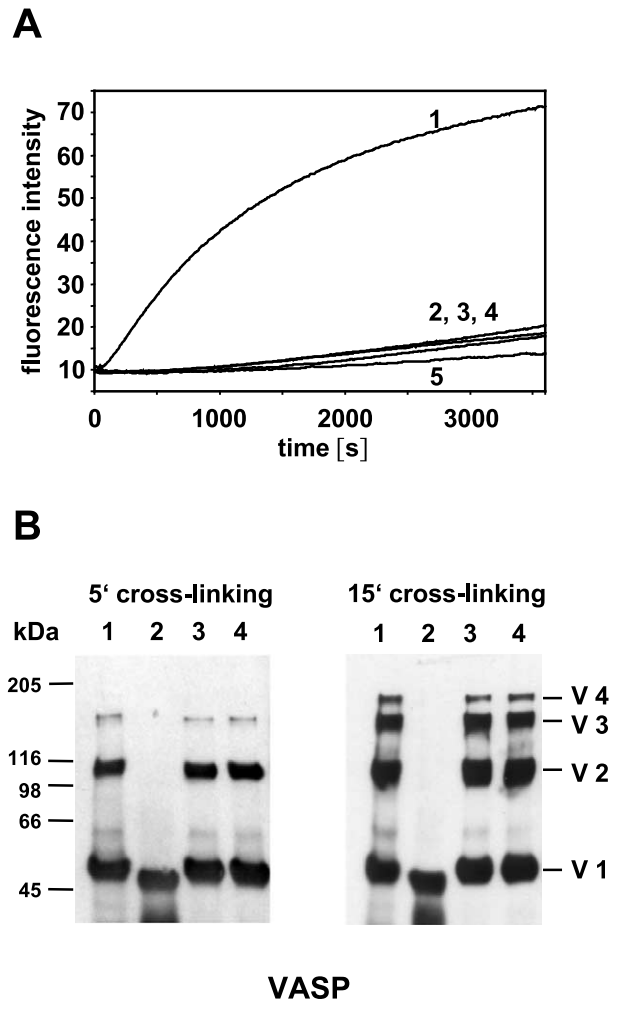

Fig. 2. VASP-stimulated actin polymerisation depends on both, an intact KLRK motif and VASP oligomerisation. A: Fluorescence spectroscopy with pyrenyl-labelled actin in the absence (3) or presence of recombinant his-tagged VASP wild type (1); VASP-ΔC (2); VASP-KLGE (4) and VASP-KLEE (5). Final concentrations were 1 μM actin and 0.25 μM of the VASP constructs. B: Chemical crosslink of VASP oligomers. Crosslinking reactions were terminated after 5 min and 15 min, respectively. Lanes: 1 = wild type VASP; 2 = VASP-ΔC; 3 = VASP-KLGE; 4 = VASP-KLEE.

Probing for ECP-actin with an anti-actin antibody (Fig. 3B) revealed that it remained as a monomer. With wild type VASP, heterodimers with actin were already present after 5 min crosslinking (Fig. 3B, left panel) while complexes with VASP-ΔC and both KLRK mutants were seen only after 15 min and in significantly smaller amounts as compared to wild type VASP. The delayed heterodimer formation for VASP-ΔC was somewhat surprising, since the KLRK motif is intact in this protein, showing that deletion of the C-terminal oligomerisation domain lowers the affinity for binding to monomeric actin which suggests an allosteric effect on the G-actin binding site. Except when VASP-ΔC was used, ECP-actin was also present in aggregates of higher molecular masses after 15 min, indicating that monomeric ECP-actin may also associate with VASP oligomers (Fig. 3B, right panel). The presence of VASP in hetero-oligomeric complexes was confirmed by analysing the same samples for VASP proteins after 5 min of chemical crosslinking (Fig. 3C). Heterodimer formation was confirmed for the wild type as indicated by an

