Localization of the N-terminal and C-terminal ends of triadin with respect to the sarcoplasmic reticulum membrane of rabbit skeletal muscle

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Antibodies were raised against synthetic peptides corresponding to the N-terminal (residues 2–17) and C-terminal (residues 691–706) ends of rabbit skeletal muscle triadin, a 95 kDa protein located in the sarcoplasmic reticulum membrane at the triad junction. The specificity of the antibodies generated was tested by ELISA and Western blot analysis. These tests demonstrated the ability of the antibodies to react specifically with the proteins. The anti-N-terminus antibodies bound to sarcoplasmic reticulum vesicles, indicating that the N-terminal end of the membrane-embedded triadin is exposed on the cytoplasmic side of the vesicles. In contrast, the anti-C-terminus antibodies were able to react with sarcoplasmic reticulum vesicles only after permeabilization of the vesicles with a detergent, indicating that the C-terminal end is exposed on the luminal side of the vesicles. These immunological data were complemented by proteolysis experiments using carboxypeptidases and endoproteinase Arg C. A mixture of carboxypeptidases A, B and Y was used to induce degradation of the C-terminal end of triadin in sarcoplasmic reticulum vesicles. This degradation, and a concomitant loss of reactivity of the anti-C-terminus antibodies in Western blots, was observed only when the vesicles were permeabilized, providing further evidence for the luminal localization of the C-terminal end of triadin. Treatment of sarcoplasmic reticulum vesicles with endoproteinase Arg C resulted in the removal of the N-terminal end of triadin, probably due to cleavage after Arg-34. This is a further indication of the cytoplasmic localization of the N-terminal end of triadin (and of its first 34 amino acids). When the proteolysis with endoproteinase Arg C was carried out with permeabilized vesicles, the cleavage occurred after Arg-141 or Arg-157, indicating that at least one of these residues is luminal.

INTRODUCTION

The mechanism coupling excitation of the muscle cell to its contraction (E–C coupling) is still an intense field of research. Two proteins have been identified which play a crucial role in E–C coupling: the dihydropyridine (DHP) receptor (rDHP), and the ryanodine receptor (RyR) [1]. The rDHP is a voltage-dependent L-type Ca^{2+} channel localized in the T-tubule membranes, and is the sensor of their depolarization [2,3]. The RyR is a Ca^{2+} channel localized in the terminal cisternae of the sarcoplasmic reticulum (SR) which allows the massive release of Ca^{2+} from the SR [4–6] and hence the resulting muscle contraction. The link between these two proteins is not fully established. In cardiac muscle the linkage is thought to be chemical, via a second messenger. Ca^{2+} has been proposed to be this second messenger, and the E–C coupling to be the result of a 'Ca^{2+}-induced Ca^{2+} release' [7–9]. In skeletal muscle, however, evidence in favour of a mechanical coupling [10], through a physical link between the two proteins, has been provided by electron microscopy data [11] and by more direct biochemical studies [12]. In the search for this link, a protein of 95 kDa has been identified [13,14], and has been named triadin because of its specific localization in the triad membrane [15]. This protein has been shown to interact with the rDHP and with the RyR [13–15], and could constitute the missing link between these two proteins. The sequence of triadin has been determined [16], and a first theoretical model for the topography of the protein proposed. According to the hydropathy plot, the protein is predicted to contain only one transmembrane α-helix in the N-terminal region, the N-terminal end of the protein should be external, and the bulk of the protein should be internal. On the basis of this model, Knudson et al. [16] suggested that triadin cannot be a link between RyR and rDHP, and would instead anchor calsequestrin, the luminal protein of Ca^{2+} sequestration, to the triad junction. The presence of triadin in heart muscle has been shown recently [17] and, on the basis of Northern blot analysis with two different probes, it has been suggested that triadin of skeletal muscle and heart muscle have an identical N-terminus and different C-termini.

In the present study, the two peptides corresponding to the N-terminal and C-terminal parts of skeletal muscle triadin have been synthesized, and used to raise anti-N-terminus and anti-C-terminus specific antibodies. These antibodies were used for topological studies on heavy SR (HSR) vesicles, alone or in combination with proteinases [carboxypeptidases and endoproteinase Arg C (endo-Arg C)]. The results obtained with the different techniques allow us to determine the localization of the two extremities of triadin with respect to the SR membrane.

