α1-Syntrophin has distinct binding sites for actin and calmodulin

Yuko Iwata, Yan Pan, Tomokazu Yoshida, Hironori Hanada, Munekazu Shigekawa*

Department of Molecular Physiology, National Cardiovascular Center Research Institute, Fujishiro-dai 5, Suita, Osaka 565, Japan

Received 19 December 1997

Abstract Overlay and co-sedimentation assays using recombinant α1-syntrophin proteins revealed that two regions of α1-syntrophin, i.e. aa 274–315 and 449–505, contain high-affinity binding sites for F-actin (K_d 0.16–0.45 μM), although only a single high-affinity site (K_d 0.35 μM) was detected in the recombinant full-length syntrophin. We also found that actomyosin fractions prepared from both cardiac and skeletal muscle contain proteins recognized by anti-syntrophin antibody. These data suggest a novel role for syntrophin as an actin binding protein, which may be important for the function of the dystrophin-glycoprotein complex or for other cell functions. We also found that α1-syntrophin binds calmodulin at two distinct sites with high (K_d 15 nM) and low (K_d 0.3 μM) affinity.

Key words: Syntrophin; Actin binding; Calmodulin binding; Dystrophin; Cardiac muscle; Skeletal muscle

1. Introduction

Syntrophin is a peripheral membrane protein first identified in the postsynaptic membrane of *Torpedo* electric organ [1]. Subsequently, it was found to be associated with dystrophin, the protein product of the Duchenne muscular dystrophy gene [2–4]. In skeletal muscle, dystrophin and syntrophin are present at the peripheral sarcolemma and neuromuscular junction, whereas they are associated with the peripheral sarcolemma and T-tubule membranes in cardiac muscle [5–7]. In these striated muscles, dystrophin and syntrophin form a tight complex with other dystrophin-associated proteins, many of which are integral membrane glycoproteins [5,8]. The function of the dystrophin complex is not known, but it is thought to mechanically stabilize sarcolemma by linking the actin cytoskeleton with the extracellular matrix, since dystrophin and α-dystroglycan, an extracellular component of the complex, bind actin and laminin, respectively [9–11].

The syntrophins are a multigene family of homologous proteins comprising three isoforms, α1, β1, and β2, which share ~50% amino acid identity [4,12]. α1-Syntrophin is expressed primarily in striated muscle, whereas β-syntrophins are ubiquitous in mammalian tissues. All known syntrophins contain two pleckstrin homology (PH) domains, a PDZ domain homologous to the postsynaptic density 95 kDa protein, and the syntrophin unique domain (SU) [4,12]. The PDZ domain is inserted into and interrupts the first PH domain. This domain structure suggests that syntrophins may associate with the membrane or membrane cytoskeleton and play an important role in the signal transduction involving the dystrophin-glycoprotein complex or related structures. However, the precise function of syntrophins is currently unknown.

Recently syntrophin has been reported to interact with several proteins. It binds to a protein sequence derived from exons 73 and 74 of dystrophin as well as to homologous segments of utrophin and dystrobrevin [2–4]. Syntrophin self-associates or associates with other syntrophin [13]. It interacts with nitric oxide synthase via its PDZ domain in skeletal muscle [14] and has been reported to bind CaM [15]. In the present study, we present evidence that F-actin binds to syntrophin. We identified the binding sites for actin and CaM within the α1-syntrophin molecule.

