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Cation effects on the conformations of muscle and non-muscle α-actinins

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We examined the effects of changing KCl concentration on the secondary structures of α-actinins using circular dichroism (CD), 1,1′-bis(4-anilino) naphthalene-5,5′-disulfonic acid (bisANS) fluorescence and proteolysis experiments. Under near-physiological conditions, divalent cations also were added and changes in conformation were investigated. In 25 mM KH_2PO_4, pH 7.5, increasing KCl from 0 to 120 mM led to decreases in α-helix conformation for brain, platelet and heart α-actinins (40.5–30.2%, 65.5–37.8% and 37.5–27.8%, respectively). In buffered 120 mM KCl, 0.65 mM calcium produced small changes in the CD spectra of both brain and platelet α-actinin, but had no effect on heart α-actinin. bisANS fluorescence of all three α-actinins also showed significant changes in conformation with increasing KCl. However, in buffered 120 mM KCl increasing concentrations of Ca^{2+} or Mg^{2+} did not have significant effects on the bisANS fluorescence of any α-actinin. Digestion of brain, platelet and heart α-actinins with α-chymotrypsin showed an increase of proteolytic susceptibility in 120 mM KCl. These experiments also showed that increasing the concentration of Ca^{2+} or Mg^{2+} led to greater changes in digestion fragment patterns in the absence of KCl than in the presence of 120 mM KCl. The results suggest that α-actinins exist in different conformations depending on the ionic strength of the medium, which could explain the differences in calcium and F-actin binding results obtained from different α-actinins.

Keywords: α-actinin, bisANS fluorescence, calcium effects, circular dichroism, secondary structure

Introduction

After the complete sequences of chick (Baron *et al.* 1987a,b) and *Dictyostelium discoideum* (Noegel *et al.* 1987) α-actinins were published, the domain structure of α-actinin began to be revealed. α-Actinin can be divided into three regions: (i) the N-terminal domain, which contains the actin binding site; (ii) four internal 'spectrin like' repeats, each approximately 122 amino acids long; and (iii) the C-terminal region, which includes two putative EF-hand calcium binding motifs (Blanchard *et al.* 1989). This structure of α-actinin is homologous with the erythroid and non-erythroid spectrins (Baron *et al.* 1987a,b; Travé *et al.* 1995) and dystrophin (Davison & Critchley 1988).

Using circular dichroism (CD) Goll *et al.* (1969) reported 30 and 26% α-helix conformations for impure preparations of A and Z band rabbit skeletal muscle α-actinins. Since then, many conflicting reports have emerged on the secondary structure of α-actinins. Suzuki *et al.* (1973) reported that rabbit skeletal muscle α-actinin showed two extremes at 208 and 220 nm, similar to the CD spectra of polypeptides that are largely α-helical (Greenfield & Fasman 1969). They reported that approximately 59% of the polypeptide chain in purified skeletal muscle α-actinin is α-helical. Another report on rabbit skeletal muscle α-actinin showed 74% α-helix, 26% random coil and no β-sheet structure (Suzuki *et al.* 1976). Duhaiman & Bamburg (1984) reported corrected α-helical contents of 48.0, 44.8 and 49.3% for skeletal, smooth muscle and brain α-actinins, respectively. Sixty-three percent α-helix was reported for rat heart α-actinin and 73% for dog heart α-actinin (Malhotra *et al.* 1986).

At least part of the explanation for these differences may be due to the solution composition of the preparations. For example, the effects of divalent cations on the secondary structure of macrophage α-actinin have been investigated. Tyr (270 nm) and Trp (290 nm) fluorescence was examined after successive additions of calcium: a decrease in the relative intensity of emission of both Trp and Tyr residues was found without any shift in the wavelength (Pacaud & Harricane 1993). It was shown that addition of Mg^{2+} also led to a

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decrease in fluorescence, which was not as great as that observed with Ca^{2+}. Under physiological conditions (140 mM KCl), however, these calcium- and magnesium-induced changes were no longer detectable. We previously showed that changes in structure of spectrin were detectable by 1,1′-bis(4-anilino) naphthalene-5,5′-disulfonic acid (bisANS) fluorescence (Wallis *et al.* 1993), and a systematic investigation of the effects of KCl, $MgCl_2$ and $CaCl_2$ on gizzard α-actinin structure (Wenegieme *et al.* 1994) revealed that structural changes caused by 120 mM KCl eliminated $^{45}Ca^{2+}$ binding. Because spectrins and α-actinins are similar in structure, we used this approach to investigate the structural changes with additions of cations to various muscle and non-muscle α-actinins. In this report, using CD, bisANS fluorescence and proteolysis, we describe the effects of increasing concentrations of KCl, $CaCl_2$ and $MgCl_2$ on the secondary structures of brain, platelet and heart α-actinins.

