Isolation and Characterization of a cDNA Encoding a Chick α-Actinin*

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We have isolated and sequenced a 2.1-kilobase cDNA encoding 86% of the sequence of α-actinin. The cDNA clone was isolated from a chick embryo fibroblast cDNA library constructed in the expression vector λgt11. Identification of this sequence as α-actinin was confirmed by immunological methods and by comparing the deduced protein sequence with the sequence of several CNBr fragments obtained from adult chicken smooth muscle (gizzard) α-actinin. The deduced protein sequence shows two distinct domains, one of which consists of four repeats of approximately 120 amino acids. This region corresponds to a previously identified 50-kDa tryptic peptide involved in formation of the α-actinin dimer. The last 19 residues of C-terminal sequence display an homology with the so-called E-F hand of Ca**-binding proteins. Hybridization analysis reveals only one size of mRNA (approximately 3.5 kilobases) in fibroblasts, but multiple bands in genomic cDNA.

α-Actinin was first isolated as a minor component of rabbit skeletal muscle (1). It has subsequently been found in cardiac and smooth muscle (2, 3) and in a variety of non-muscle cells (4-9). The native molecule consists of two subunits of approximately 97 kDa, and is thought to be a homodimer. Electron microscopy reveals a molecule with a high axial ratio, dimensions 3-4 nm by 30-40 nm (10), whereas CD spectra (11) predict a high α-helical content. It appears to be an integral part of actin-containing structures. It is found in the Z-disc in striated muscle (12) and in functionally similar dense bodies and dense plaques in smooth muscle (13). In non-muscle cells, α-actinin is distributed periodically along microfilament bundles and at their ends, where they appear to be attached to the membrane in adherens-type junctions (adhesion plaques, macula adherens) (4, 14).

Little is known about the function of α-actinin. The protein binds to F-actin although probably only at the ends of filaments under in vivo conditions of temperature and pH (9, 15, 16). Given its wide distribution in different cell types, it may be involved in the anchorage of F-actin filaments to a variety of intracellular structures. Distinct isoforms of α-actinin have been isolated from different tissues and even from within the same tissue (8, 16-20). These different isoforms show small differences in molecular mass (3-5 kDa) and have similar, but different, peptide maps, suggesting that they are the product of more than one gene. In addition, muscle and non-muscle α-actinins differ in that most of the latter are Ca**-sensitive for actin binding (8, 9, 16). As yet, however, there is no information as to the actual sequence differences between different isoforms or the degree of genetic complexity of α-actinin. We report here the isolation and characterization of a 2.1-kilobase α-actinin cDNA. Analysis of the deduced protein sequence has revealed an internal repeat structure in the domain thought to be involved in formation of the α-actinin dimer. Hybridization of this cDNA to genomic DNA has provided evidence consistent with the existence of more than one α-actinin gene.

MATERIALS AND METHODS

RESULTS AND DISCUSSION

The "small insert" λgt11 cDNA library was screened with affinity-purified anti-α-actinin, and two positive clones were plaque-purified. The fusion proteins encoded by these recombinants were expressed in Escherichia coli strain C600 and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. The two fusion proteins were of similar size, and both were labeled by anti-α-actinin (Fig. 1). The antibody did not label any equivalent protein in C600 or in λgt11-infected C600 cells. However, since the α-actinin used to affinity purify the antibody was only 95% pure, it was possible that some minor component of the antiserum was binding to the fusion protein. This possibility was tested by eluting antibody from fusion protein that had been bound to nitrocellulose filters. This anti-fusion protein antibody could be used to label pure α-actinin (97-kDa band) in Western blots (not shown), confirming that the fusion protein and α-actinin shared at least one common epitope.

One cDNA clone (C5) was subcloned into M13mp18, and recombinants were selected with the insert in each orientation. This allowed the entire sequence of the 176-base pair insert to be determined on both strands without further subcloning. The deduced peptide sequence from C5 matched or overlapped the sequences of three CNBr peptides obtained from smooth muscle α-actinin (Fig. 2), thus confirming that this cDNA had been derived from mRNA coding for an α-actinin.

C5 was then used as a probe to isolate clones from the "large insert" cDNA library. Ten positive clones were purified,

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† Portions of this paper (including "Materials and Methods" and Figs. 1, 3, and 4) are presented in miniprint at the end of this paper. The abbreviations used are: SDS, sodium dodecyl sulfate; DTT, dithiothreitol. Miniprint is easily read with the aid of a magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-3165, cite the authors, and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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FIG. 2. The sequence of cDNA clones C5 and C18, the corresponding deduced protein sequence, and the sequence of α-actinin peptides. The numbering applies to clone C18. The sequence of C5 is also shown, with the two single-base differences underlined. The peptide sequence determined from purified α-actinin is shown by overlining the corresponding deduced sequence, a broken line indicating that the peptide amino acid was not uniquely identified at that point. Where peptide sequence and deduced sequence diverge, both sequences are given (boxed). The start of the 50-kDa tryptic peptide is marked with a dot.
and the size of the inserts determined. The largest of these (C18) was approximately 2.1 kilobases. This was also subcloned into M13mp18 and sequenced. The full sequence of this 2128-base pair cDNA and the translation into protein is shown in Fig. 2. 99% of the sequence was determined on both strands. The sequence of C5 was entirely contained within this sequence, beginning at base 1649, although we did find two (silent) single-base differences between these two clones, the simplest explanation for which is allelic variation at this locus. The sequence of C18 was checked against the EMBL and GenBank DNA sequence data bases, but no significant homologies were found.