2. Materials and methods

2.1. Plasmid construction, and protein expression and purification

For the isolation of α1-syntrophin cDNA, poly(dT)$_{13}$ primed first strand cDNA was synthesized using a SuperScript reverse transcriptase (Gibco BRL) from total RNA (2 μg) prepared from rabbit skeletal muscle and then amplified by PCR using the following primers: sense, 5′-TCGGTTGCGACCCGGGGGCTC-3′ (nt −31 to −11 of α1-syntrophin [16]) and antisense, 5′-GGCTCTCTGAGCACCTCA-3′ (nt 1623–1644 of α1-syntrophin [16]). The PCR product was then cloned into the pGEM-T vector (Promega) and its DNA sequence determined. A series of MBP or GST fusion protein constructs (see Fig. 2A) were generated by PCR using α1-syntrophin cDNA as a template and appropriate primers. Except for the primers described below, sense primers were designed to exogenously contain a *Bam*HI restriction site, whereas antisense primers were designed to contain an in-frame stop codon (TAA) and an exogenous *Sal*I or *Xho*I site. In the case of G178–273 and G178–505 fusion protein constructs, an endogenous *Eco*RI site of rabbit cDNA was used as the sense primer. For a Δ316–365 fusion protein construct, two PCR products corresponding to aa 274–315 and aa 366–448 were first generated using an antisense primer exogenously containing a *Hin*dIII restriction site (for aa 274–315) and a sense primer exogenously containing a *Hin*dIII restriction site (for aa 366–448) and then they were ligated with each other. After restriction enzyme digestion, all these fusion protein constructs were inserted into the *Bam*HI-*Sal*I site of pMAL-c vector (New England Biolabs) or the *Bam*HI-*Sal*I or *Eco*RI-*Xho*I site of pGEX-4T3 vector (Pharmacia). Soluble fusion proteins were purified from transformed *Escherichia coli* (HB101 strain from Takara) according to the manufacturer's protocol.

2.2. Preparation of actomyosin and actin

Actomyosin from hamster ventricle was isolated from the post-microsomal supernatant from high salt extract of muscle homogenates. Briefly, ventricle (0.2 g) was homogenized for 30 s three times with Physcotron NS-10 at 25 000 rpm in 2 ml of buffer A (0.6 M NaCl and 10 mM $NaHCO_3$ containing a mixture of protease inhibitors [17]), kept for 15 min at room temperature, and then centrifuged at 5500×*g* for 20 min. The supernatant was recentrifuged at 100 000×*g* for 40 min. The supernatant was then diluted 10-fold with ice-cold H_2O and the resultant pellet was solubilized with buffer A. Actomyosin-containing fractions were purified by repeating this dilution/solubilization procedure three times. The actomyosin fraction was also prepared from hamster skeletal muscle by the same method. Purified actin was prepared from rabbit skeletal muscle acetone powder as described by Spudich and Watt [18]. This actin was used in all experiments except for the one shown in Fig. 1. For the latter, we used actin prepared from hamster ventricle muscle acetone powder.

*Corresponding author. Fax: (81) (6) 872-7485.
E-mail: shigekaw@ri.ncvc.go.jp

Abbreviations: MBP, maltose binding protein; GST, glutathione *S*-transferase; CaM, calmodulin

PII S0014-5793(98)00085-4

2.3. F-actin co-sedimentation assay

F-actin (2.5 μM) was incubated with various concentrations of GST or MBP fusion proteins in 60 μl of actin binding buffer (2 mM Tris-HCl, pH 8.0, 1.0 mM ATP, 0.2 mM $CaCl_2$, 2 mM $MgCl_2$, 100 mM NaCl, and 0.5 mM β-mercaptoethanol) for 30 min at room temperature. For the full-length syntrophin fusion protein (M1–505), 0.5% Triton X-100 was included in actin binding buffer to avoid protein aggregation. The samples were then subjected to centrifugation at 100 000 × *g* for 40 min. Supernatants were carefully decanted and pellets redissolved in an equivalent volume of actin binding buffer. To quantify the fusion proteins bound to F-actin, equal volumes of aliquots were taken from the supernatants and resuspended pellets, respectively and were subjected to SDS-PAGE on a 9% gel. CBB-stained gels were analyzed on a densitometer (Pharmacia LKB, Ultroscan II).

2.4. Other procedures

Immunoblot analysis and blot overlay assay were performed as previously described [19]. In the latter assay, bound fusion proteins or actin were detected with anti-MBP or anti-actin antibody, respectively. Binding of fusion proteins to CaM-Sepharose beads and fluorescence measurement of dansyl-CaM were measured as described in detail previously [20]. Data fitting was performed by least squares analysis using the GraphPad Prism software.

2.5. Antibodies and other materials

Monoclonal anti-syntrophin antibody SYN1351, which recognizes all three isoforms of syntrophin, was a gift from Dr. Stanley C. Froehner [21]. Preparation of polyclonal anti-β-dystroglycan (pAb43) antibody was described in [19]. We also used anti-dystrophin (NCL-DYS2) and anti-α-actin (BioMakor) monoclonal antibodies and anti-GST (Sigma) and anti-MBP (New England Biolabs) polyclonal antibodies. Peptides corresponding to aa 87–105 and aa 106–126 of α1-syntrophin were synthesized. These peptides were >95% pure as determined by high pressure liquid chromatography.

