A discrete amino terminal domain of Kv1.5 and Kv1.4 potassium channels interacts with the spectrin repeats of α-actinin-2

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Abstract The interaction between the amino terminus of Kv1-type potassium channels and α-actinin-2 has been investigated. Using a combination of yeast two-hybrid analysis and in vitro binding assays, α-actinin-2 was found to bind to the N-termini of both Kv1.4 and Kv1.5 but not to the equivalent segments of Kv1.1, Kv1.2 or Kv1.3. Deletion analysis in the in vitro binding assays delineated the actinin binding region of Kv1.5 to between amino acids 73 and 148 of the channel. The Kv1.5 binding sites in α-actinin-2 were found to lie within actinin's internal spectrin repeats. Unlike the reported interaction between actinin and the NMDA receptor, calmodulin was found to have no effect on actinin binding to Kv1.5. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Potassium channel; Kv1.5; Actinin-2; Cytoskeleton

1. Introduction

Made up of three fundamental kinds of protein filaments, microtubules, intermediate filaments and actin filaments, the cytoskeleton is a complex group of structures involved in virtually every cellular process [1,2]. Actin filaments provide mechanical strength to the cell, and, like the microtubules, are important in cytokinesis. Forming a band just beneath the plasma membrane, the actin cytoskeleton serves also to anchor large proteins in the membrane.

Among the proteins likely anchored to the actin cytoskeleton are a number of ion channels. The NR2 subunit of the NMDA receptor, muscle sodium channels, and the voltage-regulated potassium channel Kv1.5 have all been shown to associate with molecules linked to the actin cytoskeleton [3–5]. Calcium-mediated actin depolymerization reduces NMDA receptor activity [6] and cytochalasin affects the expression of sodium channels [7,8], and a number of potassium channels [9–12], including Kv1.5 [5]. Using a yeast two-hybrid system, we have previously shown that Kv1.5 binds to the actin cytoskeleton linking protein, α-actinin-2. We also demonstrated that the channel and α-actinin-2 are closely associated when co-expressed in HEK cells, that the two could be co-immunoprecipitated, and that disruption of their association caused significant increases in Kv1.5 current sizes. In this study, we have used in vitro techniques to demonstrate that α-actinin-2 binds a specific region in the Kv1.4 and Kv1.5 N-termini via its central spectrin repeats. We further show that calcium and calmodulin have no effect on the association and that the α-actinin-2 interaction is specific to Kv1.5 and its close homolog, Kv1.4. No such binding of α-actinin-2 occurs with Kv1.1, Kv1.2 or Kv1.3. These results confirm our previous findings and provide further insight into the role of the actin cytoskeleton in the regulation of Kv1.5.

2. Materials and methods

2.1. Deletion constructs

DNA encoding the Kv1.5 N-terminus, and internal deletions, were cloned as *Nco*I fragments into either pET42 or pET28 vectors (Novagen, Madison, WI, USA) as appropriate. Sequence-confirmed PCR-derived segments encoding N-terminal fragments of Kv1.1 (aa 1–167), Kv1.2 (aa 1–164), Kv1.3 (aa 1–182) and Kv1.4 (aa 1–305) were cloned as *Eco*RI–*Sal*I fragments into pGBT9 or pGAD424. The Kv1.1 and Kv1.4 N-termini were similarly cloned into pET42. Deletions in Kv1.5-encoding segments were made by restriction digests or internal digestion followed by incubation with nuclease Bal31 for varying times. Digestions were stopped by addition of 20 mM EGTA, and the DNA was ligated, then recovered after transformation into *Escherichia coli*. The presence of in-frame fusions with the glutathione-S-transferase (GST)-tag was confirmed by DNA sequencing. α-Actinin-2 deletions were made in appropriate pET21 or pET42 vectors by digestion with restriction endonucleases followed by religation in the absence of deleted sequences.

2.2. Preparation of GST- and T7-tagged proteins

Recombinant proteins were expressed in *E. coli* strain BL21(DE3) and purified using BugBuster Extraction Reagent (Novagen). Proteins detected in the soluble fraction by Coomassie staining of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels were purified using T7-tag affinity-purified kit and GST bind resin and buffer kit (Novagen). Proteins expressed in the insoluble fraction (inclusion bodies) were washed, and solubilized with 1 × solubilization buffer with 0.3% *N*-lauroylsarcosine according to Novagen's recommendations. They were then dialyzed in 0.02 M Tris–HCl to remove residual detergent.