EXPERIMENTAL

Materials

Monoclonal anti-(rabbit calsequestrin) antibody was from Affinity BioReagents (Neshanic Station, NJ, U.S.A.). ^{125}I-labelled anti-(rabbit IgG) was from Amersham. Proteinases (endo-Arg C and carboxypeptidases A, B and Y) were from Boehringer Mannheim.

Abbreviations used: A-Cter antibody, anti-(C-terminal peptide) antibody; A-Nter antibody, anti-(N-terminal peptide) antibody; DFP, di-isopropyl fluorophosphate; DHP, dihydropyridine; E–C coupling, excitation–contraction coupling; endo-Arg C, endoproteinase Arg C; HSR, heavy sarcoplasmic reticulum; rDHP, dihydropyridine receptor; RyR, ryanodine receptor; SR, sarcoplasmic reticulum.

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Membrane preparation

HSR vesicles were prepared from rabbit skeletal muscle according to the procedure of Kim et al. [18], slightly modified as described previously [19].

Peptide synthesis

The two peptides corresponding to the N-terminal and C-terminal ends of triadin were synthesized chemically by the stepwise solid-phase method [20], using an Applied Biosystems 430A automated synthesizer. The N-terminal peptide (Thr-Glu-Ile-Thr-Ala-Glu-Gly-Asn-Ala-Ser-Thr-Thr-Thr-Thr-Val-Ile-Tyr) corresponds to residues 2–17 of the rabbit skeletal muscle triadin [16], with an extra Tyr added at the C-terminus. The C-terminal peptide (Tyr-Pro-Gly-Glu-Ser-Ser-Gly-Lys-Pro-Asn-Ser-Pro-Gly-Pro-Lys-Gln) corresponds to residues 691–706. The *t*-butyloxycarbonyl group was used for protection of the α-amino group of all amino acids. All couplings were performed by the dicyclohexylcarbodi-imide/1-hydroxybenzotriazole method, using *N*-methylpyrrolidone and dimethyl sulphoxide as coupling solvents, according to the protocol defined by Applied Biosystems. Amino groups left unreacted at the end of each coupling cycle were capped with acetic anhydride. Deprotection and cleavage of the peptides from the resin was performed with trifluoromethane sulphonic acid. Both peptides were purified by reverse-phase HPLC, using a 300 Å Deltapak C_{18} column (1.9 cm × 30 cm) (Waters Associates). Each purified peptide was characterized by fast atom bombardment mass spectrometry, as described previously [21].

Coupling of peptides to ovalbumin and generation of anti-peptide antibodies

The N- and C-terminal peptides were coupled with bisdiazobenzidine to ovalbumin through the hydroxyl group of the Tyr residue added at the C-terminus of the N-terminal peptide or present at the N-terminus of the C-terminal peptide. The coupling reaction was performed as described by Tamura and Bauer [22].

Rabbits were immunized with three intradermal injections at 3-week intervals followed by three intramuscular injections at 1-day intervals of 300 μg of the conjugated peptide. The rabbits were bled 3 weeks after the last injection.

Calsequestrin extraction

Calsequestrin was extracted from the HSR vesicles according to the procedure described by Ikemoto et al. [23], modified as follows. HSR vesicles were washed with 0.4 M NaCl/20 mM Pipes (pH 7), and collected by centrifugation. The pellet was resuspended in the same buffer containing 1 mg/ml sodium cholate, at a protein concentration of 10 mg/ml. After 15 min of incubation at room temperature, the vesicles were centrifuged (15 min at 200000 ***g***) and the solubilized proteins were collected in the supernatant. The supernatant contained mainly calsequestrin. Calsequestrin was coated after dilution at 500 ng/ml on microtitration plates for ELISA or used as a competitor with anti-calesquestrin antibodies.

