

Calcium-sensitive non-muscle α -actinin contains EF-hand structures and highly conserved regions

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The F-actin crosslinking molecule α -actinin from the slime mould *Dictyostelium discoideum* carries two characteristic EF-hand structures at the C-terminus. The calcium-binding loops contain all necessary ligand-binding oxygens and most likely form the structural basis for the calcium sensitivity of strictly calcium-regulated non-muscle α -actinins. Furthermore, the sequence exhibits at the N-terminal site of the molecule a high degree of homology to chicken fibroblast α -actinin. This stretch of amino acids appears to have remained essentially constant during evolution and might represent the actin-binding site. The findings have led us to propose a model for the inhibitory action of Ca^{2+} on non-muscle α -actinins.

Actin-binding protein; Cytoskeleton; EF-hand structure; Evolution; Sequence homology; (*Dictyostelium discoideum*)

1. INTRODUCTION

α -Actinin, an actin filament crosslinking protein, can be found in a wide variety of organisms in both muscle and non-muscle cells. It is a homodimer whose subunits assemble in an anti-parallel fashion to form a rod-like structure. The crosslinking activity of non-muscle α -actinin is completely inhibited by calcium at the micromolar level. This calcium sensitivity distinguishes muscle and non-muscle α -actinin [1,2]. Using a partial genomic clone of the *Dictyostelium discoideum* α -actinin gene, we have previously shown that *D. discoideum* harbours a single α -actinin gene that codes for a mRNA of approx. 3.0 kb. This mRNA is present during all developmental stages of the *D. discoideum* life cycle [3]. Screening of a cDNA library [4] with this clone led to the isolation of

cDNA clones carrying the complete sequence for *D. discoideum* α -actinin. Here, we present the first complete sequence of a non-muscle α -actinin, that of *D. discoideum*. Two sequences at the C-terminus are characteristic EF-hand structures and most likely form the structural basis for the calcium sensitivity. Furthermore, the *D. discoideum* sequence exhibits a high degree of homology with a recently reported partial sequence of chicken fibroblast α -actinin [5]. The most striking homology is located at the N-terminal site of the molecule. It is possible that during evolution the conserved structure of actin forced a similarly constant complementary region in the actin-binding site of α -actinins.

2. MATERIALS AND METHODS

α -Actinin from *D. discoideum* strain AX2-214 was purified essentially as in [6]. The isolation of cDNA clones containing α -actinin-specific sequences is described by Noegel et al. [4]. Since the clones did not contain a poly(A) sequence, the cDNA library was rescreened with an *EcoRI* fragment that was located near the carboxy-terminus.

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The isolated clones were identical to those previously described and harboured in addition a poly(A) sequence. They hybridized to a single mRNA species of approx. 3.0 kb that was previously shown to be the α -actinin message [3]. Overlapping fragments of the cloned cDNAs were subcloned into vectors M13mp18/19 or into pUC18/19 [7]. The inserts were sequenced by the dideoxy chain termination method of Sanger et al. [8]. The sequences were analyzed with programs of the University of Wisconsin Genetic Computer Group (UWGCG).

3. RESULTS AND DISCUSSION

In fig.1 the cDNA sequence and derived protein sequence are shown. The protein sequence consists of 862 amino acids; the calculated molecular mass is 97.6 kDa and the N-terminus is blocked. Secondary structure prediction according to Chou-Fasman [9] suggests an α -helical content of about 50% over the entire protein. The region upstream of the methionine start codon exhibits a high AT content, a characteristic feature of non-coding DNA regions in *D. discoideum* [10].

The asterisks beneath the amino acids in regions 733–763 and 769–799 (fig.1) indicate calcium-binding loops whose functional features are in agreement with the EF-hand model for calcium-modulated proteins as proposed by Kretsinger [11]. Fig.2 aligns the sequences of the putative calcium-binding loops of *D. discoideum* α -actinin (rows e,f) with the corresponding regions of typical members from the calmodulin, troponin C, myosin light chain and parvalbumin families. Both domains contain a calcium-binding loop with all the necessary liganding oxygens and are close enough that their hydrophobic surfaces could impart mutual stability [11]. The search of the protein sequence database of the protein identification resource (PIR) showed in both cases the highest alignment scores for *Trypanosoma* calmodulin. *D. discoideum* calmodulin [12] does not exhibit a closer relationship to α -actinin; for EF-hand 1 in

α -actinin it scores slightly better than *Chlamydomonas* [13] or spinach [14] calmodulin, but for EF-hand 2 worse than these lower and higher plant calmodulins. The alignment scores suggest that α -actinin assumed the EF-hand structures very early in evolution, most likely before the separation of slime moulds and higher plants.