2.3. Protein–protein interactions by the yeast two-hybrid system

N-terminal fragments of Kv1.1, Kv1.2, Kv1.3 and Kv1.4 fusions to the GAL4 DNA binding domain in pGBT9 were tested in yeast strain PJ69-4a [13] against the α-actinin-2 fragment in pactGAD [5]. Methods were as previously described [5].

2.4. In vitro binding assays

Approximately 2 μg (normalized by comparison to standards on Coomassie-stained SDS–PAGE gels) of GST, GST N-terminus of Kv1.1, GST N-terminus of Kv1.4, GST N-terminus of Kv1.5 and other GST-fused Kv1.5 fragments (B, C, D, E, F, G and H) were combined with 2 μg of T7 fusion α-actinin-2 fragments (clones 1, 2, 3, and 4) in binding buffer (2 mM Tris–Cl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, 0.1% Triton X-100) [14]. The mixtures were incubated at room temperature for 1 h with periodic mixing.

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Glutathione-Sepharose beads 4B (Amersham Pharmacia) washed in the binding buffer were added to each tube and incubated with mixing for 30 min at room temperature. The mixtures were spun for 5 min at 1000 rpm and pelleted beads were washed and repelleted four times in wash buffer (25 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.5 mM dithiothreitol) [14]. The pelleted bead–protein complexes were boiled in SDS sample buffer for 5 min. Aliquots containing 0.2 μg of the GST fusion were then resolved by SDS–PAGE. The proteins were transferred to PVDF membranes (Amersham) and probed as Western blots with monoclonal T7 antibodies (dilution 1:10 000, Novagen). The T7-tag was deleted in the cloning of fragment 4 of α-actinin-2 for this work, so polyclonal anti-α-actinin antibodies (dilution 1:500, Sigma A3144) were used to detect the protein in Western blots where this fragment was used. Antibody binding in all cases was detected using horseradish peroxidase-labeled secondary antibodies and a chemiluminescent reagent (ECL, New England Nuclear). To check equivalent loading of constructs, identical quantities of GST fusion proteins were subjected to PAGE on a separate gel and stained with Coomassie blue. The same procedure was used in the reverse experiments in which the binding of GST-fused α-actinin-2 fragments (5, 6, 7, 8, and 9) was tested for their abilities to bind a T7-tagged N-terminus of Kv1.5.

2.5. Calmodulin binding experiments

GST-tagged Kv1.5 N-terminus was incubated with T7-tagged α-actinin-2 in binding buffer containing 2 mM Tris–Cl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, 0.1% Triton X-100. Calcium was added, where appropriate, to a nominal concentration of 2.5 mM. 1.2 μM calmodulin was included in calmodulin competition experiments. Detection of bound protein was as described in Section 2.4, with anti-T7 (1:10 000 dilution). Calmodulin was obtained from Calbiochem (La Jolla, CA, USA).

3. Results

3.1. α-Actinin-2 interacts with Kv1.5 and Kv1.4 but not with Kv1.1, Kv1.2 or Kv1.3

Since Kv1.5 is a member of a large subfamily of *Shaker*-type potassium channels with at least seven members, Kv1.1–Kv1.7, the possibility that the N-termini of related channels might also bind α-actinin-2 was investigated. A yeast two-hybrid system [13] was used to screen for interactions between α-actinin-2 and Kv1.1–Kv1.5, channels whose physiological roles are well understood. Fragments comprising the N-termini of Kv1.1, Kv1.2, Kv1.3 and Kv1.4 cloned in frame to the DNA binding domain of the GAL4 protein in pGBT9 were screened for interaction with the α-actinin-2 as described [5]. As expected, co-transformation with pactGAD and the Kv1.5 N-terminus used in the original studies yielded yeast transformants capable of growth on media lacking adenine and histidine (−ade, −his), indicating activation of the reporter genes (Fig. 1A). However, similar co-transformation of pactGAD with Kv1.1, Kv1.2 or Kv1.3 GAL4 binding domain fusions failed to yield transformants capable of growth on −ade, −his. The Kv1.4 fusion construct yielded transformants capable of growth on −ade, −his even in the absence of pactGAD. Since α-actinin-2 fused to the GAL4 DNA binding domain also activates the reporter genes (data not shown), the yeast two-hybrid system could not indicate to us whether Kv1.4 and α-actinin-2 interact.