Materials and methods

All water used in these experiments was deionized, glass distilled and then passed through a MilliQ water purification system to a resistivity of 10 mΩ or better. Fresh horse brains and hearts were obtained from Beltex Corporation (Fort Worth, TX). Week-old human platelet concentrates were obtained from Carter Blood Center (Fort Worth, TX). Platelets were washed and stored at −70°C as described by Landon & Olomucki (1983).

Preparation of α-actinins

All α-actinin preparations were purified to greater than 95% as demonstrated by densitometry (ISCO Model 1312) of Coomassie Blue-stained SDS–polyacrylamide gels (Figure 1). The only detectable contaminant was a 42 kDa protein, probably actin.

Heart α-actinin

α-Actinin was prepared from fresh horse hearts according to the method previously described for gizzard α-actinin (Landon & Olomucki 1983).

Brain α-actinin

A sample of 100 g of fresh horse brain was homogenized in 1 l of cold purified water and phenylmethylsulfonyl fluoride (PMSF) was added to 0.5 mM. The solution was centrifuged at 8000 *g* (7000 r.p.m.) in a GSA rotor for 30 min at 4°C and the supernatant was discarded. This was repeated and the resulting pellet was extracted for 30 min in 1 l of buffer [10 mM Tris–HCl, pH 7.5, 1 mM NaN_3, 0.5 mM dithiothreitol (DTT)] and PMSF was added to 0.5 mM. Following centrifugation as described above, the supernatant was saved and the pellet was re-extracted in the same manner. The supernatants resulting from both extractions were pooled, PMSF was added (0.5 mM) and the crude α-actinin solution was loaded onto a DE53 column (2.5 × 17 cm) pre-equilibrated in extraction buffer. The protein was then eluted from the column using a 0–0.3 M KCl gradient. Further purification was achieved on hydroxyapatite and Ultrogel AcA34 columns (see platelet α-actinin purification, below). The protein eluted from the AcA34 column purified to about 99% with a yield of 0.8–1 mg per 100 g of fresh brain. Frozen brain gave unsatisfactory results.

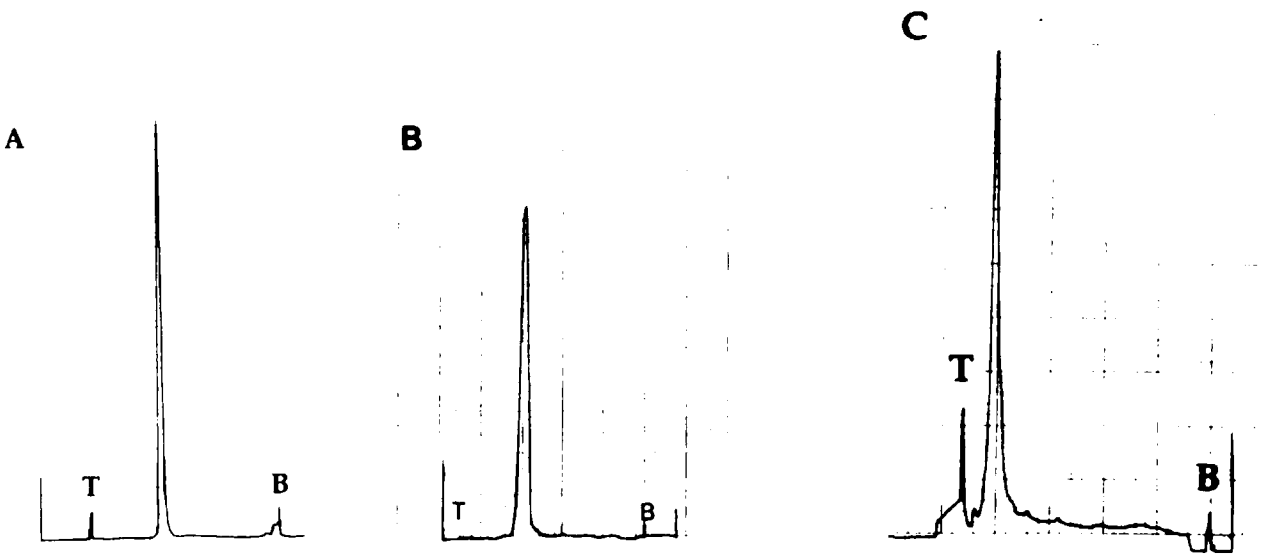

Figure 1. Densitometric scans of (A) heart, (B) brain and (C) platelet α-actinins. Proteins were prepared and run on 10% (A and B) or 15% (C) SDS–polyacrylamide gels as described in the Materials and methods. T indicates the top of the separating gel and B indicates the methylene blue dye front in each case.