3. Results

We isolated an actomyosin fraction from post-microsomal supernatants from high salt extracts of hamster ventricular muscle homogenates. This fraction predominantly contained myosin (~200 kDa) and actin (~40 kDa), but other proteins were also present as seen in the CBB-stained gel (Fig. 1). Immunoblot analysis revealed that this fraction contained 57 and 60–62 kDa proteins recognized by anti-syntrophin antibody (Fig. 1, IB:syn). These proteins have electrophoretic mobilities equivalent to those of syntrophins present in the dystrophin-glycoprotein complex purified from hamster ventricular muscle [19]. In the same actomyosin fraction, however, no protein band reactive with anti-dystrophin or anti-β-dystroglycan antibody was detected (data not shown), indicating that the dystrophin-glycoprotein complex is not present in this fraction. We obtained essentially the same results with the actomyosin fraction obtained from hamster skeletal muscle

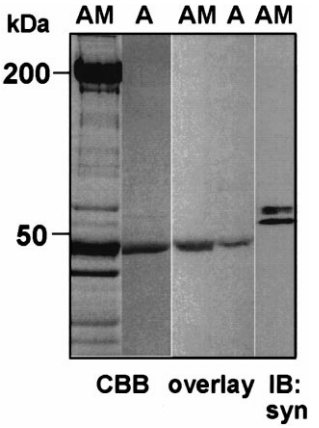

Fig. 1. Syntrophin binds actin in actomyosin. Actomyosin fraction (AM) and actin (A) purified from hamster ventricle muscle were subjected to SDS-PAGE on a 9% gel followed by blot overlay assay with a MBP fusion protein (M1–505) (see Fig. 2A) or by immunoblot analysis with anti-syntrophin antibody.

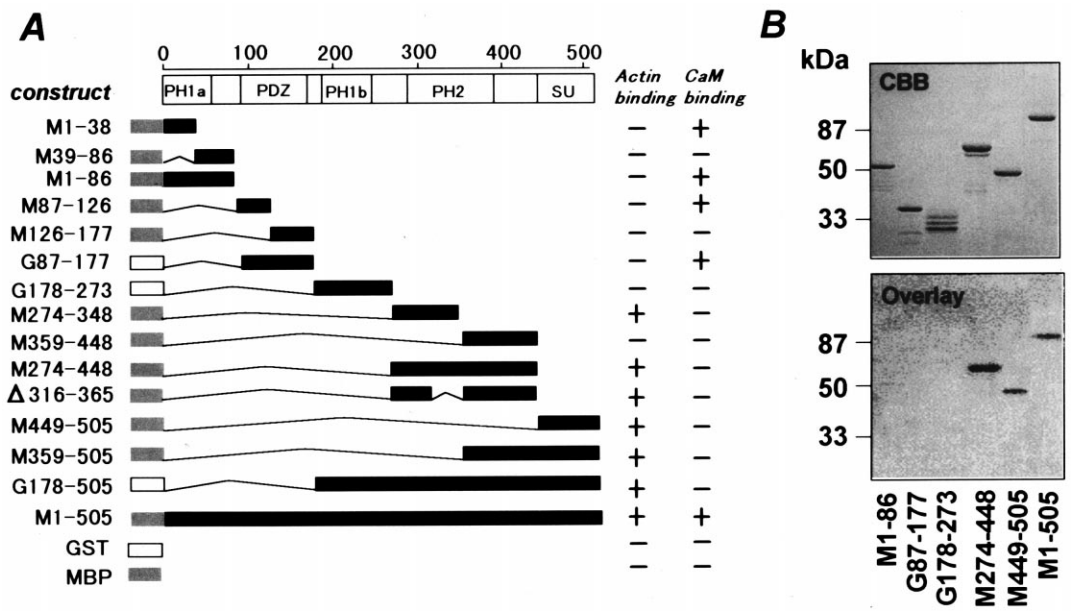

Fig. 2. Schematic representation of fusion protein constructs and overlay assay of their actin binding. A: Syntrophin sequences produced as MBP or GST fusion proteins are shown together with the domain structure of α1-syntrophin. Numbers represent those for the amino acids of α1-syntrophin. The results from co-sedimentation assay for actin binding and from CaM-Sepharose binding assay are summarized on the right for each fusion protein. B: Typical SDS-PAGE patterns for representative fusion proteins stained with CBB (CBB) and the results of overlay assay with 5 μg/ml F-actin (Overlay) are shown.