A partial amino acid sequence of chicken fibroblast α -actinin that was recently published [5] shows strong homology to the N-terminal third of *D. discoideum* α -actinin (residues 52–250). These two proteins are nearly identical between residues 97 and 151, where out of 55 amino acids 48 residues are identical and 5 residues are conservative replacements. It is possible that the complete sequence of the chicken fibroblast α -actinin will show the entire N-terminal portions of the molecules to be highly homologous. This strong homology of two α -actinins from unrelated organisms is also evident at the DNA level although to a lower extent (nucleotides 350–750). Using a probe from the *D. discoideum* α -actinin gene containing the highly conserved DNA stretch to test cross-hybridization to chicken DNA, we found three bands of about 1.35, 1.75 and 3.0 kb. These may represent different isoforms of chicken α -actinin or other crosslinking proteins with homologous F-actin-binding regions. Similar conclusions were drawn from the characterization of an actin-binding peptide that seems to be conserved in rat hepatic actinogelin, rat skeletal muscle and chicken gizzard α -actinins [15,16]. Limited similarities between *D. discoideum* α -actinin (residues 485–560) and non-erythroid α -spectrin [17] (residues 395–470) were found and possibly reflect functional sites for dimerization rather than common actin-binding sites. No significant sequence homologies to the actin-severing protein gelsolin [18], myosins or actins (taken from the Computer Genbank, EMBL, Feb.1987) were detected. These proteins apparently bind to sites different from the α -actinin-binding site on the actin molecule.

An important question is how the actin-binding

Fig.1. Nucleotide and deduced amino acid sequence of *D. discoideum* α -actinin cDNA. The underlined amino acids denote the peptides which were isolated from a tryptic digest of α -actinin and sequenced by Edman degradation [3]. At the 3'-end of the coding region a polyadenylation signal (underlined nucleotide sequence) overlaps with the last codon followed by a poly(A) stretch 36 nucleotides downstream from the stop codon. The EF-hand regions are indicated with stars.

A)

a)		0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0												
b)				E	L'			L'L'			L'D'	D'	D'G	I'D'		E	L'			L'L'		L'												
c)				*	*			*			*	*	*	*		*	*			*		*												
d)											X	Y	Z	-Y	-X		-Z																	
e)	E	E	L	S	E	F	K	A	C	F	S	H	F	D	K	D	N	D	N	K	L	N	R	L	E	F	S	S	C	L	K	S	I	G
CaM	E	Q	I	S	E	F	K	E	A	F	S	L	F	D	K	D	G	D	G	T	I	T	T	K	E	L	G	T	V	M	R	S	L	G
TnC	E	M	I	A	E	F	K	A	A	F	D	M	F	D	T	D	G	G	G	D	I	S	T	K	E	L	G	T	V	M	R	M	L	G
MLC	E	Q	Q	D	E	F	K	E	A	F	L	L	Y	D	R	T	G	D	S	K	I	T	L	S	Q	V	G	D	V	L	R	A	L	G

B)

b)				E	L'			L'L'			L'D'	D'	D'G	I'D'		E	L'			L'L'		L'												
c)				*	*			*	*		*	*	*	*	*	*	*	*		*	*													
d)											X	Y	Z	-Y	-X		-Z																	
f)	L	T	E	E	Q	L	N	Q	V	I	S	K	I	D	T	D	G	N	G	T	I	S	F	E	E	F	I	D	Y	M	V	S	S	R
CaM	L	T	D	E	E	V	D	E	M	I	R	E	A	D	V	D	G	D	G	Q	I	N	Y	E	E	F	V	K	M	M	M	S	K	*
TnC	P	T	K	E	E	L	D	A	I	I	E	E	V	D	E	D	G	S	G	T	I	D	F	E	E	F	L	V	M	M	V	R	Q	M
S-100	K	E	Q	E	V	V	D	K	V	M	E	T	L	D	S	D	G	D	G	E	C	D	F	Q	E	F	M	A	F	V	A	M	I	T
Parv.	L	T	D	K	E	T	K	D	L	L	I	K	G	D	K	D	G	D	G	K	I	G	V	D	E	F	T	S	L	V	A	E	S	*