Platelet α-actinin

α-Actinin was purified from platelets as described previously (Landon *et al.* 1977), except for these modifications: the DE53 eluate was subjected to 15–40% ammonium sulfate precipitation. The precipitate was dissolved in a minimum volume of phosphate buffer (5 mM, pH 6.8 containing 0.5 mM DTT) and the clear protein solution was loaded onto a hydroxyapatite column (2.5 × 3 cm) equilibrated with the same buffer. The column was washed extensively with 50 mM phosphate buffer and a gradient of 50–150 mM potassium phosphate, pH 6.8, was employed to elute α-actinin. Fractions containing α-actinin were pooled, concentrated and loaded on an Ultrogel AcA34 column (1.25 × 90 cm) in 10 mM Tris–HCl, 0.5 mM DTT, 1 mM NaN_3, pH 7.0. The α-actinin concentration was determined by absorbance using $A_{280}^{1\%} = 12.6$ (Landon *et al.* 1977) and reconfirmed by protein estimation by the method of Bradford (1976).

Before all experiments investigating structural changes of α-actinins, proteins were dialyzed into the appropriate buffer and centrifuged at 37 000 g_{ave} for 30 min to remove protein aggregates.

SDS–PAGE

SDS–PAGE was performed according to the discontinuous system of Laemmli (1970). Either 10 or 15% gels were used with a 4% stacking gel. Samples were mixed with 0.5 vol solubilization buffer (10 mM Tris, pH 8.0, 10% β-mercaptoethanol, 20% glycerol, 6% SDS plus trace amounts of bromophenol blue) and heated for 5 min. Gels were stained with 0.04% Coomassie brilliant blue in 40% methanol and 10% acetic acid, and then destained with 7% acetic acid and 40% methanol.

Chymotrypsin digestion

α-Actinins (0.5 mg ml^{-1}) in 10 mM Tris–HCl, pH 7.5, 0.5 mM EDTA, 1 mM β-mercaptoethanol were digested with α-chymotrypsin at 1:25 w/w enzyme:substrate ratio at 37°C for 5 min. Various concentrations of KCl, $MgCl_2$ or $CaCl_2$ were added to the proteins to equilibrate at 4°C for 1 h before digestion. The reaction was terminated by heating in solubilization buffer for 5 min.

Removal of calcium

α-Actinins were dialyzed against 25 mM KH_2PO_4, pH 7.5 with 8–10 g l^{-1} Chelex-100 for at least 72 h at 4°C. We cannot be sure that this extended Chelex treatment removes every bound divalent cation, but we showed previously that divalent cations are removed by this treatment from high-affinity sites (Wallis *et al.* 1992, 1993).

CD

α-Actinins (1 mg ml^{-1}), dialyzed and Chelex-100 treated in 25 mM KH_2PO_4 (pH 7.5), were diluted to 0.15 mg ml^{-1} using Chelex-100-treated buffer. Concentrated KCl in 25 mM KH_2PO_4, pH 7.5, to give 60 or 120 mM KCl was added to the protein in 25 mM KH_2PO_4 (pH 7.5) and at 120 mM KCl indicated concentrations of Mg^{2+} and Ca^{2+} were added. UV (190–250 nm) CD spectra of purified α-actinin and the prepared samples were obtained with an Aviv CD Spectrophotometer, model 62DS (Lakewood, NY), equipped with computerized data collection. A 0.1 cm path length cuvette was used and the instrument was continually purged with nitrogen throughout the measurements. The data were analyzed using the PROSEC program. A molecular weight of 200 000 was assumed (Endo & Masaki 1982) with a mean residue weight of 112 calculated from the gizzard α-actinin sequence (Baron *et al.* 1987a,b).

Fluorescence

These experiments were performed as described previously (Wallis *et al.* 1993, Wenegieme *et al.* 1994). Protein concentrations were 0.05–0.23 μM and 16–56 μM bisANS were added to achieve saturation. Data were obtained with a Shimadzu RF-0500 recording spectrofluorophotometer (Kyoto, Japan) equipped with an FDU-3 floppy disk unit. The excitation wavelength was 365 nm and the emission wavelength range was 400–650 nm.