To further investigate the binding of Kv1.5 to α-actinin-2, a GST fusion protein affinity binding method was employed. The N-terminal 240 amino acids of Kv1.5 were cloned in frame with the GST-tag coding sequence in pET42 and expressed in *E. coli*. Full-length α-actinin-2 was cloned, with no GST-tag, in pET21d and also expressed in *E. coli*. In essence, the Kv1.5 GST fusion protein, coupled to glutathione-Sepharose, was used to assay for α-actinin-2 binding. α-Actinin-2

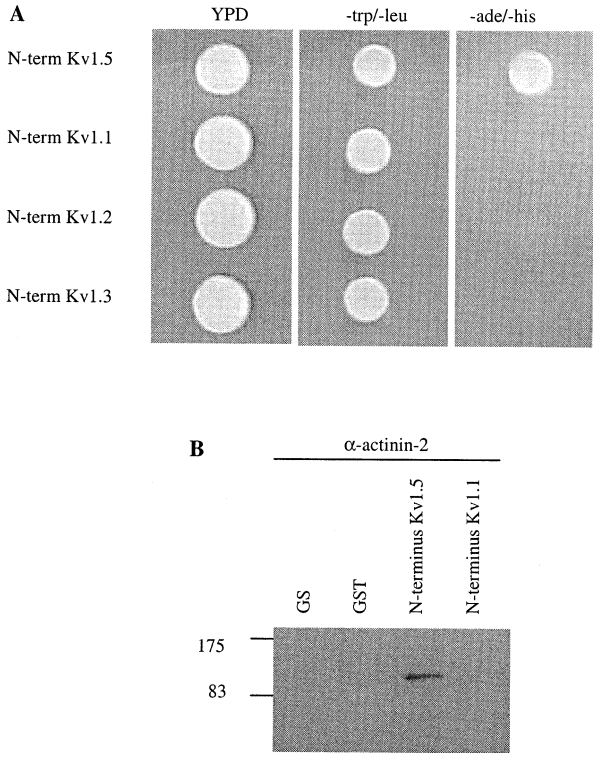

Fig. 1. Yeast two-hybrid assays demonstrate actinin binding specific to Kv1.5. A: Yeast two-hybrid assay for the interaction of α-actinin-2 (aa 583–694) with various Kv1 channel N-termini. Patches were made as labeled. Kv1.5 (aa 1–209), Kv1.1 (aa 1–168), Kv1.2 (aa 1–164), Kv1.3 (aa 1–182). The Kv channel sequences were expressed as fusions to the DNA binding domain of the yeast GAL4 protein. The actinin fragment was a fusion to the GAL4 transcriptional activator domain. Growth on SD −leu, −trp assays for the presence of the plasmids carrying the genes. Growth on SD −ade, −his indicates a detectable interaction. B: Comparison of Kv1.1 and Kv1.5 N-terminal binding to α-actinin-2 in an in vitro binding assay. Purified GST-tag alone or GST fusions to Kv1.1 or Kv1.5 or no GST-tag protein were incubated with T7-tagged purified α-actinin-2 and glutathione-Sepharose beads. Following incubation, the beads were pelleted, washed extensively, then subjected to SDS–PAGE. Western blots were performed using anti-T7 to detect α-actinin-2.

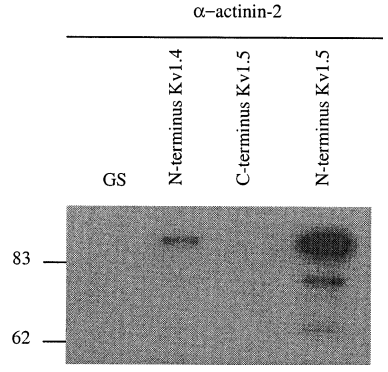

Fig. 2. Kv1.4 N-terminus also binds α-actinin-2. Purified GST-tagged Kv1.4 N-terminus was incubated with T7-tagged purified α-actinin-2 and glutathione-Sepharose beads. Kv1.5 N-terminal and C-terminal fusions were included as positive and negative controls, respectively. Following incubation, the beads were pelleted, washed extensively, and subjected to SDS–PAGE. Western blots were performed using anti-T7 antibody to detect α-actinin-2. GS refers to actinin incubated with glutathione-Sepharose in the absence of a GST fusion protein.