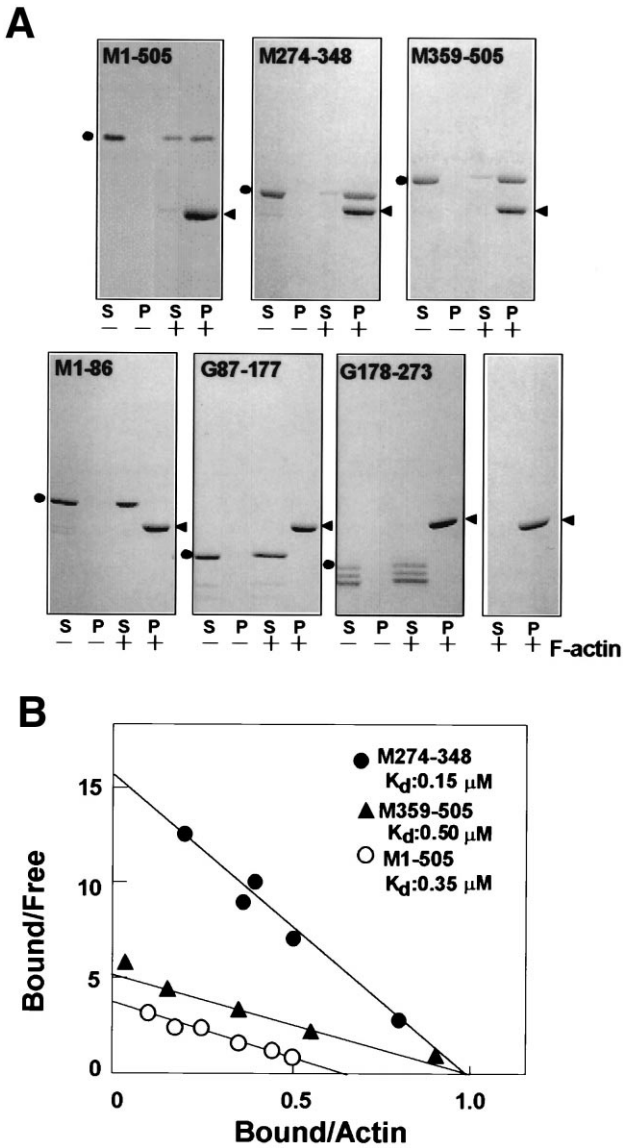

Fig. 3. Co-sedimentation of syntrophin fusion proteins with F-actin. A: Typical CBB-stained gel patterns of the supernatants (S) and the pellets (P) that were obtained by centrifugation of syntrophin fusion proteins (0.2 mg/ml each) incubated with or without 4 μM F-actin. The result obtained with F-actin alone is also shown in the lower right panel. The positions of fusion protein (●) and actin (◀) are indicated. B: Scatchard plots for binding of MBP1–505 (0.3–3 μM), MBP274–448 (0.3–2.7 μM) and MBP359–505 (0.5–3 μM) to F-actin (2.5 μM). The amounts of free and bound proteins were quantified by densitometry of CBB-stained gels (see A).

(data not shown). We found that a recombinant full-length α1-syntrophin specifically bound to a protein of 40 kDa in the actomyosin fraction (Fig. 1, overlay). This 40 kDa protein is actin, because the same fusion protein bound to actin purified from cardiac (Fig. 1, overlay) or skeletal muscle (data not shown) and because conversely actin bound to the fusion protein (Fig. 2B, M1–505). These data provide evidence that α1-syntrophin binds actin.