Results

Cation effects on CD spectra of α-actinins

Structural changes upon cation binding to brain, platelet and heart α-actinins were examined by CD. After recording spectra in buffer without KCl, 60 mM KCl was added to the Chelex-treated proteins and spectra were re-recorded. Then more KCl was added to bring the concentration to 120 mM. After each addition, the protein was left to incubate for 30 min at 20°C. At 120 mM KCl, increasing concentrations of Ca^{2+} or Mg^{2+} were added. As KCl concentrations were increased from 0 to 60 to 120 mM KCl in the brain α-actinin solution, there was a decrease in α-helix, an increase in turns and no significant change in random coil conformation (Figure 2A). Increasing concentrations of KCl led to a decrease in α-helix conformation and an increase in turns and random coil conformation for platelet α-actinin (Figure 2B). Heart α-actinin showed a decrease in α-helix, a smaller decrease in turns, and an increase in random conformation with increasing KCl (Figure 2C). No β-sheet conformation was observed for any of the three α-actinins under these conditions. These data are quantified in Table 1. At 120 mM KCl, addition of 0.65 mM $CaCl_2$ led to a slight change in the secondary structure of both brain and platelet α-actinins, but did not affect the secondary structure of heart α-actinin significantly (Table 1). Also, there was no significant change in circular dichroic spectra of any α-actinin when Ca^{2+} was removed by the initial Chelex-100 treatment in buffer without any KCl (data not shown) suggesting that little calcium was bound initially to the purified proteins.

Cation effects on the bisANS fluorescence of α-actinins

After titrating Chelex-treated α-actinins with bisANS to saturation, cations were added and changes in bisANS