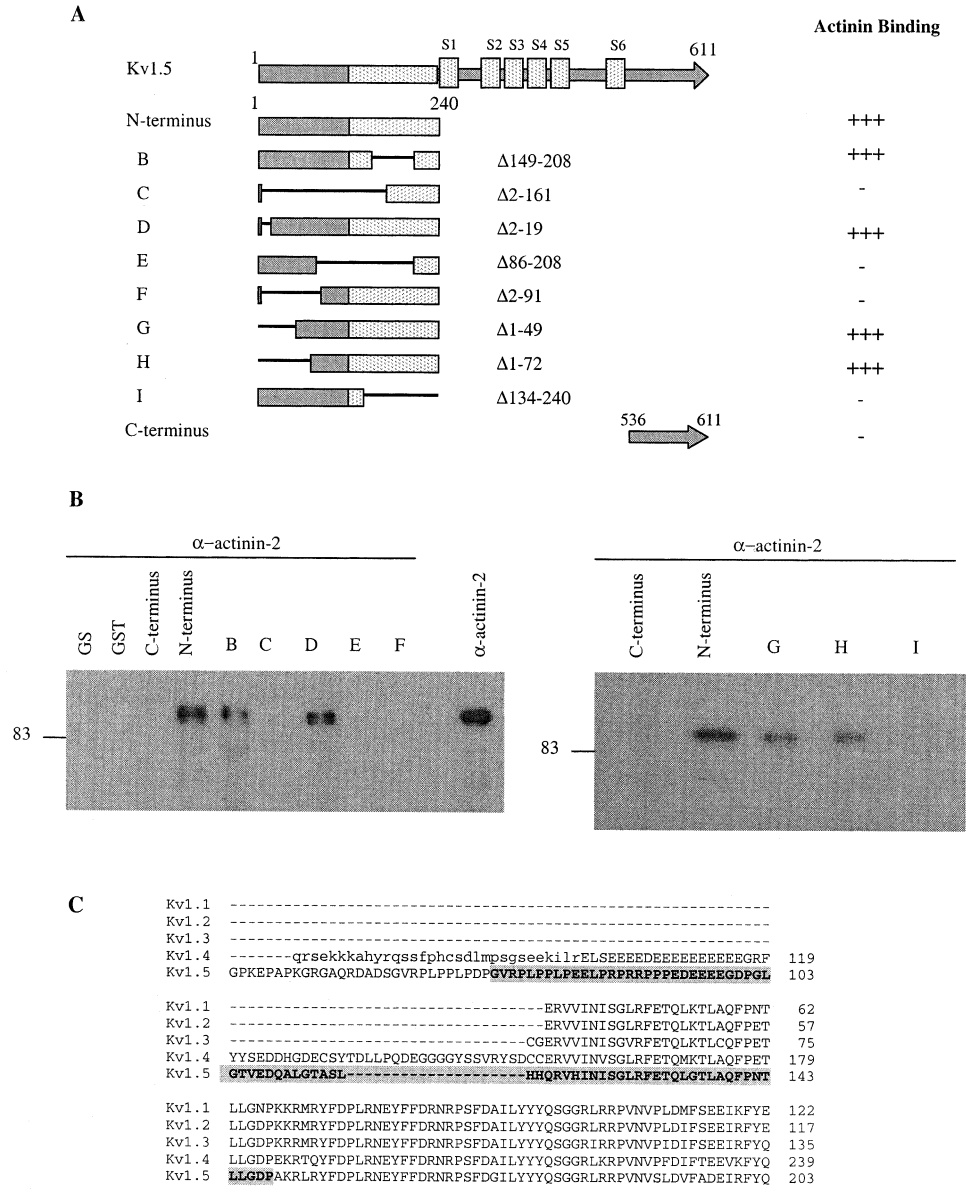

Fig. 3. Analysis of α-actinin-2 binding to GST-tagged Kv1.5 N-terminal constructs. A: Schematic diagram of the various Kv1.5 N-terminal constructs. In the top line, putative transmembrane domains S1–S6 are located in appropriate positions along the sequence, and the stippled upstream region represents the T1 assembly domains. Solid lines in other constructs represent deleted amino acids which are numbered alongside. The actinin-2 binding activity of the constructs is indicated in the column on the right. B: Representative Western blot showing actinin binding by the various constructs. Lanes correspond to the diagrammatic representation in A. All constructs were GST fusions. The fusions were individually incubated with T7-tagged purified α-actinin-2 and glutathione-Sepharose beads. Following incubation, the beads were pelleted, washed extensively, then subjected to SDS–PAGE. Western blots were performed using anti-T7 to detect α-actinin-2. GS refers to actinin incubated with glutathione-Sepharose in the absence of a GST fusion protein; GST refers to actinin incubated with GST-tag alone. Purified α-actinin-2 was loaded in the lane labeled 'α-actinin-2' as a control. C: Multiple sequence alignment. Homologous amino acids are depicted in vertical synchrony. The putative α-actinin-2 binding region is boxed. The lowercase letters in the Kv1.4 sequence represent the additional amino acids present in the Kv1.4 fragment tested for binding that are not homologous to Kv1.5.

