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Primary structure of the cDNA 5′-terminal region encoding the N-terminal domain of the rabbit muscle α-actinin subunit

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A number of cDNA clones have been obtained in summary encoding the N-terminal domain containing 286 amino acid residues of the rabbit skeletal muscle α-actinin subunit. α-Actinin cDNA clones were isolated from specific cDNA libraries using the primer extension method for synthesis of the first cDNA chain. A strong stop signal for AMV reverse transcriptase in the 5′-terminal region of mRNA of α-actinin was found. It seems there is a G+C rich region (93% G+C nucleotides) including a continuous sequence of 23 G and C nucleotides encoding 6 glycine residues.

α-Actinin; Rabbit skeletal muscle; 96-Fold degenerate oligonucleotide mix; AMV reverse transcriptase strong stop signal

1. INTRODUCTION

α-Actinin is a minor protein of contractile systems. It connects F-actin strands by cross-bridges and enhances ATPase activity and superprecipitation of actomyosin in vitro [1,2].

α-Actinin consists of 2 identical subunits (M_r of each is about 100 kDa) [3]. During mild trypsinolysis each of the subunits divides into 2 parts, the N-terminal monomer (M_r ~ 30 kDa) and the C-terminal dimer (M_r ~ 70 kDa of each chain). The N-terminal domain of each subunit is able to bind F-actin [4–7].

The primary structures of α-actinins from different objects have been published [8–12]. In this work we present the primary structure of the N-terminal domain of rabbit muscle α-actinin deduced from the nucleotide sequence of cDNA. Cloning of this cDNA was carried out as in [13] by creation of specific cDNA libraries.

The strong stop signal for AMV reverse transcriptase has been found during synthesis of α-actinin cDNA. As in [13] this signal seems to be the G+C rich region of mRNA (in this case richer in G+C content than in [13]). To overcome this problem we used, as in [13], the specific oligonucleotide primer just before the G+C rich region of mRNA.

2. MATERIALS AND METHODS

2.1. *Materials*

We used dNTP (Pharmacia), restriction endonucleases, the 5′-end-labelling kit (Boehringer Mannheim), [^{3}H]dCTP (32 Ci/mmol), RNAsin, *E. coli* DNA polymerase I, ribonuclease H, T_4 DNA polymerase, T_4 DNA ligase, M13 cloning kit, M13 sequencing kit, terminal deoxynucleotidyltransferase (Amersham) and avian myeloblastosis virus reverse transcriptase (Vostok, USSR).

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2.2. *Synthesis of oligonucleotides*

Oligonucleotides I and II were synthesized in accordance with the primary structure of rabbit muscle α-actinin fragments [5], and oligo III in accordance with the sequence, determined in this work, of α-actinin cDNA (Fig. 1). Synthesis was performed on the Beckman System I DNA synthesizer.

2.3. *Isolation of α-actinin cDNA clones*

Specific libraries were created and screened as in [13], using oligo I and oligo III as primers and oligo II as a probe. The *Pst*I–*Eco*RI restriction α-actinin cDNA fragment from plasmid pTZ-ARI-3a (Fig. 2: fr727) and the short DNA fragment obtained by PCR. Amplification as in [14] was done using single strand M13mp10 with insertion of the fr727 as a matrix, and reverse M13 primer and oligo III as primers.

2.4. *Nucleotide sequence determination*

DNA sequencing was carried out on both strands by the dideoxy chain termination method [15]. Oligo II (96-fold degenerated oligonucleotide mix) was used as a sequencing primer.

3. RESULTS AND DISCUSSION

Oligos I and II were designed according to the primary structure fragments of α-actinin previously determined [5]. Using oligo I as a primer we created a specific library of 1500 clones and screened it using the probe oligo II (Fig. 1 and 2). Only the clone containing the 727 bp α-actinin cDNA (fr727) was isolated and sequenced as in [15]. The use of degenerated oligonucleotide mixes (up to 64-fold) for DNA sequencing has been previously published [16]. We successfully used the 96-fold degenerated oligonucleotide mix oligo II as sequencing primer (data not shown).