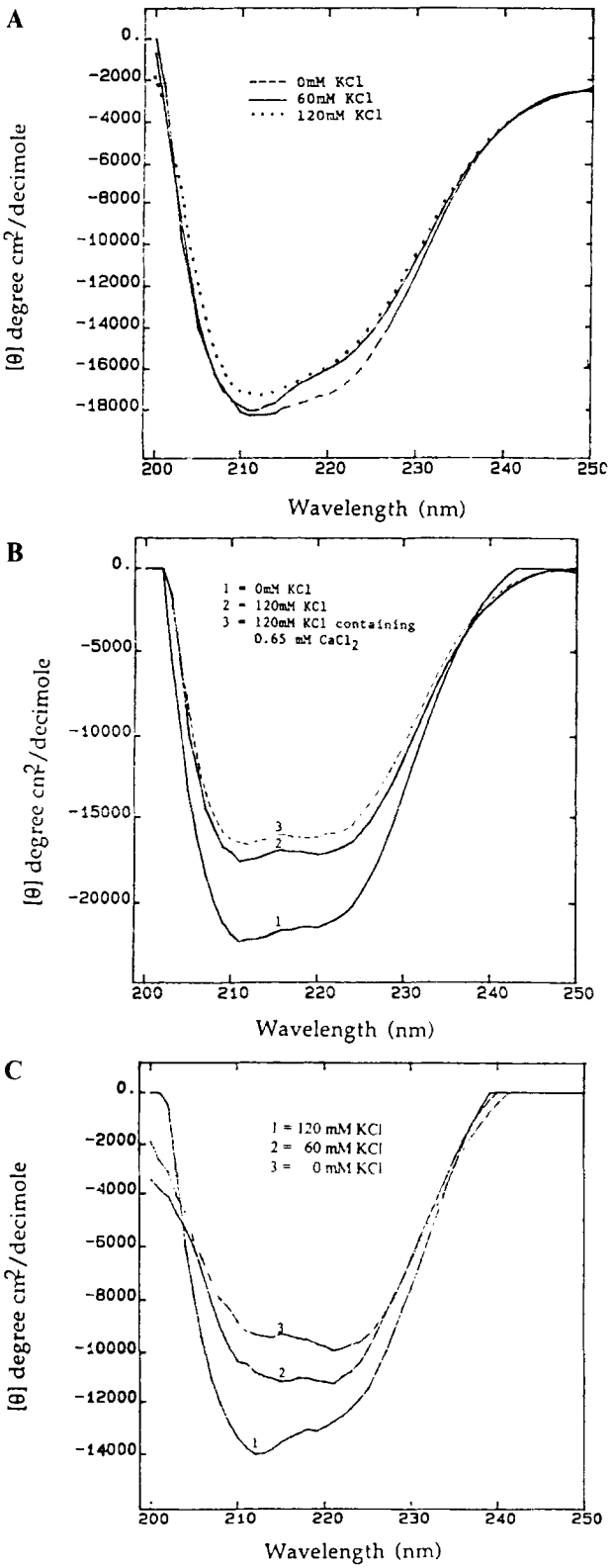

Figure 2. Effects of KCl on the circular dichroic spectra of α-actinins. α-Actinins were dialyzed against Chelex-100 in 25 mM KH_2PO_4, pH 7.5, to remove endogenous cations. Circular dichroic measurements were taken at 0 mM, 60 mM and 120 mM KCl. Circular dichroic spectra of (A) brain α-actinin; (B) platelet α-actinin; and (C) heart α-actinin.

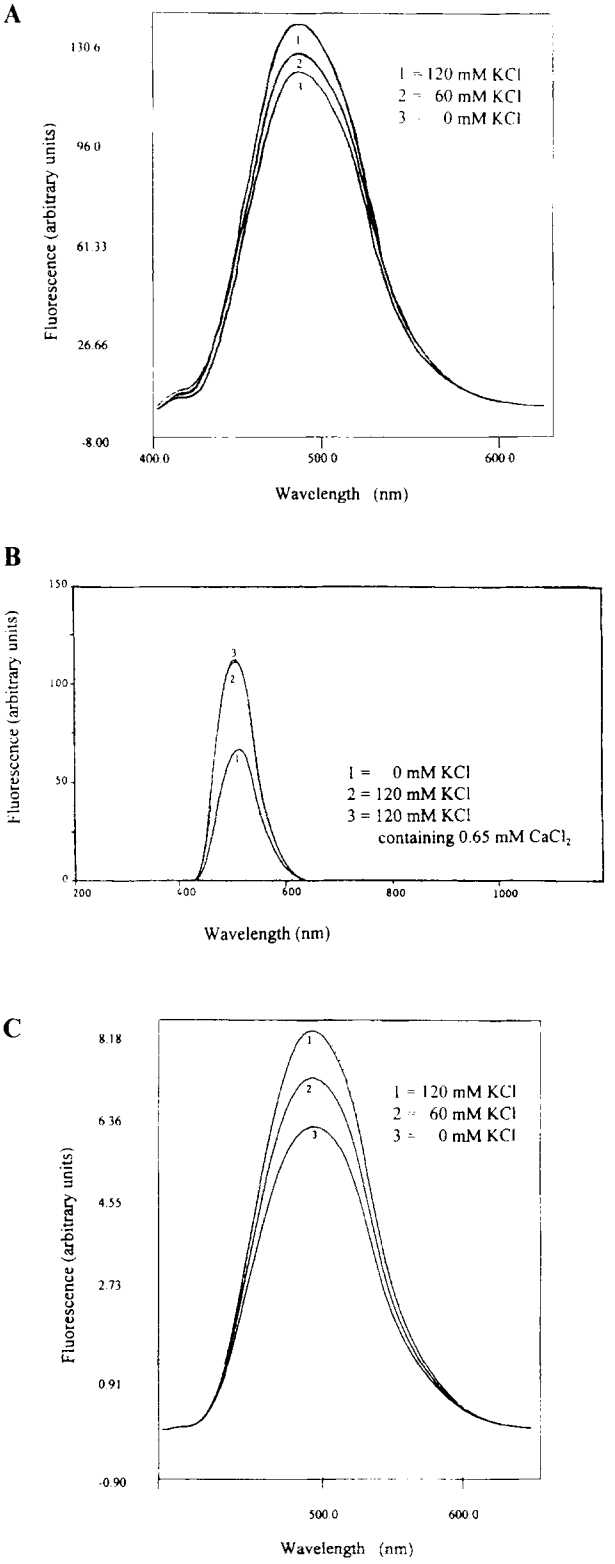

Figure 3. Effects of KCl on the bisANS fluorescence of α-actinins. α-Actinins were dialyzed against Chelex-100 in 25 mM KH_2PO_4, pH 7.5, to remove endogenous cations. bisANS was added to the protein beyond saturation, after which changes in fluorescence were measured with 60 or 120 mM KCl additions. (A) Brain α-actinin, (B) platelet α-actinin and (C) heart α-actinin.