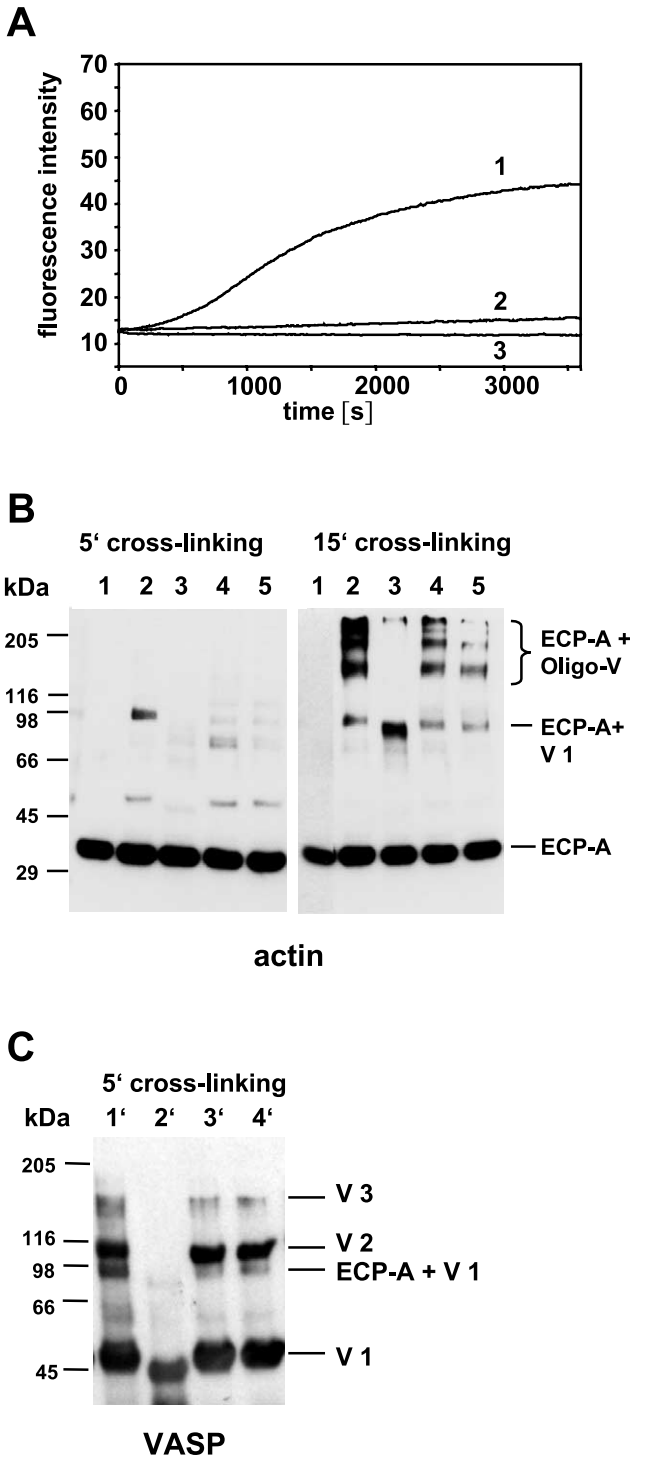

Fig. 3. Direct binding of VASP to G-actin involves the KLRK motif. A: Polymerisation kinetics of ECP-actin (supplemented with 10% pyrenyl-labelled actin) in the absence (3) or presence (1, 2) of equimolar amounts of wild type VASP. VASP-induced ECP-actin polymerisation occurred in the presence of Mg^{2+} (1) but not Ca^{2+} (2) ions. B: Western blot analysis of ECP-treated Ca^{2+}-actin (2 μM) in the absence or presence of VASP proteins (1 μM) crosslinked for 5 or 15 min. ECP-actin controls (lane 1), VASP wild type (lane 2), VASP-ΔC (lane 3) VASP-KLGE (lane 4), VASP-KLEE (lane 5). C: Detection of VASP constructs after chemical crosslinking to EPC-actin after 5 min. Lane 1′ = VASP wild type; lane 2′ = VASP-ΔC; lane 3′ = VASP-KLGE; lane 4′ = VASP-KLEE. For interpretation see text.