After screening several specific cDNA libraries (total

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Oligonucleotides		Sequence	
Oligo I, 17 mer.		Asn Gln Glu Asn Glu Lys	
32-fold degen.	3′	TTP-GTQ-CTQ-CTP-CTQ-TT	5′
Oligo II, 17 mer.		Phe Ala Ile Gln Asp Ile	
96-fold degen.	5′	TTQ-GCN-ATM-CAP-GAQ-AT	3′
Oligo III,		Ile Glu Glu Asp Phe Arg	
17 mer.	3′	AG-CTC-CTC-CTG-AAG GCG	5′

Fig. 1. Amino acid sequence of protein fragments of rabbit muscle α-actinin and the oligonucleotides encoding them. Q=C or T; P=A or G; N=T, C, G or A; and M = C, T or A.

3000 clones), created in the same manner as before, 20 positive clones were isolated (Fig. 2d: clones series pUC-ARI). All these clones had α-actinin coding sequences with lengths shorter than the first cloned fragment fr727, and synthesis of 15 was interrupted near the same point as the fr727. We assumed that interruption of AMV reverse transcriptase synthesis of cDNA was due to the G+C rich region analogous to that observed in [13]. The oligo III, complementary to mRNA of α-actinin, was synthesized (Figs 2 and 3). The distance between oligo III and the 5′-terminus of fr727 was 87 bases. The specific cDNA was synthesized using this primer, then cloned and screened by the short cDNA fragment (~130 bp) containing the 5′-terminal 87 bp of fr727 obtained by PCR. Eight α-actinin cDNA clones were isolated from the library of only 200 clones. The lengths of cDNA of these clones ranged from ~200 bp to ~250 bp (Fig. 2e; clones series pUC-ARIII). After sequencing these fragments the primary structure of the whole N-terminal domain was derived. The initiator Met was determined as the first one encountered in the sequence. The length of the established amino acid sequence is in good accordance with the lengths of tryptic peptides in [5]. In total, the sequence of 286 amino acid residues of the rabbit muscle α-actinin N-terminus was determined (Figs 2b and 3).

Analysis of the nucleotide sequence behind the point of interruption of synthesis of the first chain cDNA shows a G+C rich sequence of this region (95% G+C nucleotides) including a continuous sequence of 23 G+C bases, encoding 6 Gly residues (Fig. 3). It seems that the regions with a high content of G+C bases are often a strong stop signal during cDNA synthesis [13]. Using oligonucleotide primers complementary to the mRNA just prior to the G+C rich regions solves these problems [13].

The deduced amino acid sequence of rabbit muscle α-actinin is in good accordance with the data on the N-terminal amino acid sequence of tryptic fragments T_1–T_9 of α-actinin obtained in the work of Simonidze et al. [5].

A comparison of the primary structure of the N-terminal domain of rabbit muscle α-actinin in this work with the primary structures from other objects [8–12] revealed the high homology of these proteins with the exception that the N-terminal sequence (from the first to the twenty-fourth amino acid residue) is completely different. This is probably connected with the species or tissue specificity of the given kind of α-actinin.

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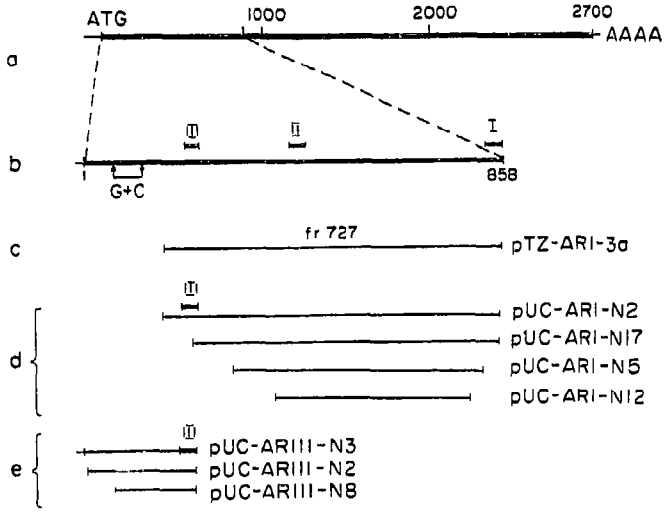