Table 1. CD changes of platelet and heart α-actinins with increasing concentrations of KCl and $CaCl_2$

KCl (mM)	$CaCl_2$ (mM)	f_A	f_B	f_T	f_R
Brain α-actinin					
0	0	0.405 ± 0.001	0	0.144 ± 0.008	0.449 ± 0.007
120	0	0.320 ± 0.013	0	0.250 ± 0.011	0.444 ± 0.002
120	0.65	0.261 ± 0.003	0	0.291 ± 0.004	0.448 ± 0.001
Platelet α-actinin					
0	0	0.655 ± 0.002	0	0.092 ± 0.007	0.252 ± 0.007
120	0	0.378 ± 0.001	0	0.289 ± 0.006	0.332 ± 0.004
120	0.65	0.339 ± 0.004	0	0.333 ± 0.005	0.326 ± 0.001
Heart α-actinin					
0	0	0.375 ± 0.015	0	0.340 ± 0.021	0.285 ± 0.013
120	0	0.278 ± 0.019	0	0.289 ± 0.018	0.432 ± 0.012
120	0.65	0.280 ± 0.020	0	0.301 ± 0.015	0.419 ± 0.024

Samples were dialyzed against Chelex-100 to remove endogenous divalent cations and then against 25 mM KH_2PO_4, pH 7.5. f_A, f_B, f_T and f_R indicate fractions of α-helix, β-sheet, turns and random coil, respectively. Results are averages of three to four measurements on two preparations ± SD.

fluorescence were measured. Similar amounts of bisANS were added to each sample, but measurements were made at different band passes, so results are recorded as relative increases. Increasing the concentration of KCl always led to an increase in the bisANS fluorescence of brain, platelet and heart α-actinins (Figure 3). Increasing the concentration of $CaCl_2$ or $MgCl_2$ in buffered 120 mM KCl did not lead to any significant changes in the bisANS fluorescence of any α-actinin.

Cation effects on the proteolysis of α-actinins

α-Actinins were incubated with increasing concentrations of KCl, $CaCl_2$ or $MgCl_2$ and digested with α-chymotrypsin for 5 min. When KCl concentrations were increased from 0 to 60 to 120 mM, brain α-actinins became more susceptible to chymotrypsin (Figure 4). The same results were found with platelet α-actinin, but not heart α-actinin (data not shown). The C90 and C70 digestion peptides disappeared faster with 120 mM KCl, and the intensities of C55 (the central repeat region) and C36 (the N-terminal end) decreased with increasing concentrations of KCl. There is also a corresponding increase in the lower molecular weight fragments with increasing KCl concentration (Figure 4). More changes in the peptide pattern were observed when the concentration of magnesium was increased from 1 to 5 mM in the absence of KCl (Figure 4, lanes 5 and 6) than in the presence of KCl (Figure 4, lanes 7 and 8). In the absence of KCl and with increasing $MgCl_2$, the C90 and C70 peptides almost disappeared. The C55 and C36 peptides also showed a greater decrease in intensity with increasing $MgCl_2$. In 120 mM KCl, however, the effects of increasing $MgCl_2$ from 1 to 5 mM were less obvious, e.g. the C55 intensity decrease was smaller and the C36 fragment was not affected. Increasing $CaCl_2$ from 0.3 to 0.5 mM both in the absence (Figure 4, lanes 9 and 10) and presence of KCl (Figure 4, lanes 11 and 12) gave effects similar to the $MgCl_2$ increases.