Antibody assays by ELISA

The ability of the antisera to react with the synthetic peptides, and with the membrane-bound triadin in HSR vesicles, was tested by ELISA, using microtitration plates (Nunc ref. 4-42404), as previously described [19], except that the incubation with primary antibodies was followed by an incubation with Protein A–horseradish peroxidase conjugate (Sigma), diluted 4000-fold. The immune complex was revealed with 3,3′,5,5′-tetramethylbenzidine as a substrate for peroxidase, and the extent of the reaction was determined by measurement of the absorbance at 450 nm, after addition of H_2SO_4.

For back-titration experiments, the HSR vesicles were incubated overnight at 4 °C with the antibodies in PBS (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_3HPO_4, pH 7.4) supplemented with 1% BSA (PBS/BSA) and, after centrifugation at 20000 ***g*** for 3 min, the supernatants were collected. The unreacted antibodies present in the supernatant were assayed by ELISA against the corresponding peptide or protein coated on to microtitration plates as described above. In experiments using permeabilized HSR vesicles, the vesicles were diluted at 1 mg/ml in PBS/BSA supplemented with 2.5 mg/ml CHAPS and, after 1 h of incubation at 0 °C, the vesicles were incubated overnight at 4 °C with the antibodies in PBS/BSA at the chosen dilution, as described above.

Western blot analysis

The reactivity of the anti-peptide antibodies was tested against triadin or its proteolytic fragments using the Western blot technique [24]. HSR proteins were loaded on a 8.5% polyacrylamide gel. After electrophoretic separation, the proteins were transferred to a nitrocellulose sheet for 2 h at 1 A. After saturation of the remaining binding sites with BSA, the nitrocellulose sheets were incubated overnight at 4 °C with the antibodies (dilution 1:5000), then with ^{125}I-labelled anti-rabbit antibodies, and the reactive proteins were detected by autoradiography.

Proteolytic digestion

Carboxypeptidases

HSR vesicles were diluted to 3 mg/ml in a medium composed of 0.27 M sucrose and 50 mM Pipes (pH 7.0) at 37 °C. Carboxypeptidase A, carboxypeptidase B and carboxypeptidase Y were added at zero time, at proteinase/total protein ratios of 1:100 and 1:10 (w/w) for each proteinase. After 10 min, 1 h, 2 h and 4 h, a portion of the reaction medium was withdrawn and the reaction stopped by addition of EDTA (5 mM final concentration; pH 6) and di-isopropyl fluorophosphate (DFP) (100 mM); 5 min later, Laemmli's dissociation medium (62.5 mM Tris, pH 6.8, 2% SDS, 5% β-mercaptoethanol, 10% glycerol, Bromophenol Blue) was added. The samples were heated for 1 min at 100 °C, and then stored at −20 °C for further analysis by immunoblotting. In control experiments, HSR vesicles were incubated for 4 h under the same conditions, but in the absence of proteinase. For the proteolytic degradation in permeabilized vesicles, the HSR vesicles were diluted in sucrose medium supplemented with CHAPS at a concentration of 1 mg/ml, and proteolysis was achieved as described above.

Endo-Arg C

HSR vesicles were diluted to 1 mg/ml in a medium composed of 0.27 M sucrose and 50 mM Tris, pH 8.3, at 30 °C. Endo-Arg C was added at 1:10 (proteinase/total proteins, w/w) at zero time. The experiment was carried out as described for carboxypeptidases, except that the reaction was stopped by addition of 100 mM DFP.

RESULTS

Reactivity and specificity of the antibodies

The reactivity of the anti-(C-terminal peptide) (A-Cter) and anti-(N-terminal peptide) (A-Nter) antibodies was first checked using an ELISA test against the corresponding peptide. The results are presented in Figure 1, and demonstrate good reactivity of both antibodies. If each antibody was first incubated with its corresponding peptide as a competitor, the signal was fully abolished in subsequent ELISA (results not shown).