Fig. 2. (a) Scheme of poly(A)$^+$ mRNA of rabbit muscle α-actinin. (b) Scheme of the cDNA 5′-terminal region encoding the N-terminal domain of the rabbit muscle α-actinin subunit. Arrows indicate G+C rich region. I, II, III are regions corresponding to oligonucleotides I, II and III. (c) The α-actinin 727 bp cDNA fragment from the pTZ-ARI-3a clone. (d) α-Actinin cDNA from clones series pUC-ARI. Four clones of the 20 studied are shown. (e) α-Actinin cDNA from clone series pUC-ARIII. Three clones of the 8 studied are shown.

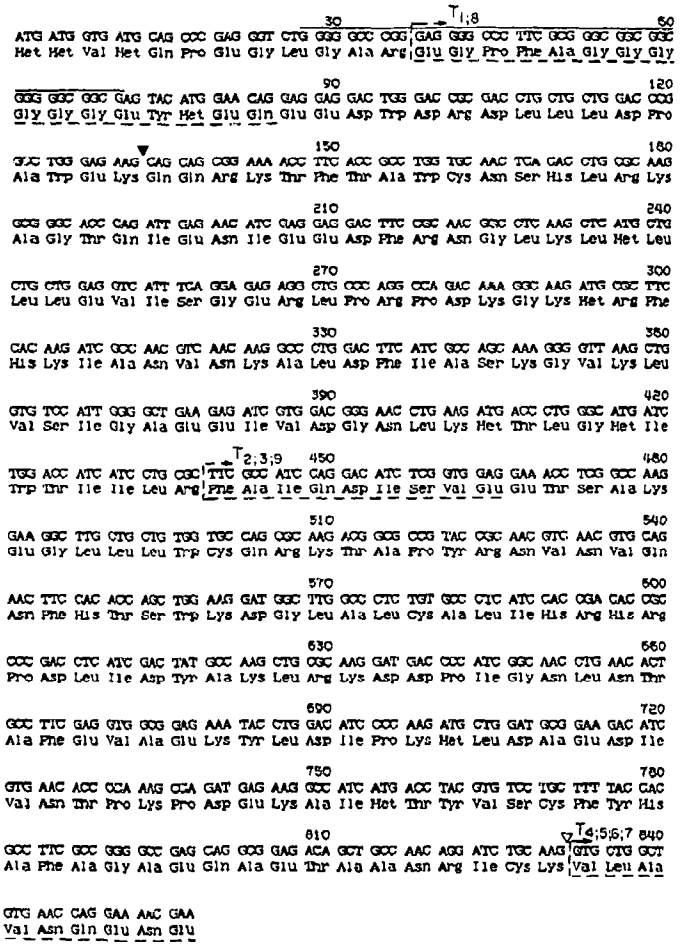

Fig. 3. Primary structure of the 5'-terminal region cDNa encoding the N-terminal domain of the rabbit muscle α-actinin subunit. ▼ = 5'-end of cDNA fragment of clone pTZ-ARI-3a; ▽ = Site of α-actinin cleaving at mild tryptic hydrolysis (C-terminal end of the N-terminal domain [9]). Dashed lines indicate nucleotide sequences encoding the N-terminal part of the T_1–T_9 tryptic fragments [9]. The continuous line above the sequence shows the G+C rich region (93% G+C).

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