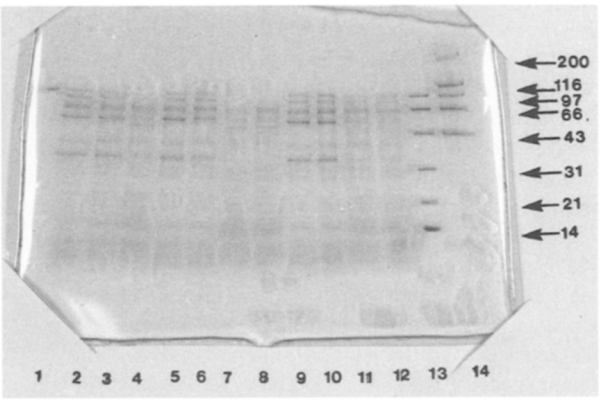

Figure 4. Effects of KCl, $MgCl_2$ or $CaCl_2$ on chymotryptic digestion of brain α-actinins. Brain α-actinin in 10 mM Tris–HCl, pH 7.5, 0.5 mM EDTA, and 1 mM β-mercaptoethanol was digested with α-chymotrypsin at a 1:25 w/w ratio for 5 min in concentrations of KCl, $MgCl_2$ and $CaCl_2$ as indicated. The reaction was terminated as described in 'Materials and methods'. Lane 1, purified brain α-actinin; lane 2, 0 mM KCl; lane 3, 60 mM KCl; lane 4, 120 mM KCl; lane 5, 0 mM KCl, 1 mM $MgCl_2$; lane 6, 0 mM KCl, 5 mM $MgCl_2$; lane 7, 120 mM KCl, 1 mM $MgCl_2$; lane 8, 120 mM KCl, 5 mM $MgCl_2$; lane 9, 0 mM KCl, 0.3 mM $CaCl_2$; lane 10, 0 mM KCl, 0.5 mM $CaCl_2$; lane 11, 120 mM KCl, 0.3 mM $CaCl_2$; lane 12, 120 mM KCl, 0.5 mM $CaCl_2$; lanes 13 and 14, low and high molecular weight markers (high: myosin, 200 000 Da; β-galactosidase, 116 000; phosphorylase b, 97 000; bovine serum albumin, 66 000; ovalbumin, 42 000; low: phosphorylase b, 97 000; bovine serum albumin, 66 000; ovalbumin, 42 000; carbonic anhydrase, 31 000; trypsin inhibitor, 21 000; lysozyme, 14 000).

Discussion

The UV CD spectra of purified brain, platelet and heart α-actinins show two minima, near 208 and 222 nm (Figure 2). These are similar to the spectra of α-helical polypeptides (Greenfield & Fasman 1969). Duhaiman & Bamburg (1984) previously reported minima at 222 nm for gizzard, skeletal

muscle and brain α-actinins, with corresponding α-helix values of 28.8, 30.3 and 30.7%, corrected to 44.8, 48.0 and 49.3%, for the three proteins in the absence of KCl. While we used the PROSEC computer program to determine the percent α-helix, β-sheet, random coil and turn conformations, Duhaiman & Bamburg used $-35\,000$ deg dmol^{-1} for the minimum at 222 nm given by Holzworth & Doty (1965) as 100% α-helix. Using that method for calculating our data for α-helix, we find values of 49.9% for brain α-actinin, 60.1% for platelet α-actinin and 27.1% for heart α-actinin. We previously reported a value of 30.0% for gizzard α-actinin (Wenegieme *et al.* 1994). While the values for the muscle α-actinins are similar to the uncorrected values reported by Duhaiman & Bamburg (1984), those for the non-muscle α-actinins differ significantly due to possible MARCKS protein contamination of the brain α-actinin they studied. We purified these proteins thoroughly (Figure 1) and saw only occasional slight contamination by a 42 kDa protein (probably actin), not MARCKS or phosphatidylinositol 3-kinase (Shibasaki *et al.* 1994). Both the values we report here and those of Duhaiman & Bamburg (1984) are similar to those previously described for muscle α-actinins (Goll *et al.* 1969) but are lower than those reported by other researchers (Suzuki *et al.* 1973, 1976). The α-helix contents for rat and dog heart α-actinin were reported to be 63 and 73%, respectively (Malhotra *et al.* 1986). In every case these results were obtained in the absence of KCl, but differences in calculation methods may explain some of these differences.

We investigated the effects of increasing ionic strength (K^+ ion) on the CD spectra of buffered brain, platelet and heart α-actinins, and saw that increasing the concentration of potassium ion led to significant changes in the CD spectra of α-actinins. Without KCl, we obtained 40.5, 65.5 and 37.5% α-helix values for brain, platelet and heart α-actinins, respectively, which decreased to 32.0, 37.8 and 27.8% in 120 mM KCl. We chose K^+ rather than Na^+ or another cation because the effects we observed would have the greatest physiological relevance. This supports our hypothesis that approaching physiological ion concentrations produces a change in the secondary structure of the α-actinin molecule, leading to a decrease or loss (Wenegieme *et al.* 1994) of high-affinity calcium binding. Addition of 0.65 mM $CaCl_2$ in buffered 120 mM KCl yielded more significant changes with platelet and brain α-actinins, whereas there were only small changes with heart α-actinin, as we observed previously for grizzard α-actinin (Wenegieme *et al.* 1994). The results reported here should help to reconcile the widely varying secondary structure fractions calculated previously under varying ionic conditions.