We used GST or MBP fusion proteins containing various portions of α1-syntrophin to define actin binding sites in this protein. The fusion protein constructs used are schematically shown in Fig. 2A along with functional domains of α1-syntrophin proposed previously [4,12]. Fig. 2B shows CBB staining of representative fusion proteins after SDS-PAGE (CBB) and the results of the overlay assay with F-actin (Overlay). Actin bound to the fusion proteins containing the PH2 (M274–448) or SU domains (M449–505), whereas it did not interact with fusion proteins containing PH1a, PDZ, or PH1b domains (M1–86, G87–177, or G178–273). GST or MBP protein alone did not associate with actin (data not shown).

F-actin co-sedimentation assay confirmed these results. M1–505, M274–348 or M359–505 was specifically co-precipitated with F-actin (Fig. 3A). Fig. 3B shows Scatchard plots of the binding of these fusion proteins to F-actin. From the observed linear plots, single classes of binding site with maximal binding of 0.60 ± 0.09, 0.97 ± 0.04 and 1.04 ± 0.06 mol/mol actin ($n = 3$) and apparent dissociation constants (K_d) of 0.35 ± 0.10, 0.16 ± 0.01 and 0.45 ± 0.07 μM ($n = 3$) were estimated for M1–505, M274–348 and M359–505, respectively. Thus these fusion proteins bind actin at a stoichiometry of about 1:1 with high affinity, although the stoichiometry for M1–505 is smaller (0.6). It appears that only a single site is available in the recombinant full-length syntrophin. We performed F-actin co-sedimentation assay of fusion proteins containing shorter segments of M274–348 and M359–505 and the results are summarized in Fig. 2A together with those obtained with other fusion proteins. We found that Δ316–365, but not M359–448, binds F-actin and that M449–505 also binds F-actin (Fig. 2A, data not shown). These data indicate that the N-termini of the PH2 domain (aa 274–315) and the SU domain (aa 449–505) of α1-syntrophin contain binding sites for F-actin (Fig. 2A).

Many actin binding proteins contain CaM binding sites. Using the above fusion proteins, we tested whether α1-syntrophin binds CaM. Fusion proteins containing full-length α1-syntrophin (M1–505), PH1a (M1–86), and PDZ domain (G87–177) bound to CaM-Sepharose in the presence of Ca^{2+} and then were eluted with EGTA (Fig. 4A). We also analyzed binding of CaM to fusion proteins by measurement of fluorescence emission spectra of dansyl-CaM. M1–505, M1–86, and G87–177 increased the dansyl-CaM fluorescence with a concomitant blue shift of spectrum only in the presence of Ca^{2+} (Fig. 4B and data not shown). In contrast, G178–273, M274–448, and M449–505 did not bind to CaM-Sepharose or dansyl-CaM (Fig. 4A and data not shown).

To localize the CaM binding sites more precisely, shorter segments of M1–86 and G87–177 were tested for their binding to CaM-Sepharose and the results are summarized in Fig. 2A together with data obtained with other fusion proteins. We found that M1–38 and M87–126 bound to CaM-Sepharose and also increased dansyl-CaM fluorescence (data not shown). Fig. 4C shows Scatchard plots of the binding of M1–38 and M87–126 to dansyl-CaM as monitored by an increase in the fluorescence intensity in 0.1 mM $CaCl_2$. M87–126 bound CaM with high affinity (K_d 15 nM) (Fig. 4C). A similar K_d value of 20 nM was obtained from another experiment. With M1–38, the maximal fluorescence was not reached even at the highest concentration used (2 μM) (data not shown), indicating its low affinity for CaM. If we assume that the maximal fluorescence induced by M1–38 was the same as that by M87–126, K_d values of 0.30 and 0.33 μM for CaM could be estimated with M1–38 in two different experiments. To further narrow down the CaM binding site in M87–126, we prepared two synthetic peptides (peptides 87–105 and 106–126) based