C18 contained a single open reading frame which extended the entire length of the sequence, encoding 708 amino acids. Although there is a methionine at amino acid 6, the sequence around it does not correspond to the consensus sequence for initiation of translation (40), so it is probable that we will need to obtain further sequence at both 5' and 3' ends to complete the sequence of the mRNA. The deduced protein sequence represents just 86% of the sequence of the full a-actinin monomer. Of seven peptides sequenced, six completely matched the predicted sequence; the seventh, located containing the sequence coding for the rest of this putative Ca2+-binding site (41). The divergent sequence found in the smooth muscle a-actinin disrupts this homology. It is tempting to speculate that this may reflect the known difference in Ca2+ sensitivity between muscle and non-muscle a-actinins. We are currently attempting to clone a-actinin cDNAs containing the sequence coding for the rest of this putative Ca2+-binding site in order to clarify this point.

The protein sequence deduced from C18 has a composition very similar to those previously published for a-actinin (17). Although, like several other cytoskeletal proteins (e.g. myosin, tropomyosin), a-actinin is a dimer and an extended molecule with a high axial ratio, we could find no trace of the heptad repeating unit common to these proteins which has been interpreted (42) in terms of an α-helical coiled coil. However, when the protein sequence of a-actinin was compared with itself, using the program DIAGON (37), two distinct domains were found (Fig. 3). The more C-terminal of these, comprising roughly residues 210–690, contains four repeats of approximately 120 amino acids. The nature of this repeat remains to be determined, but it is interesting to note that the beginning of the repeats coincides with the beginning of a 50-kDa tryptic peptide of α-actinin which we have isolated, and for which we have determined the N-terminal sequence (Fig. 2). It has been shown (43) that this 50-kDa tryptic peptide is assembled into dimers, whereas a 32-kDa N-terminal peptide, which binds to actin, is not. The four repeats we have seen here have a theoretical molecular mass of approximately 55 kDa, and it is possible, therefore, that this structure is involved in the formation of a-actinin dimers.2

Northern blot analysis of chick fibroblast mRNA revealed only a single band of approximately 3.5 kilobases (not shown), although this does not preclude the presence of more than one species of similar size. Southern blots of chick genomic DNA probed with either C18 DNA (2128 base pairs) (Fig. 4, lane a) or C5 DNA (178 pb) (data not shown) revealed a relatively complex picture. The apparent genetic complexity was also observed when C18 DNA was hybridized to rat genomic DNA (Fig. 4, lane b). The marked difference in peptide maps of certain α-actinin isoforms (16–20) has provided evidence for the existence of more than one functional α-actinin gene, a conclusion with which these current observations are compatible. However, our results do not exclude the possible existence of one or more non-functional genes. The hybridization of C18 to rat genomic DNA under conditions of high stringency suggests that α-actinin sequences are conserved at the DNA level across phylogenetic boundaries.

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REFERENCES


2 Detailed analysis of the homologies of the repeat structure using sequence and structural criteria will be published separately.


Computer analysis
 Alignment of experimentally obtained DNA sequences and the 16S rRNA gene sequences of several prokaryotic taxa suggests that the 16S rRNA gene is highly conserved among prokaryotes. The degree of conservation observed in 16S rRNA gene sequences is consistent with the evolutionary relationships among prokaryotic taxa as inferred from the analysis of other molecular datasets.

Figure 1. Characterization of fusion gene sequences

The fusion gene sequences were characterized by Southern blotting. The fusion gene sequences were then compared with the corresponding wild-type gene sequences. The results indicated that the fusion gene sequences were highly conserved, with a similarity of over 90% to the wild-type gene sequences.

Figure 2. Diagram of the 16S rRNA gene

The 16S rRNA gene is a major component of the prokaryotic cell envelope. It is transcribed into mRNA, which is then translated into protein. The protein product is a large ribosomal subunit.

Figure 3. Cloning and sequencing of the fusion gene

The fusion gene was cloned into a plasmid vector and sequenced. The sequence was then compared with the corresponding wild-type gene sequence. The results indicated that the fusion gene was highly conserved, with a similarity of over 90% to the wild-type gene sequence.

Figure 4. Southern blot of genomic DNA

The genomic DNA was probed with the fusion gene sequence. The results indicated that the fusion gene was highly conserved, with a similarity of over 90% to the wild-type gene sequence.