Masaki & Takaiti (1969) showed a slower sedimenting component of their chicken skeletal muscle α-actinin when the ionic composition was decreased below 10 mM KCl. All our solutions used to examine CD changes contained 25 mM K_2HPO_4 (pH 7.5) at a minimum, so the changes we observed are not likely due to a changing ratio of the slower and faster sedimenting species. Also, we have obtained preliminary results, using dynamic light scattering, indicating that additions of KCl and $CaCl_2$ to a buffered solution of platelet α-actinin did not significantly change the state of aggregation of the protein because the mass of protein aggregates with molecular weights above 200 000 in these solutions remained at 3% or less (data not shown). Finally, aggregation should decrease, not increase, α-actinin susceptibility to chymotrypsin cleavage. Therefore, the CD changes we observe probably are due to conformational changes caused by changing electrostatic interactions, not aggregation. However, at least two reports of muscle α-actinin self-association have appeared (Sanger *et al.* 1984, Taylor & Taylor 1994), so that could be a fruitful area for more experimentation.

Changes in bisANS fluorescence of brain, platelet and heart α-actinins also suggest changes in secondary structure upon increasing potassium ion concentration. These changes could be due to unfolding of the α-actinin molecule, indicated by a decrease in α-helix and increases in turn and coil conformations and by increased susceptibility to chymotrypsin digestion, creating more hydrophobic sites that were not previously available to bisANS. Previous reports have shown that increasing the calcium or magnesium concentration in the absence of K^+ or Na^+ ions led to decreases in relative intensity of both Trp and Tyr residue fluorescence, but these calcium- or magnesium-induced changes disappeared in the presence of 140 mM KCl (Pacaud & Harricane 1993). This is in agreement with our data showing that in 120 mM KCl, no significant calcium- or magnesium-induced fluorescence changes were detectable for any α-actinin.

Proteolysis experiments frequently are used to detect changes in protein conformation, e.g. Kuznicki *et al.* (1995). Chymotryptic digestion of gizzard and brain α-actinins revealed that α-actinin becomes more susceptible to proteolysis in the presence of 120 mM KCl (Figure 4). This finding, like the bisANS fluorescence and CD changes, indicates changes in secondary structure that result in exposing digestion sites not previously accessible in the absence of KCl. Increasing concentrations of Ca^{2+} or Mg^{2+} led to greater changes in the absence of KCl than in the presence of 120 mM KCl, which supports the fluorescence and previous data (Pacaud & Harricane 1993). The Ca^{2+} effects on the non-muscle α-actinins were greater than its effects on muscle α-actinins (Wenegieme *et al.* 1994 and unpublished observations). Kuroda *et al.* (1994) reported a decrease in proteolytic susceptibility of skeletal muscle α-actinin with increasing K^+ ion concentration. This is different from our observations for all three α-actinins. We think this difference could be due to aggregation or the presence of cross-linked dimers during preparation, indicated by the bands on the SDS–polyacrylamide gels above the 105 kDa polypeptide.

Previous reports have shown that the repeat regions common to spectrins, α-actinins and dystrophin are capable of changes in flexibility (Yan *et al.* 1993) and degrees of 'twisting' (Taylor & Taylor 1993). The intersegment flexibility of α-actinin may even be greater than for spectrin because the extra amino acids in the α-actinin repeat may be located in the intersegment region (Gilmore *et al.* 1994, Speicher & Ursitti 1994). The crystal structure of the repeat-segments of spectrin reported by Yan *et al.* (1993) showed that a segment consists of a three-helix bundle. They

reported that hydrophobic interactions between segments may constrain intersegment flexibility, indicated by the model of the interface between two tandem segments. The projection image of smooth muscle α-actinin from two-dimensional crystals showed that the ends of the molecule appear different, indicating that the molecule is twisted about the long axis (Taylor & Taylor 1993). They suggested that differences in calcium sensitivities of α-actinin could be due to the proximity of the C-terminal and actin-binding domains that differ depending on the degree of twisting of the central repeats. Our data support these findings that cation changes could lead to structural changes in the repeat region of α-actinins including dissociation of the three helix bundles and, possibly, an unfolding of the helices in the repeat regions.

We show here that the concentration of KCl in the buffer is very important for the conformation of α-actinin from both muscle and non-muscle sources because the conformations under near-physiological conditions (120 mM KCl + 25 mM K_2HPO_4) differ from those in the absence of KCl. Also, calcium causes small, but significant, conformational changes in platelet and brain α-actinins in this solution, but has no significant effect on heart α-actinin. These findings could possibly explain the differences in results of: (i) calcium binding properties of α-actinins; (ii) F-actin–α-actinin binding experiments; and (iii) F-actin–α-actinin gelation studies.

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