The specificity of the antibodies was then evaluated using Western blot analysis. Among all the proteins of the HSR vesicles, both antibodies reacted strongly with a protein of 95 kDa (Figure 2, lanes 1 and 3). The low-molecular-mass bands (about 60 kDa) observed with A-Nter antibodies (Figure 2, lane 1) correspond to the bands described by Caswell et al. [15] and Knudson et al. [16] and proposed to be proteolytic fragments of triadin. If the samples were not fully reduced, the antibodies recognized higher-molecular-mass proteins (results not shown), previously identified as oligomers of triadin [15]. The reactivity of both antibodies was completely abolished when the corresponding free peptide was added as a competitor (Figure 2, lanes 2 and 4).

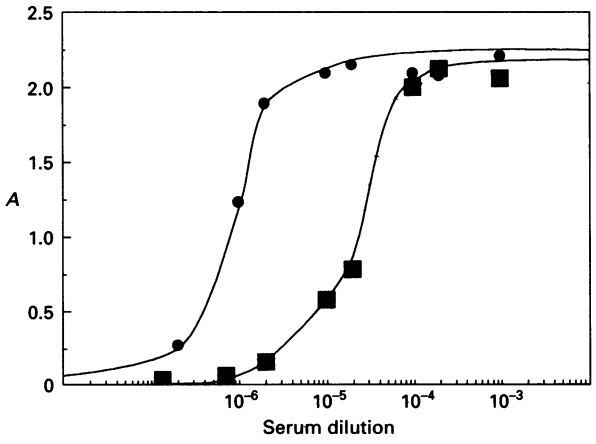

Figure 1 Assessment by ELISA of the reactivity of anti-peptide antibodies against the corresponding peptides

The microtitration plates were coated with the N-terminal or C-terminal peptide diluted at 100 ng/ml in PBS. The corresponding anti-serum [A-Nter (■) or A-Cter (●)] was added at different dilutions and the reactivity of the antisera was measured by a chromogenic reaction, as described in the Experimental section.

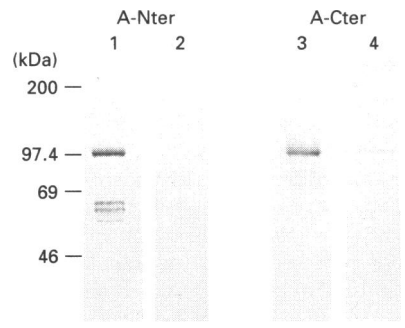

Figure 2 Western blot analysis of the reactivity of A-Nter and A-Cter antibodies against the proteins of HSR vesicles

HSR proteins (20 μg) were separated by SDS/PAGE, and electrotransferred on to a nitrocellulose sheet. The sheets were incubated with: lane 1, A-Nter antibodies (1:5000); lane 2, A-Nter antibodies preincubated with the N-terminal peptide (5 μg/ml) as a competitor; lane 3, A-Cter antibodies (1:5000); lane 4, A-Cter preincubated with the C-terminal peptide (5 μg/ml). Immunoreactive proteins were detected by autoradiography after incubation with ^{125}I-labelled anti(rabbit IgG).

Reactivity of the antibodies with HSR vesicles

Once the specificity and the excellent reactivity of the antibodies had been demonstrated, we tested their accessibility to triadin in HSR vesicles. The HSR vesicles have an orientation similar to that of the SR in the muscle cell: the outside of the vesicles corresponds to the cytoplasmic side of the SR, and the inside of the vesicles corresponds to the luminal side of the SR [25,26].

We performed back-titration experiments: after incubation of the antibodies with HSR vesicles, the unbound antibodies were titrated against the corresponding antigen coated on to the microtitration plate (peptide for anti-triadin antibodies, or purified calsequestrin for anti-calsequestrin antibody). When all the antibodies react with the HSR vesicles, no residual reactivity is observed in the subsequent ELISA test, whereas when the antibodies do not react with HSR vesicles, the signal in the ELISA test is high, corresponding to a large amount of untrapped antibodies. These tests were performed either on intact HSR vesicles or on permeabilized vesicles. The permeabilization was achieved by pre-incubation of HSR vesicles with CHAPS, and was controlled by the reactivity of antibodies against a luminal protein, calsequestrin. The results are presented in Figure 3.