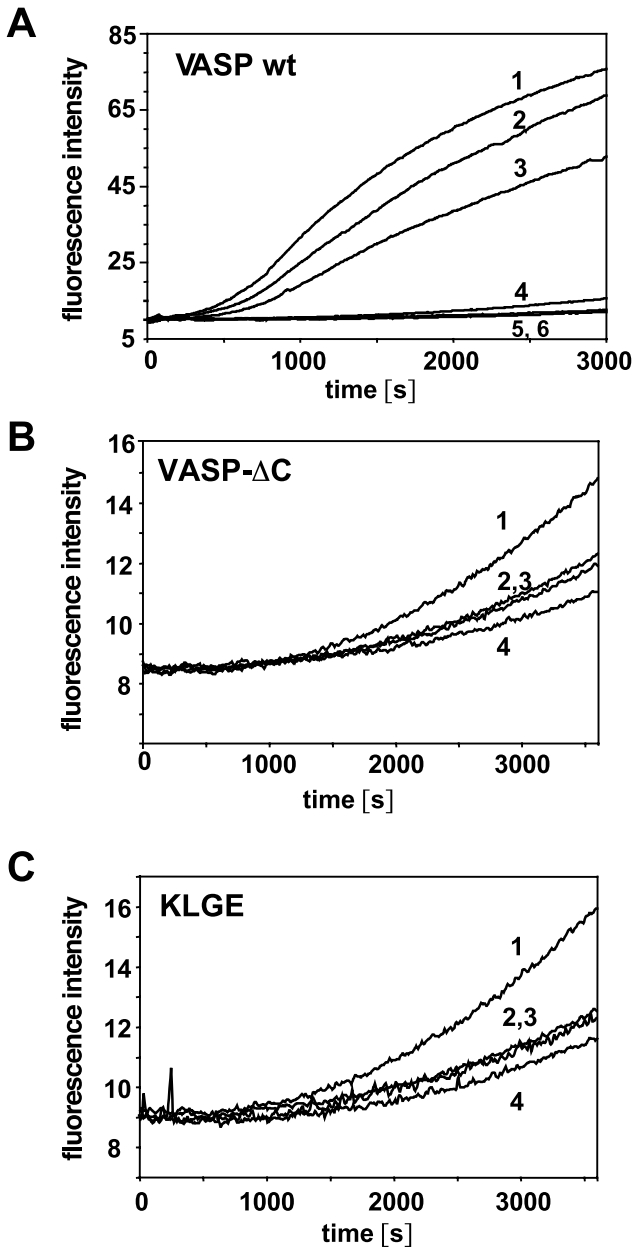

Fig. 4. Profilin–actin complexes do not substitute for defective G-actin binding by the mutant VASP proteins. A–C: Kinetics of actin filament formation from profilin–actin in the absence or presence of VASP proteins. Equimolar amounts of actin and profilin I/profilin IIa (1 μM) were incubated prior to the addition of any VASP construct to allow profilin–actin complex formation. 0.25 μM VASP wild type (A), VASP-ΔC (B) or VASP-KLGE (C) were then added to the samples and actin polymerisation was monitored by fluorescence spectroscopy. A: Induction of actin polymerisation by wild type VASP (3) is enhanced in the presence of either profilin IIa (1) or profilin I (2) while in the absence of VASP both profilins suppress actin filament formation (5,6) below the actin control (4), due to a slight sequestering effect. B: VASP-ΔC cannot substitute for wild type VASP. (1) Actin control; (2) actin and profilin I; (3) actin and VASP-ΔC; (4) actin, VASP-ΔC and profilin I. C: The KLGE mutant fails to promote profilin–actin-based actin polymerisation. (1) Actin control; (2) actin and profilin I; (3) actin, VASP-KLGE and profilin I; (4) actin and VASP-KLGE.

additional band below the VASP homodimer. In addition, faint signals indicative of heterodimers that had not been observed when probing for ECP-actin were also detectable for VASP-ΔC, KLGE and KLEE (Fig. 3, compare B with C). This discrepancy may reflect different affinities of the antibodies. After 15 min of crosslinking, VASP/actin complexes of higher molecular masses were also detected. However these complexes could not be well resolved, since the presence of VASP oligomers in addition to VASP–actin hetero-oligomers resulted in a broad smear in the upper part of the Western blot (data not shown). The binding of VASP to immobilised ECP-actin under physiological conditions was also tested in a solid phase binding assay (data not shown) comparable to the assay depicted in Fig. 5 for VASP and G-actin. Taken together, these experiments demonstrate that VASP directly binds to G-actin with the KLRK motif being involved in this interaction.

3.4. *Recruitment of profilin–actin complexes does not rescue the actin polymerisation deficiencies of mutant VASP proteins*

VASP may recruit profilin–actin complexes [12,13,15]. To test if this recruitment was sufficient for filament formation independently of the KLRK motif, we analysed VASP-induced actin polymerisation from pre-formed actin–profilin complexes (Fig. 4). Actin nucleation and polymerisation by wild type VASP were enhanced in the presence of profilin I and profilin IIa. In the absence of VASP, there was a slight sequestering effect (cf. [14]) due to the formation of profilin–actin heterodimers resulting in a net decrease of free actin monomers (Fig. 4A). The addition of neither VASP-ΔC (Fig. 4B) nor the KLRK mutants (Fig. 4C, and data not shown) to profilin I–actin solutions resulted in actin filament formation, again arguing for oligomerisation and a functional KLRK motif being instrumental for actin polymerisation by VASP.