Fig.2. Alignment of EF-hand regions from α -actinin and known calcium-modulated proteins. According to the EF-hand hypothesis [11] there are 16 characteristic positions (row b) building an EF-hand structure. A true EF-hand domain scores 12 or better out of 16 [20]. Row a shows the recommended numbering [11], the stars (rows c and boxed residues) indicate the correct alignment and the liganding oxygens that correspond to the octahedral vertices (rows d). Panel A aligns residues 730–763 from *D. discoideum* α -actinin (row e) with calmodulin (*Trypanosoma brucei gambiense* [21], residues 7–40), skeletal muscle troponin C (*Rana esculenta* [22], residues 17–50) and skeletal muscle myosin A1 and A2 catalytic light chains (rabbit [23], residues 47–80). Panel B shows the alignment of α -actinin (residues 766–799; row f) with the same calmodulin (residues 116–148) and troponin C (residues 53–86) as in panel A, and in addition with S-100 protein (β -chain, bovine [24], residues 48–81) and parvalbumin (*Amphiuma means* [25], residues 77–109).

activity of non-muscle α -actinin is regulated by calcium. The highly conserved sequence in the N-terminal portion of *D. discoideum* α -actinin is separated by 500 amino acids from the EF-hand structures and the two ends of the polypeptide are also separated in space. Based on EM data from α -actinin-antibody complexes, the rod-shaped α -actinin molecule consists of two filamentous subunits that extend from one end to the other in an anti-parallel fashion [19]. Thus, it is unlikely that the EF-hands interact with the N-terminal

region of the same subunit. However, as outlined in fig.3, the EF-hand of one subunit could be near the N-terminus of the other subunit and affect the binding to actin. To clarify this inhibitory mechanism we plan to change the structure of the putative actin-binding site or the calcium-binding domains by genetic engineering. Using a strain that does not produce α -actinin, we hope to construct transformants that express an α -actinin which is not regulated by calcium.

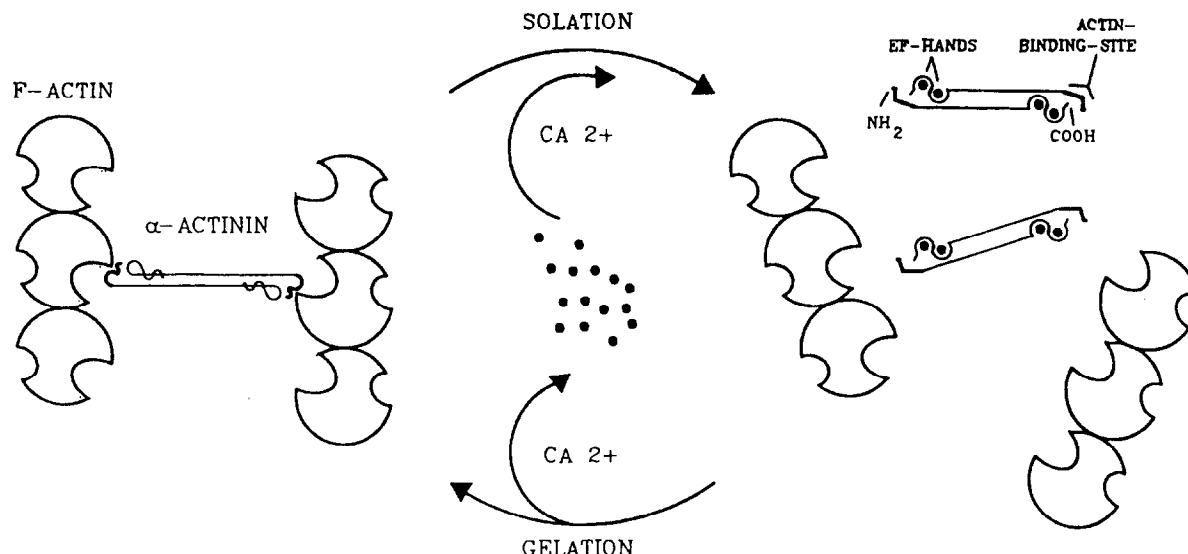


Fig.3. Proposed model for the putative interaction between the calcium- and actin-binding sites in *D. discoideum* α -actinin. The α -actinin molecule is drawn as a homodimer, whose anti-parallel subunits form a rod-shaped molecule. The binding site to F-actin resides in the N-terminal portion of the polypeptide (bold region), and the EF-hand structures at the C-terminus. In analogy to studies based on the crystal structures of troponin C [26] and calmodulin [27], the calcium-binding domains of α -actinin may wrap around the central part of the α -actinin subunit provided calcium is not present. Following an increase in the internal calcium concentration each EF-hand structure binds one Ca^{2+} and changes its conformation by folding back from the central part of the subunit, thus disturbing the interaction of the actin-binding site of the neighbouring subunit with F-actin.

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