The A-Nter antibodies (Figure 3a) reacted with intact HSR vesicles, and the permeabilization of the vesicles with CHAPS did not increase this reactivity. At a high concentration of HSR vesicles, all the antibodies were trapped and the signal was identical to that obtained with antibodies pre-incubated with the free peptide as a competitor. This indicates that the N-terminal end of triadin is localized on the external side of the vesicles. In contrast, the A-Cter antibodies (Figure 3b) showed low reactivity with the intact vesicles, but reacted strongly after permeabilization of the vesicles with CHAPS. In this case, the signal reached that obtained when all the antibodies were first trapped by the free peptide. This suggests that the C-terminal end of triadin is localized on the internal side of the vesicles, and is accessible only after permeabilization. The low reactivity observed with non-permeabilized vesicles could be due to a small number of vesicles being already permeable to antibodies. In order to test this hypothesis, and to check that CHAPS really does induce permeabilization of the vesicles, the reactivity of the HSR vesicles with antibodies against an internal protein, calsequestrin [27,28], was measured before and after permeabilization (Figure 3c). The same results were obtained with the anti-calsequestrin antibodies and with the A-Cter antibodies: strong reactivity of anti-calsequestrin was observed only after permeabilization of the vesicles. The similar behaviour of A-Cter and anti-calsequestrin strongly suggests that the epitopes for both antibodies are located on the same side of the vesicles, i.e. the luminal side, whereas the N-terminal part of triadin is located on the external surface of the vesicles, i.e. the cytoplasmic side.

Accessibility of the two ends of triadin to proteinases

In order to confirm the localization of the two extremities of triadin, we evaluated their accessibility to proteinases. We used two kinds of proteinase: (1) a mixture of carboxypeptidases A, B and Y, in order to degrade sequentially the C-terminal end of

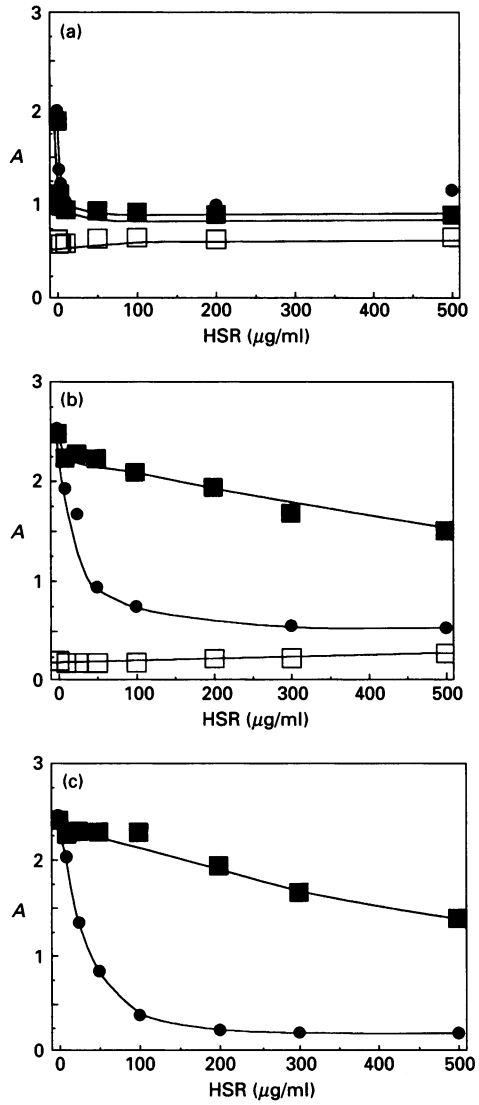

Figure 3 Reactivity of A-Nter, A-Cter and anti-calsequestrin antibodies towards HSR vesicles: back-titration by ELISA

Increasing concentrations of HSR vesicles, either intact (■) or permeabilized by CHAPS (●), were incubated overnight with the antibodies (**a**, A-Nter, 1:100000; **b**, A-Cter, 1:1000000; **c**, anti-calsequestrin, 1:10000), in the absence (■, ●) or in the presence (□) of the corresponding free peptide (5 μg/ml). The vesicles were sedimented by centrifugation, and the unbound antibodies present in the supernatant were assessed by ELISA against the corresponding peptide or protein (coated at 200 ng/ml for Nter, 100 ng/ml for Cter and 500 ng/ml for calsequestrin).

triadin, and (2) an endopeptidase specific for arginine residues, endo-Arg C.