3.5. *Binding of VASP to monomeric actin is regulated by phosphorylation*

As VASP phosphorylation reduces its interaction with F-actin [14] chemical crosslinks were performed with non-polymerisable ECP-actin and VASP that had been phosphorylated by PKA for 60 min (Fig. 5A). Under the conditions used, the physiological phosphorylation site S235 that neighbours the KLRK motif (Fig. 1A) is phosphorylated to almost stoichiometric levels and VASP stimulation of actin polymerisation is abolished [14]. Western blots probing for ECP-actin (Fig. 5A, left two panels) and VASP (Fig. 5A, right panel) showed that after 5 min of crosslinking, heterodimers were only observed for non-phosphorylated VASP (Fig. 5A, left panel). Heterodimer formation was notable for phosphorylated VASP after 15 min, but to a lesser extent than for the wild type (Fig. 5A, middle panel). Probing for VASP proteins (Fig. 5A, right panel) revealed no difference in dimerisation between both VASP populations. The decrease in heterodimer formation was due to a reduced binding to G-actin as seen in a solid phase binding assay (Fig. 5B). Immobilised G-actin was incubated with increasing amounts of VASP or phospho-VASP under physiological conditions. The G-actin conformation was demonstrated by analysing the binding of profilin I to immobilised G-actin (data not shown). While dephosphorylated VASP bound to G-actin was readily detect-

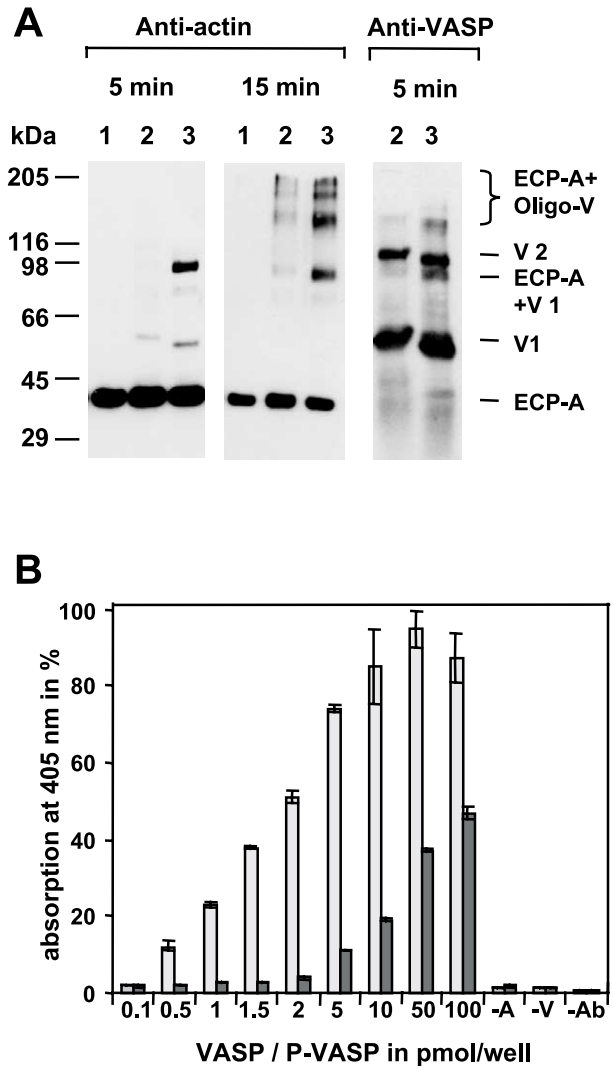

Fig. 5. Phosphorylation of VASP significantly diminishes binding of VASP to monomeric actin. A: Chemical crosslinking of ECP-actin and VASP before and after phosphorylation (60 min) by PKA. Western blot analyses of actin after 5 and 15 min of crosslinking (left two panels) and (right panel). 1 = Actin control; 2 = PKA-phosphorylated VASP+actin; 3 = non-phosphorylated VASP+actin. See text for analysis. B: Solid phase binding assay (ELISA) of G-actin and VASP. G-actin (50 pmol) was coated onto ELISA-plates and incubated with increasing amounts of non-phosphorylated (grey bars) and phosphorylated VASP protein (black bars) under physiological conditions (phosphate-buffered saline). Controls: –A = no actin coated; –V = no VASP protein added; –Ab = no primary antibody.

able at 0.5 pmol protein per well and increased with higher amounts of VASP protein (Fig. 5B, grey bars), phospho-VASP was detected as a ligand for G-actin only above 5 pmol VASP per well, confirming that binding of VASP to G-actin is weakened and can thus be regulated by phosphorylation.