was mixed with the Kv1.5 GST and, after extensive washing, the complexes were pelleted, boiled in sample buffer and used for Western analysis, probing for α-actinin-2. Controls were included to ensure that actinin was binding specifically to the N-terminus, and not binding to GST or glutathione-Sepharose. As shown in Fig. 1B, α-actinin-2 did bind Kv1.5 in this assay. When a similarly GST-tagged Kv1.1 N-terminus was used in place of Kv1.5 in otherwise identical in vitro binding assays, no α-actinin-2 binding could be detected. Thus, it would appear that α-actinin-2 binding is not a universal property of Kv channels and may be specific to only a few. The same approach was used to determine whether the Kv1.4 N-terminus also bound α-actinin-2. Most likely due to codon-usage problems, we were unable to express the full Kv1.4 N-terminus, but a GST fusion to amino acids 90–305 of this channel was expressed and was tested in the in vitro system. The fragment includes all of the Kv1.4 amino acids with apparent homology to the Kv.15 actinin binding domain defined by deletion analysis (see below). As shown in Fig. 2, this fusion protein did bind α-actinin-2, albeit more weakly than

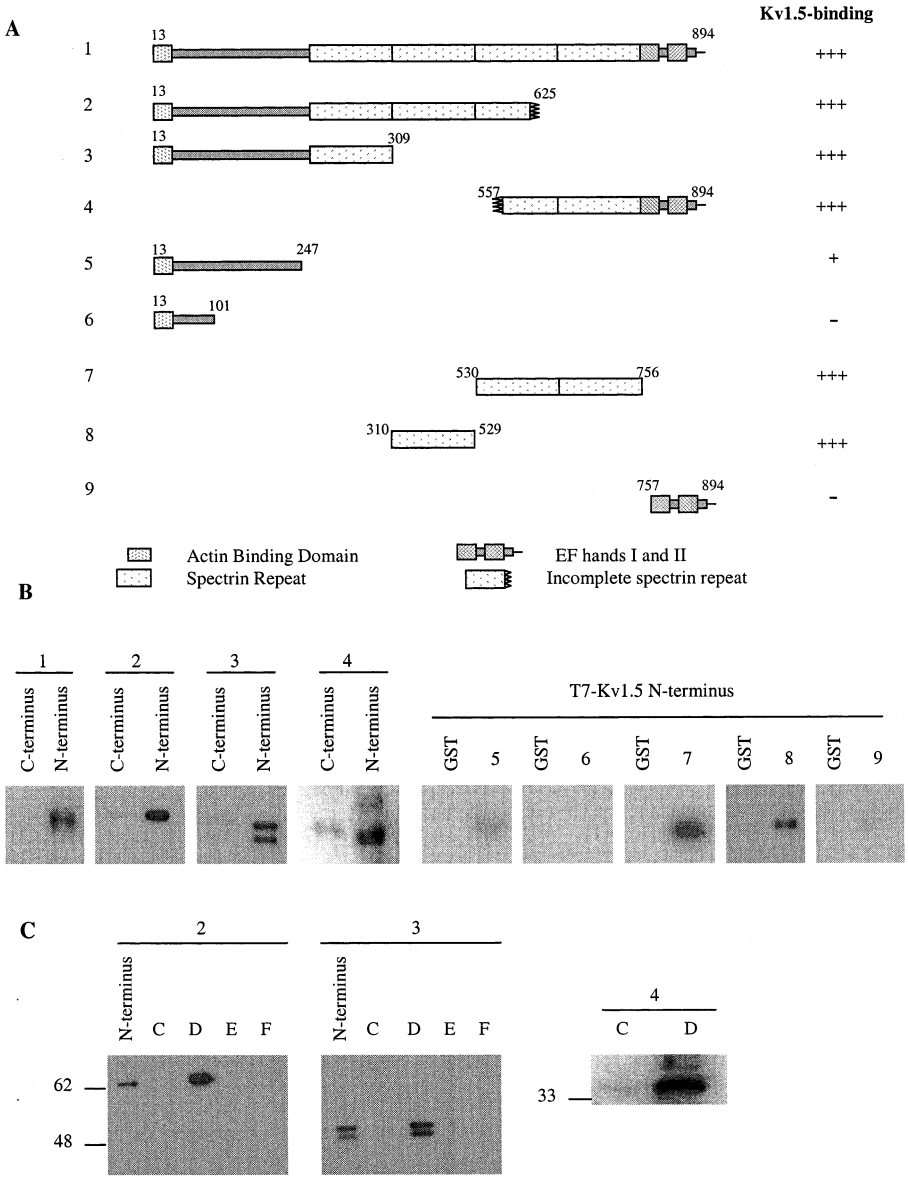

Fig. 4. Analysis of Kv1.5 N-terminus binding to α-actinin-2 deletion constructs. A: Schematic diagram of the various α-actinin-2 constructs. The binding activity of the constructs to Kv1.5 is indicated in the column on the right. B: Representative Western blots showing Kv1.5 binding by the various constructs. GST-tagged Kv1.5 C- or N-termini were incubated separately with full-length α-actinin-2, and constructs 2–4 of A and glutathione-Sepharose beads were added. Following incubation, the beads were pelleted, washed extensively, then subjected to SDS–PAGE. For constructs 1–3, Western blots were performed using monoclonal anti-T7 (Novagen) to visualize actinin fragments. For construct 4, polyclonal anti-α-actinin (Sigma, St. Louis, MO, USA) was used to detect the actinin fragment. Constructs 5–9 were expressed as GST fusions. Kv1.5 N-terminus was incubated with the actinin constructs or GST-tag alone. Glutathione-Sepharose beads were added, and the assay was performed as for constructs 1–4 to visualize the bound Kv1.5 fragments. C: Deletion constructs retain specificity for actinin binding region of Kv1.5. Constructs 2–4 were tested against various Kv1.5 N-terminal constructs by the method described in Fig. 2. Kv1.5 constructs are as presented in Fig. 2A. Western blots were performed using monoclonal anti-T7 (Novagen) to visualize actinin fragments. For construct 4, polyclonal anti-α-actinin (Sigma) was used to detect the actinin fragment.

the Kv1.5 N-terminus. Thus, α-actinin-2 binding is a property apparently shared by both Kv1.5 and Kv1.4.