Degradation by carboxypeptidases

Because of its sequence [16], the degradation of the C-terminal part of triadin can only be achieved with the combined use of three carboxypeptidases: carboxypeptidase B for lysine residues, carboxypeptidase Y for proline residues and carboxypeptidase A for the other residues.

After proteolysis, the extent of degradation was evaluated by Western blotting with A-Cter antibodies (Figure 4). In spite of the use of the three carboxypeptidases A, B and Y, at a

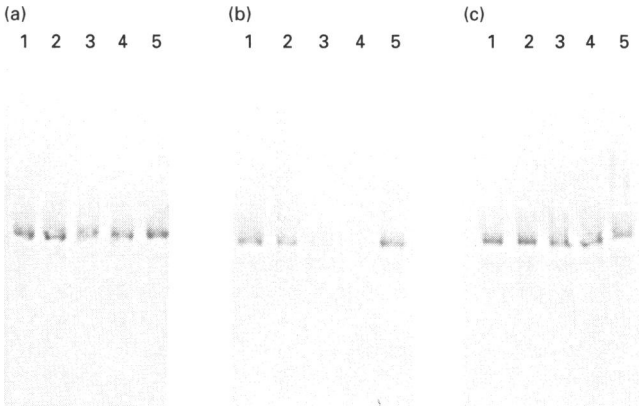

Figure 4 Kinetics of the proteolytic degradation of the C-terminal end of triadin in HSR vesicles by carboxypeptidases A, B and Y: Western blot analysis with A-Cter

HSR vesicles (3 mg/ml), either intact (**a** and **c**) or permeabilized (**b**), were incubated with carboxypeptidases A, B and Y at a proteinase/protein ratio (w/w) of 1:100 (**a** and **b**) or 1:10 (**c**) for each proteinase, as described in the Experimental section. Lanes 1 to 4, proteolytic degradation for 0, 10 min, 1 h and 4 h respectively; lane 5, control (4 h incubation under the same conditions, but without proteinases). After proteolysis, the proteins were separated by electrophoresis and analysed by Western blotting with A-Cter antibodies (diluted 1:500).

proteinase/protein ratio of 1:100 for each proteinase, no significant degradation of the C-terminal part was observed in intact vesicles (Figure 4a). If, however, the vesicles were permeabilized by addition of a detergent, CHAPS, then degradation of the C-terminal end was observed, as shown by the resulting loss of reactivity with A-Cter antibodies (Figure 4b). With a greater concentration of proteinases (ratio 1:10 for each) but in the absence of detergent, no degradation could be induced (Figure 4c), indicating that the limiting factor for proteolytic attack was indeed the accessibility and not the quantity of proteinases.

Degradation by endo-Arg C

Among the 706 amino acids comprising the sequence of triadin, there are only 10 arginine residues, at positions 34, 141, 157, 210, 271, 301, 428, 458, 617 and 634. The accessibility of these arginines, and particularly of the first and last ones, could therefore provide complementary information on the organization of the protein and of the two termini of triadin with respect to the membrane.

We induced the degradation of triadin by addition of endo-Arg C to HSR vesicles (proteinase/protein ratio = 1:10), and, as for the carboxypeptidases, evaluated the extent of degradation by measuring reactivity with the A-Nter and A-Cter antibodies (Figure 5). When the proteolytic treatment was performed on intact vesicles (Figure 5a), proteolysis induced the disappearance of the N-terminal end of the protein (as seen by the disappearance of the 95 kDa band reactive with A-Nter), and the concomitant appearance of a C-terminal reactive fragment of about 90 kDa. This indicates that triadin was degraded at its N-terminal end. The cleavage induced the loss of a N-terminal fragment of about 5 kDa, and therefore most probably occurred at Arg-34. When the degradation was carried out with vesicles permeabilized with CHAPS (Figure 5b), the disappearance of the N-terminal end was accompanied by the appearance of a C-terminal fragment of about 80 kDa. This fragment could result from cleavage at Arg-141 or Arg-157, the only arginines in the sequence that would