4. Discussion

Based on recent findings that VASP may initiate actin polymerisation, we hypothesised that VASP directly interacted with actin monomers and that a basic four amino acid motif (KLRK) located within the first conserved sequence block of the EVH2 domain [11] was the core sequence of a G-actin binding motif. We also postulated that the formation of a single VASP/actin heterodimer would not suffice for actin nuclei formation, but that oligomerisation was necessary to induce actin polymerisation. We demonstrate that VASP and G-actin interact physically and that the KLRK motif is indeed involved in complex formation. Mutations of the arginine/lysine tandem residues significantly reduced VASP binding to G-actin and prevented de novo formation of actin filaments in vitro. Furthermore, the latter also requires VASP oligomerisation, probably allowing recruitment of multiple actin monomers.

The generation of the KLRK mutants was based on a detailed analysis of the G-actin binding site in thymosin β4 by mutational analyses [24]. This study revealed that while the replacement of any lysine residue within the KLKK motif (Fig. 1A) significantly weakened thymosin β4/G-actin interactions, a complete loss of G-actin binding was only achieved by introducing negative charges, replacing the lysines for glutamic acid residues. In analogy to these findings, we generated the KLGE and KLEE mutants that are both deficient in G-actin binding (Fig. 3) and failed to promote actin polymerisation (Figs. 2 and 4). Interestingly, the physiological phosphorylation site S235/S239 of murine and human VASP, respectively [14,26] (asterisk in Fig. 1A) lies next to the KLRK motif. Phosphorylation of this residue abolishes VASP-induced actin polymerisation [14] and phosphorylation of the equivalent site in Mena has recently been shown to be crucial for its physiological function [16]. The similarity of the results obtained in our study with the KLRK mutants and with wild type VASP phosphorylated by c-AMP-dependent kinase (compare Figs. 3 and 5) indicates that binding of VASP to G-actin significantly depends on electrostatic interactions that are negatively influenced by phosphorylation.

Our data also revealed that the direct interaction of VASP with monomeric actin is crucial for stimulation of actin filament formation, since the addition of profilin–actin complexes that should bind to the GP_5 motifs in VASP [12,13] and deliver actin monomers could not rescue the function of the mutant proteins. There is also good evidence for the physiological relevance of VASP/G-actin interactions: recent analyses of Mena/VASP-deficient MV^{D7} cells revealed that the organisation of the subcortical actin filaments and lamellipodial protrusion may be regulated by Ena/VASP proteins [4]. In addition, deletion constructs of Mena lacking the KLRK containing region failed to induce the motility phenotype of cells re-expressing wild type Mena [16] arguing for a significant contribution of this motif for Ena/VASP-induced actin polymerisation in vivo.

It is noteworthy that many of the nucleating proteins (e.g. the WASP/Scar and formin proteins; cf. [27–29]) harbour their own G-actin binding sites, in addition to binding to profilin–G-actin. Possibly, the G-actin monomers, once released from profilin–actin complexes, need to be tethered to the nucleator proteins like VASP for efficient concentration or orientation during the relatively slow process of nucleus formation. It is also conceivable that this interaction might change the conformation of actin as present in the profilin–actin complex, to a slightly different structure favourable for actin nucleation and elongation (cf. [30,31]).

In conclusion, we propose that the physical interaction of VASP with G-actin is essential for the physiological function

of VASP and can be regulated by phosphorylation. Furthermore, our data favour the concept that the conformation of monomeric actin may be modulated by binding to different ligands such as profilin or VASP.