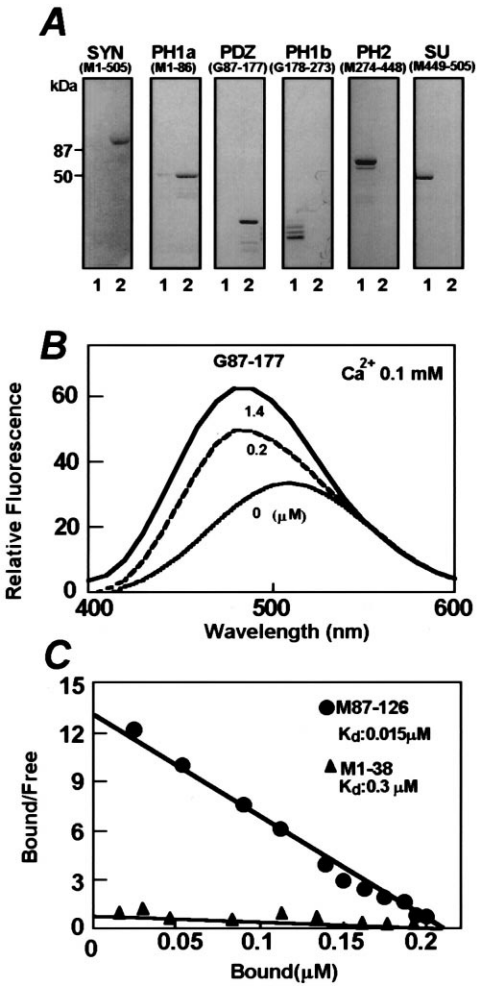

Fig. 4. CaM binding assay of fusion proteins. A: CaM-Separose beads (100 μl) were incubated with fusion proteins (10 μg each) in 100 μl of buffer containing 0.1 mM $CaCl_2$, 10 mM HEPES/Tris (pH 7.2) and 100 mM NaCl and then washed extensively. Proteins were subsequently eluted from beads with 100 μl of buffer containing 5 mM EGTA. The flow through fraction (20 μl) (lane 1) and the EGTA eluate (20 μl) (lane 2) were subjected to SDS-PAGE and stained with CBB. B: Fluorescence emission spectra of 0.2 μM dansyl-CaM in the presence of 0.1 mM $CaCl_2$ and either 0, 0.2 and 1.4 μM M87–177 measured with excitation at 340 nm. C: Scatchard analysis of binding of syntrophin fusion proteins to dansyl-CaM as monitored by an increase in the fluorescence intensity at 490 nm as previously described [20].

on the sequence of α1-syntrophin [16]. Peptide 106–126 increased fluorescence of dansyl-CaM in a concentration-dependent manner and K_d values of 15 and 16 nM, which are close to that for M87–126, were estimated for this peptide from the Scatchard analysis in two independent experiments (cf. Fig. 4C, data not shown). Peptide 87–105, however, did not increase the fluorescence of dansyl-CaM. Thus, a 20 amino acid segment in the PDZ domain (aa 106–126) and the N-terminus of the PH1a domain (aa 1–38) of α1-syntrophin were identified as high- and low-affinity CaM binding sites, respectively.

We measured binding of various concentrations of full-length α1-syntrophin fusion protein (M1–505) to dansyl-CaM and the results were analyzed as in Fig. 4C. Scatchard plots were biphasic and were fitted well by non-linear least squares analysis to a simple two-independent-site equation with K_d values of 0.02 and 0.3 μM for the two sites (data not shown). For CaM, therefore, two binding sites in aa 106–126 and aa 1–38 function independently in the recombinant full-length α1-syntrophin.

4. Discussion

The present results demonstrate that α1-syntrophin has high-affinity binding sites for F-actin and CaM. In skeletal and cardiac muscle, α1-syntrophin is known to be a component of the dystrophin-glycoprotein complex whose integrity is critically important for maintaining the viability of sarcolemma [22]. This is the first report providing evidence that α1-syntrophin binds actin isolated from either skeletal or cardiac muscle. The actin binding sites are within a short N-terminal segment containing part of a PH domain (aa 274–315) and a SU domain (aa 449–505) (Fig. 2A), and their binding affinities are 0.16 and 0.45 μM, respectively, which are comparable to or even higher than those of other actin-binding proteins such as dystrophin (K_d = 1–5 μM) [11,23], utrophin (K_d = 4 μM) [24], and α-actinin (K_d = 0.6 μM) [25]. Interestingly, these binding sites of α1-syntrophin do not exhibit homology to those from other actin binding proteins [26]. It is also interesting to note that only a single class of binding site for F-actin was detected in the fusion protein containing full-length syntrophin (Fig. 3B). Thus the two high-affinity actin binding sites described above do not work simultaneously in the full-length protein. To clarify how this happens, we need further experiments using both the recombinant and native syntrophin proteins.