3.2. α-Actinin-2 binding site in Kv1.5 localizes to a small region of the channel's N-terminus

In our previous study, using the yeast two-hybrid system, we showed that the α-actinin-2 binding site in Kv1.5 lies somewhere within the large, soluble N-terminal domain of approximately 250 amino acids [5]. Attempts to further define the actinin-2 binding site in Kv1.5 using the yeast two-hybrid system yielded inconclusive results, most likely because of problems with expression of our constructs in the yeast (data not shown). Therefore, we decided to employ the same GST fusion protein affinity binding method described above for this purpose.

A series of internal deletion mutations were constructed by digestion with restriction endonucleases followed by Bal31 digestion for varying periods. This approach generated a large number of deletions of varying sizes, of which five were chosen for further analysis. As with the full Kv1.5 N-terminus, these deletion mutants were cloned in frame with the GST-tag in pET42, expressed, purified and assayed for α-actinin-2

binding. A C-terminal fragment of Kv1.5 was similarly expressed and assayed. Confirming yeast two-hybrid studies, data in Fig. 3 show that α-actinin-2 did not bind to the Kv1.5 C-terminus GST fusion protein. α-Actinin-2 did bind separate N-terminal deletion constructs lacking amino acids 149–208 and amino acids 2–19 (Fig. 3, constructs B and D). However, a large deletion, eliminating amino acids 2–161 of Kv1.5, completely abolished actinin-2 binding, as did a smaller deletion of amino acids 86–208 (constructs C and E, respectively). α-Actinin-2 also failed to bind the Kv1.5 N-terminus lacking amino acids 2–91 (construct F), demonstrating that the α-actinin-2 binding site must be in the area of Kv1.5 amino acid 90.

To further define the α-actinin-2 binding region, three additional N-terminal truncations were constructed using convenient restriction sites. Deletion constructs lacking amino acids 1–49 (construct G) or 1–72 (construct H) bound α-actinin-2, thus delineating the maximal extent of the α-actinin-2 binding region in Kv1.5 to between amino acids 73 and 148. An N-terminal deletion construct ending in amino acid 133 of Kv1.5 (construct I) failed to bind α-actinin-2, indicating that the C-terminal extent of the binding site lies beyond amino acid 134. These findings offer a simple explanation for the differing actinin binding abilities of the various Kv1 channels. Except the extreme C-terminal of the binding region, known to be part of the Kvβ binding site, there are no homologous sequences to this region in Kv1.1, Kv1.2 or Kv1.3 (Fig. 2C). Kv1.4, on the other hand, does have limited homology to this region. Most notably, it shares with Kv1.5 a region rich in glutamate residues.