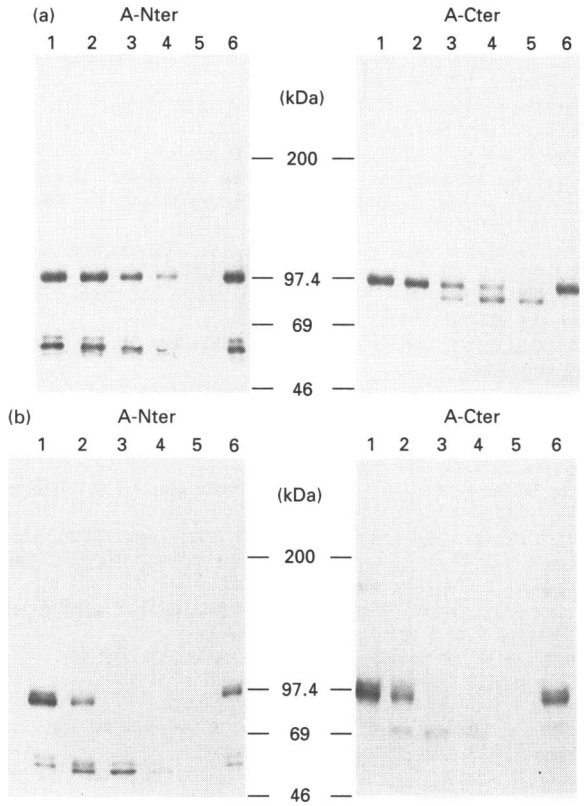

Figure 5 Kinetics of the proteolytic degradation of triadin in HSR vesicles by endo-Arg C: Western blot analysis with A-Nter or A-Cter

HSR vesicles (1 mg/ml), either intact (**a**) or permeabilized (**b**), were incubated with endo-Arg C at a proteinase/protein ratio (w/w) of 1:10, as described in the Experimental section. Lanes 1 to 5, proteolytic degradation for 0, 10 min, 1 h, 2 h and 4 h respectively; lane 6, control (4 h incubation under the same conditions, but without proteinase). After proteolysis, the proteins were separated by electrophoresis and analysed by Western blotting with A-Nter or A-Cter antibodies (diluted 1:5000).

lead to a decrease in the apparent molecular mass of the protein of about 15 kDa and produce an 80 kDa C-terminal fragment.

The results obtained with endo-Arg C indicated that Arg-34 is accessible on intact vesicles, and thus is localized on the cytoplasmic side of the SR, whereas Arg-141 or Arg-157 is accessible only on permeabilized vesicles, and hence is localized on the luminal side of the SR.

DISCUSSION

In this study we have used a combination of immunological and proteolytic techniques in order to determine the orientation of the two extremities of triadin with respect to the SR membrane. This approach has already been used successfully to study the topography of numerous membrane proteins, including the RyR of the SR [19], the GluR1 glutamate receptor subunit [29], the mitochondrial phosphate carrier [30], the multidrug transporter [31], the mitochondrial ADP/ATP carrier [32,33] and the acetylcholine receptor [34] (for a review of the different techniques and proteins studied, see [35]).

Triadin is a membrane protein, localized in the membrane of the SR of skeletal muscle at the triad junction. The sequence of triadin has recently been published [16], and a first theoretical model was proposed for the arrangement of the protein in the SR membrane. Because of the low sensitivity of the protein to proteolysis, and the presence of only one theoretical transmembrane segment starting at position 47, the major part of the protein was predicted to be luminal, with a small N-terminal part protruding into the cytoplasm [16]. Nevertheless, no experimental data have been obtained up to now on the localization of the two extremities of triadin.

For the experiments described in the present paper, two peptides corresponding to the N-terminal and the C-terminal ends of rabbit skeletal muscle triadin have been synthesized and used to produce anti-peptide antibodies. Once the specificity of these antibodies had been demonstrated, they were used to study the topology of the two ends of triadin with respect to the SR membrane, either alone or in combination with proteinases.