We found by immunoblot analysis that the actomyosin fractions isolated from hamster skeletal and cardiac muscle contain more than one protein reactive with the anti-syntrophin antibody that is able to recognize all isoforms of syntrophin (Fig. 1 and Section 3). Since these proteins have electrophoretic mobilities equivalent to those recognized by the same antibody in the dystrophin-glycoprotein complex purified from the same tissue [19], it is likely that other syntrophin isoforms also bind actin. This interpretation is consistent with the fact that at least one of the deduced actin binding sites, i.e. the SU domain, is highly conserved in all three isoforms of syntrophin [4,12].

It has been widely postulated that the dystrophin-glycoprotein complex mechanically stabilizes sarcolemma by linking the cytoskeleton to the extracellular matrix (see Section 1). The N-terminal region of dystrophin has been shown to interact directly with F-actin [10,11,23]. Our present finding may thus suggest that syntrophin by binding actin also helps strengthen the linkage between the cytoskeleton and the extracellular matrix, because it is a component of the dystrophin-glycoprotein complex. On the other hand, the actomyosin fraction contained proteins immunoreactive with anti-syntrophin antibody as described above, whereas the same fraction contained no proteins reactive with anti-dystrophin or anti-β-dystroglycan antibody (Fig. 1 and see Section 3). Since only mild procedures were used to isolate the actomyosin fraction in this study (see Section 2), the result suggests that syntrophin may normally also occur in a pool of proteins containing actomyosin which is distinct from that containing the dystrophin-glycoprotein complex. This interpretation is consistent with our recent finding that by immunofluorescence

anti-syntrophin antibody produced a regular striation pattern overlapping the staining with anti-α-actinin antibody in longitudinal sections of hamster skeletal muscle (Y. Iwata et al., unpublished observation). This latter finding suggests that syntrophin colocalizes with α-actinin on the Z lines. Independent behavior of dystrophin and syntrophin (M_r 58 000 protein) was also noted previously by Butler et al. [6] when each was immunoisolated using a specific antibody from Triton X-100 extracts of *Torpedo* postsynaptic membranes.

We identified two CaM binding sites in α1-syntrophin, one (aa 106–126) with high affinity and localized within a PDZ domain and the other (aa 1–38) with low affinity and localized in a PH (PH1a) domain (Fig. 2A). These binding sites function independently in the recombinant full-length α1-syntrophin because the binding curve could be fitted by assuming two classes of independent binding site with K_d values of the above high- and low-affinity sites (see Section 3). Both these CaM binding sites have all the features expected of a typical CaM binding segment; by using the algorithm developed by Erickson-Viitanen and DeGrado [27], the hydrophobic moments of amino acid sequences aa 17–34 and aa 108–125 were calculated to be 0.64 and 0.66, respectively and the mean hydrophobicity of these segments to be −0.03 and −0.06, respectively. CaM binding to the sites in the PDZ and PH domains is interesting, because these modules may allow proteins containing these modules to associate with other membrane or cytosolic proteins directly or via an interaction with phosphatidylinositol lipids [28,29]. CaM could potentially modulate the function of these putative signaling modules in α1-syntrophin.

Using fusion proteins, Newbell et al. [15] have recently studied CaM binding to α1-syntrophin. They reported that an N-terminal region (aa 4–173) bound CaM both in the presence and absence of Ca^{2+} with relatively low affinity (K_d 100 nM), whereas a 24 aa segment at the C-terminus (aa 480–503) bound CaM with high affinity (K_d 20–30 nM). These data are not consistent with the present findings which show that the N-terminal region contains high- and low-affinity sites to which CaM binds in a strictly Ca^{2+}-dependent manner and that the fusion proteins containing the SU domain (M449–505) does not bind CaM. At present, we have no explanation for these differences.

Acknowledgements: We thank Drs. Hirofumi Onishi and Shin-ichirou Kojima for a generous gift of skeletal actin. We also thank Dr. Stanley C. Froehner for a gift of SYN1351. This work was supported by a grant from the Cardiovascular Research Foundation.