3.3. α-Actinin-2 binds to hKv1.5 via its spectrin repeats

A similar approach was taken to identify the Kv1.5 binding region(s) in α-actinin-2. Numerous fragments, representing three general regions, were tested for binding to the 240 amino acid Kv1.5 N-terminal fragment. These were the actin binding domain/N-terminal region, the central spectrin repeats and the C-terminal EF-hands. As illustrated in Fig. 4, the actin binding region failed to bind Kv1.5, as did the EF-hands. In contrast, all fragments that included any spectrin repeat bound Kv1.5 well. While not every individual spectrin repeat was tested in isolation, fragments containing spectrin repeat 1, spectrin repeat 2, spectrin repeats 2 and 3, and spectrin repeat 4 along with a part of spectrin repeat 3 all bound Kv1.5. A long N-terminal fragment outside the spectrin repeats (construct 5, Fig. 4B) bound Kv1.5 weakly.

To confirm the specificity of these interactions, several of the α-actinin-2 constructs were tested against Kv1.5 deletion mutants (Fig. 4C). As expected, spectrin repeat-containing constructs tested (numbers 2, 3, and 4) bound Kv1.5 construct D, lacking only amino acids 2–19, but failed to bind any Kv1.5 construct lacking an intact actinin binding region.

3.4. Neither Ca^{2+} nor calmodulin modulate Kv1.5 binding to α-actinin-2

The interaction between α-actinin-2 and another ion channel, the NR1 subunit of the NMDA receptor, is modulated by calmodulin [3]. This interplay is involved in the calcium-dependent inactivation of the channel. While there has been no evidence reported that Kv1.5 is affected by calcium, were the ion to play a role in regulating this channel, it could have profound implications for the modulation of the cardiac action potential. Therefore, the Kv1.5 N-terminus GST fusion was incubated with full-length α-actinin-2 in the presence of 0 or 500 μM Ca^{2+} plus or minus 1.2 μM calmodulin. As shown in Fig. 5, neither Ca^{2+} nor calmodulin had any effect on α-actinin-2 binding to Kv1.5.

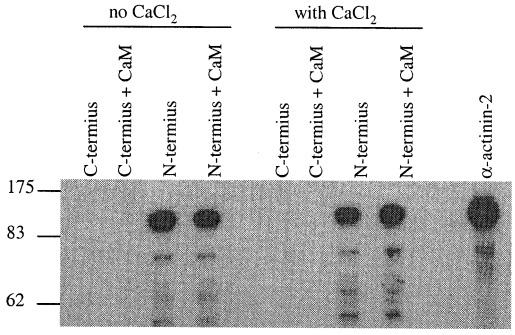

Fig. 5. Calcium and calmodulin do not affect Kv1.5 binding to α-actinin-2. GST-tagged Kv1.5 N-terminus was incubated with T7-tagged α-actinin-2 as described in Fig. 3. 1.2 μM calmodulin and 2.5 mM $CaCl_2$ were included as indicated. Visualization was with anti-T7 (Novagen). Purified α-actinin-2 was loaded in the far right lane as a control.

4. Discussion

Using in vitro binding techniques, we have delineated a region of interaction between the actin crosslinking protein α-actinin-2 and hKv1.5, a human voltage-gated potassium channel. A similar interaction occurs between α-actinin-2 and the related Kv1.4 channel, but not between actinin-2 and other Kv1-type channels. The Kv1.4 fragment employed in this study bound actinin much less well than did Kv1.5, but as we were unable to test the full Kv1.4 N-terminus for binding, it is unclear whether this weaker binding was due to an intrinsically lower affinity of this channel for α-actinin-2 or to a disruption of Kv1.4's actinin binding region. The Kv1.4 fragment tested overlaps completely the aligned Kv1.5 actinin binding region and includes a similar, if more extensive, stretch of glutamate residues. A stretch of arginines immediately upstream of the glutamates in Kv1.5, however, is notably absent in the Kv1.4 sequence. A stretch of arginines does occur immediately upstream of the Kv1.4 fragment employed in our study, so we may have inadvertently disrupted the Kv1.4 α-actinin-2 binding site. Since the spacing of the stretches differs so radically in the two proteins, it is nevertheless hard to see how the two could act similarly in α-actinin-2 binding (if the arginines are involved in at all). Further experiments will be necessary to address these possibilities.