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References

[1] Reinhard, M., Halbrugge, M., Scheer, U., Wiegand, C., Jockusch, B.M. and Walter, U. (1992) EMBO J. 11, 2063–2070.
[2] Huttelmaier, S., Mayboroda, O., Harbeck, B., Jarchau, T., Jockusch, B.M. and Rudiger, M. (1998) Curr. Biol. 8, 479–488.
[3] Rottner, K., Behrendt, B., Small, J.V. and Wehland, J. (1999) Nat. Cell Biol. 1, 321–322.
[4] Bear, J.E. et al. (2002) Cell 109, 509–521.
[5] Lanier, L.M. et al. (1999) Neuron 22, 313–325.
[6] Vasioukhin, V., Bauer, C., Yin, M. and Fuchs, E. (2000) Cell 100, 209–219.
[7] Reinhard, M., Rudiger, M., Jockusch, B.M. and Walter, U. (1996) FEBS Lett. 399, 103–107.
[8] Brindle, N.P., Holt, M.R., Davies, J.E., Price, C.J. and Critchley, D.R. (1996) Biochem. J. 318, 753–757.
[9] Drees, B., Friederich, E., Fradelizi, J., Louvard, D., Beckerle, M.C. and Golsteyn, R.M. (2000) J. Biol. Chem. 275, 22503–22511.
[10] Huttelmaier, S., Harbeck, B., Steffens, O., Messerschmidt, T., Illenberger, S. and Jockusch, B.M. (1999) FEBS Lett. 451, 68–74.
[11] Bachmann, C., Fischer, L., Walter, U. and Reinhard, M. (1999) J. Biol. Chem. 274, 23549–23557.
[12] Reinhard, M., Giehl, K., Abel, K., Haffner, C., Jarchau, T., Hoppe, V., Jockusch, B.M. and Walter, U. (1995) EMBO J. 14, 1583–1589.
[13] Jonckheere, V., Lambrechts, A., Vandekerckhove, J. and Ampe, C. (1999) FEBS Lett. 447, 257–263.
[14] Harbeck, B., Huttelmaier, S., Schluter, K., Jockusch, B.M. and Illenberger, S. (2000) J. Biol. Chem. 275, 30817–30825.
[15] Holt, M.R. and Koffer, A. (2001) Trends Cell Biol. 11, 38–46.
[16] Loureiro, J.J., Rubinson, D.A., Bear, J.E., Baltus, G.A., Kwiatkowski, A.V. and Gertler, F.B. (2002) Mol. Biol. Cell 13, 2533–2546.
[17] Fradelizi, J., Noireaux, V., Plastino, J., Menichi, B., Louvard, D., Sykes, C., Golsteyn, R.M. and Friederich, E. (2001) Nat. Cell Biol. 3, 699–707.
[18] Skoble, J., Auerbuch, V., Goley, E.D., Welch, M.D. and Portnoy, D.A. (2001) J. Cell Biol. 155, 89–100.
[19] Rudiger, M., Jockusch, B.M. and Rothkegel, M. (1997) BioTechniques 23, 96–97.
[20] Schluter, K., Schleicher, M. and Jockusch, B.M. (1998) J. Cell Sci. 111, 3261–3273.
[21] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[22] Khaitlina, S.Y., Moraczewska, J. and Strzelecka-Golaszewska, H. (1993) Eur. J. Biochem. 218, 911–920.
[23] Matveyev, V.V., Usmanova, A.M., Morozova, A.V., Collins, J.H. and Khaitlina, S.Y. (1996) Biochim. Biophys. Acta 1296, 55–62.
[24] Van Troys, M., Dewitte, D., Goethals, M., Carlier, M.F., Vandekerckhove, J. and Ampe, C. (1996) EMBO J. 15, 201–210.
[25] Khaitlina, S., Collins, J.H., Kuznetsova, I.M., Pershina, V.P., Synakevich, I.G., Turoverov, K.K. and Usmanova, A.M. (1991) FEBS Lett. 279, 49–51.
[26] Butt, E., Abel, K., Krieger, M., Palm, D., Hoppe, V., Hoppe, J. and Walter, U. (1994) J. Biol. Chem. 269, 14509–14517.
[27] Machesky, L.M. and Insall, R.H. (1999) J. Cell Biol. 146, 267–272.
[28] Evangelista, M., Pruyne, D., Amberg, D.C., Boone, C. and Bretscher, A. (2002) Nat. Cell Biol. 4, 32–41.
[29] Sagot, I., Rodal, A.A., Moseley, J., Goode, B.L. and Pellman, D. (2002) Nat. Cell Biol. 4, 626–631.
[30] Khaitlina, S. and Hinssen, H. (1997) Biophys. J. 73, 929–937.
[31] Gonsior, S.M., Platz, S., Buchmeier, S., Scheer, U., Jockusch, B.M. and Hinssen, H. (1999) J. Cell Sci. 112, 797–809.