References

[1] Froehner, S.C. (1984) J. Cell Biol. 99, 88–96.
[2] Kramarcy, N.R., Vidal, A., Froehner, S.C. and Sealock, R. (1994) J. Biol. Chem. 269, 2870–2876.
[3] Suzuki, A., Yoshida, M., Hayashi, K., Mizuno, Y., Hagiwara, Y. and Ozawa, E. (1994) Eur. J. Biochem. 220, 283–292.
[4] Ahn, A.H., Freener, C.A., Gussoni, E., Yoshida, M., Ozawa, E. and Kunkel, L.M. (1996) J. Biol. Chem. 271, 2724–2730.
[5] Ervasti, J.M. and Campbell, K.P. (1991) Cell 66, 1121–1131.
[6] Butler, M.H., Douville, K., Murnane, A.A., Kramarcy, N.R., Cohen, J.B., Sealock, R. and Froehner, S.C. (1992) J. Biol. Chem. 267, 6213–6218.
[7] Klietsch, R., Ervasti, J.M., Arnold, W., Campbell, K.P. and Jorgensen, A.O. (1993) Circ. Res. 72, 349–360.
[8] Yoshida, M. and Ozawa, E. (1990) J. Biochem. 108, 748–752.
[9] Ervasti, J.M. and Campbell, K.P. (1993) J. Cell Biol. 122, 809–823.
[10] Hemmings, L., Kuhlman, P.A. and Critchley, D.R. (1992) J. Cell Biol. 116, 1369–1380.
[11] Way, M., Pope, B., Cross, R.A., Kendrick-Jones, J. and Weeds, A.G. (1992) FEBS Lett. 301, 243–245.
[12] Adams, M.E., Dwyer, T.M., Dowler, L.L., White, R.A. and Froehner, S.C. (1995) J. Biol. Chem. 270, 25859–25865.
[13] Yang, B., Jung, D., Rafael, J.A., Chamberlain, J.S. and Campbell, K.P. (1995) J. Biol. Chem. 270, 4975–4978.
[14] Brenman, J.E., Chao, D.S., Gee, S.H., McGee, A.W., Craven, S.E., Santillano, D.R., Wu, Z., Huang, F., Xia, H., Peters, M.F., Froehner, S.C. and Bredt, D.S. (1996) Cell 84, 757–767.
[15] Newbell, B.J., Anderson, J.T. and Jarrett, H.W. (1997) Biochemistry 36, 1295–1305.
[16] Yang, B., Ibraghimov-Beskrovnaya, O., Moomaw, C.R., Slaughter, C.A. and Campbell, K.P. (1994) J. Biol. Chem. 269, 6040–6044.
[17] Iwata, Y., Nakamura, H., Fujiwara, K. and Shigekawa, M. (1993) Biochem. Biophys. Res. Commun. 190, 589–595.
[18] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[19] Iwata, Y., Pan, Y., Hanada, H., Yoshida, T. and Shigekawa, M. (1996) J. Mol. Cell. Cardiol. 28, 2501–2509.
[20] Bertrand, B., Wakabayashi, S., Ikeda, T., Pousségur, J. and Shigekawa, M. (1994) J. Biol. Chem. 269, 13703–13709.
[21] Froehner, S.C., Murnane, A.A., Tobler, M., Peng, H.B. and Sealock, R. (1987) J. Cell Biol. 104, 1633–1646.
[22] Campbell, K.P. (1995) Cell 80, 675–679.
[23] Fabbriziro, E., Bonet-Kerrache, A., Leger, J.J. and Monet, D. (1993) Biochemistry 32, 10457–10463.
[24] Winder, S.J., Hemmings, L., Maciver, S.K., Bolton, S.J., Davies, K.E., Critchley, D.R. and Kendrick-Jones, J. (1995) J. Cell Sci. 108, 63–71.
[25] Waschsstock, D.H., Schwarz, W.H. and Pollard, T.D. (1993) Biophys. J. 65, 205–214.
[26] Vandekerckhove, J. and Vancompernolle, K. (1992) Curr. Opin. Cell Biol. 4, 36–42.
[27] Erickson-Viitanen, S. and DeGrado, W.F. (1987) Methods Enzymol. 139, 455–478.
[28] Shaw, G. (1996) BioEssays 18, 35–46.
[29] Sheng, M. (1996) Neuron 17, 575–578.