The interaction of actinin-2 with Kv1.5 occurs between the central region in the N-terminus of the channel and the central spectrin repeats of α-actinin-2. Because Kv1.5 constructs lacking amino acids 1–72 and 149–208 both bind α-actinin-2 comparably well as does the full Kv1.5 N-terminus, the maximal size of the binding site is 76 amino acids, and the minimum length of the site is 45 amino acids. There is no consensus α-actinin-2 binding site reported in the literature. Binding sequences vary from 18 residue glycine-rich repeats interspersed with positively charged residues [15], to 10–20 amino acid proline-rich regions in zyxin [16] to short stretches rich in glutamate and lysine residues in β1- and β2-integrins [17,18]. The α-actinin-2 binding region in Kv1.5 has little

direct homology to any of these. There is a proline- and glutamate-rich area embedded within the binding region of Kv1.5, however. The homologous region of Kv1.4 lacks the prolines but is even richer in glutamate residues. Substantial further work will be necessary to identify the precise residues required for Kv1.5 and Kv1.4 binding to α-actinin-2. That none of Kv1.1, Kv1.2 or Kv1.3 contains sequences homologous to the glutamate-rich region very probably underlies the failure of these latter channels to bind α-actinin-2.

The situation in the α-actinin-2 protein seems comparably complex. We have found multiple binding sites for Kv1.5, most likely amounting to one binding site per spectrin-like repeat. Although only spectrin-like repeat number 2 was tested in isolation, from experiments with the other α-actinin-2 deletion constructs it is apparent that at least spectrin repeats 1, and 3 or 4 also bind the Kv1.5 N-terminus (Fig. 3). Like all spectrin repeats, the repeats in α-actinin-2 are highly degenerate, overall exhibiting only about 20% amino acid identity with each other. With this degree of degeneracy, it will likely be difficult to define the precise residues required for binding to Kv1.5, especially if residues of like charge suffice in this regard. Replacement of the spectrin repeat-defining residues, such as the conserved tryptophan in helix A and the leucine residue near the carboxyl end of helix C [19], may yield information as to whether it is the spectrin repeat structure or some other feature of these sequences that underlies the interaction. That a portion of α-actinin-2 upstream of the spectrin repeats also bound Kv1.5, albeit weakly, was initially surprising. A comparison with spectrin repeat 2, however, showed that the latter 60 amino acid portion of this region is quite homologous to the spectrin repeat, at least as homologous, in fact, as the repeat is to those in spectrin. Whether this homology is responsible for this region's binding to Kv1.5, or whether some other feature is involved, will require further investigation.

To gain insight into another possible role for α-actinin-2 in Kv1.5 regulation, we tested whether calmodulin could compete with actinin for binding to the channel. Calmodulin is known to compete with α-actinin-2 for binding to another ion channel, the NR1 subunit of the NMDA receptor [3,20], thereby mediating both Ca^{2+}-dependent inactivation of the channels [21,22] and their localization [23]. It would be highly significant if calmodulin similarly competed with α-actinin-2 for Kv1.5 binding. Like actinin binding sites, no consensus exists for those binding calmodulin [24]. Thus, the fact that Kv1.5 lacks a region homologous to the actinin/calmodulin binding portion of the NMDA receptor is not informative. Furthermore, the presence of EF-hands in α-actinin-2 suggests the possibility of a role for calcium independent of calmodulin in regulating Kv1.5 binding. However, in our system, no evidence for a calmodulin or calcium effect on the association of Kv1.5 with actinin could be found. Thus, while we cannot exclude the possibility of a role for another molecule in mediating a calcium effect, we have found no evidence that calmodulin can regulate Kv1.5 in the same manner as it does the NMDA receptor.

In summary, we have found that α-actinin-2, via its central spectrin-like repeats, binds to a proline- and glutamate-rich region in the N-terminus of Kv1.5 and to a similarly glutamate-rich Kv1.4 N-terminus. Unlike the interaction of actinin with the NMDA receptor, the actinin interaction with Kv1.5 is not affected by calcium or calmodulin. Cytochalasin and antisense experiments suggest that the actinin-2 is involved in regulating channel expression [5]. In addition, actinin may be involved in targeting Kv1.5 to specific surfaces on the cell. This might accomplish in concert with other molecules, such as PSD-95 [25,26] known to cluster Kv-type channels in neurons. Alternatively, α-actinin-2 might serve merely to anchor Kv1.5 in the membrane and prevent it from being internalized as the plasma membrane is cycled through the cell.

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