The A-Nter antibodies were able to react with HSR vesicles, and the permeabilization of these vesicles with CHAPS did not modify this reactivity. This is a good indication of the external localization of the N-terminal part of triadin. In contrast, the A-Cter antibodies reacted only slightly with the HSR vesicles, but the reactivity was greatly increased after permeabilization of the vesicles with CHAPS, strongly suggesting that the C-terminal end of triadin is internal. We performed the same experiments with antibodies directed against an internal protein, calsequestrin. We obtained the same results with anti-calsequestrin antibodies as with A-Cter antibodies: a slight reactivity, greatly increased by the addition of CHAPS to the vesicles. This result confirmed that the effect of CHAPS was indeed to allow access of the antibodies to intravesicular epitopes, and not to unmask parts of the protein that were poorly accessible because of protein folding, for example. We concluded, therefore, that the C-terminal end of triadin is probably internal.

These results were complemented by proteolysis experiments. Using a mixture of carboxypeptidases A, B and Y we observed degradation of the C-terminal end of triadin in HSR vesicles, but only if the vesicles were permeabilized with CHAPS. This is a further indication of the luminal localization of the C-terminus of triadin. The action of endo-Arg C on unpermeabilized HSR vesicles probably results in cleavage at Arg-34, indicating that this residue is accessible and hence located on the cytoplasmic side. When degradation by endo-Arg C was carried out with permeabilized vesicles, we observed the appearance of a new C-terminal fragment, probably resulting from cleavage after Arg-141 or Arg-157. This residue (Arg-141 and/or Arg-157) is thus located on the luminal side of the HSR vesicles.

The results presented here constitute the first experimental data on the topography of triadin. On the basis of our experimental data, Figure 6 shows a refined model for the topography of triadin in the SR membrane, complementing the theoretical model of Knudson et al. [16].

The identification of the organization of the protein in and around the membrane can be a clue to the understanding of the precise function of triadin. Because of its interaction with either the RyR or rDHP, triadin has been proposed to be the link between these two proteins that are involved in E–C coupling [13–15]. Nevertheless, according to the model proposed recently [16], being mostly luminal, triadin cannot be easily imagined to interact between two proteins on the cytoplasmic side of the SR. Knudson et al. [16] proposed that triadin constitutes a link between the RyR and calsequestrin, and anchors calsequestrin to the triad junction. Furthermore, as these authors could not detect the presence of triadin in cardiac muscle, they proposed that it could have a function specific to skeletal muscle. However, the presence of triadin in heart, previously detected by biochemical techniques [36], has been confirmed recently using molecular biology techniques [17]. The cardiac muscle isoform

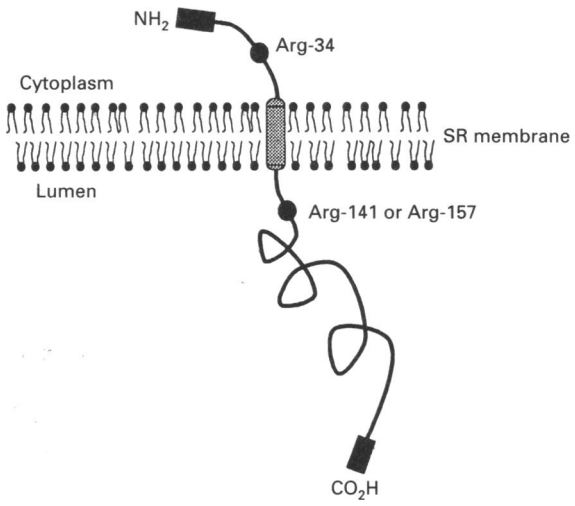

Figure 6 Model for the topography of triadin in the SR membrane

The theoretical model proposed by Knudson et al. [16] has been modified in the light of our experimental data on the localization of the N-terminal and C-terminal ends of triadin, and of Arg-34 and Arg-141/157.

shares with the skeletal muscle isoform a common N-terminal part, while they differ in their C-termini. The C-terminal part of triadin could therefore be involved in the type of coupling, which differs in cardiac and skeletal muscles. In this context, the exact localization of this part of the protein is of major interest, and our anti-N-terminus and anti-C-terminus antibodies could be valuable tools for further investigations in this field.

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