Self-aggregation of triadin in the sarcoplasmic reticulum of rabbit skeletal muscle

ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · MAY 1999

Impact Factor: 4.66 · DOI: 10.1016/S0005-2736(99)00024-3 · Source: PubMed

ELSEVIER

Biochimica et Biophysica Acta 1418 (1999) 197–205

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Self-aggregation of triadin in the sarcoplasmic reticulum of rabbit skeletal muscle

Gabriele R. Froemming, Brendan E. Murray, Kay Ohlendieck *

Department of Pharmacology, National University of Ireland, University College Dublin, Belfield, Dublin 4, Ireland

Received 10 December 1998; accepted 5 February 1999

Abstract

The 95 kDa transmembrane glycoprotein triadin is believed to be an essential component of excitation–contraction coupling in the junctional sarcoplasmic reticulum of skeletal muscle fibers. It is debatable whether triadin mediates intraluminal interactions between calsequestrin and the ryanodine receptor exclusively or whether this junctional protein provides also a cytoplasmic linkage between the Ca^{2+}-release channel and the dihydropyridine receptor. Here, we could show that native triadin exists as disulfide-linked homo-polymers of above 3000 kDa. Under non-reducing conditions, protein bands representing the α_1-dihydropyridine receptor and calsequestrin did not show an immunodecorative overlap with the extremely high-molecular-mass triadin clusters. Following chemical crosslinking, the ryanodine receptor and triadin exhibited a similarly decreased electrophoretic mobility. However, immunoblotting of diagonal non-reducing/reducing two-dimensional gels clearly demonstrated a lack of overlap between the immunodecorated bands representing triadin, the α_1-dihydropyridine receptor, the ryanodine receptor and calsequestrin. Thus, in native membranes triadin appears to form large self-aggregates primarily. Although triadin exists in a close neighborhood relationship to the Ca^{2+}-release channel tetramers, it does not seem to be directly linked to the other main triad components implicated in the regulation of the excitation–contraction–relaxation cycle and Ca^{2+}-homeostasis. This agrees with a proposed role of triadin in the maintenance of overall triad architecture.

Keywords: Triadin; Sarcoplasmic reticulum; Excitation–contraction coupling; Calcium homeostasis; (Skeletal muscle)

1. Introduction

In contrast to the proposed Ca^{2+}-induced Ca^{2+}-release mechanism underlying cardiac excitation–contraction coupling [1], signal transduction at skeletal muscle triads is thought to occur by means of direct physical interactions between the transverse tubular voltage-sensor and the Ca^{2+}-release units of the sarcoplasmic reticulum [2–4]. The elucidation of crucial protein–protein interactions between triad components and the topology of membrane complexes involved in the regulation and stabilization of this unique cell biological process is under intense investigation [5–7]. Many of the key components of the excitation–contraction–relaxation cycle have been identified, i.e. the voltage-sensing α_1-dihydropyridine receptor [8,9] and its auxiliary α_2/δ-β-γ subunits [10,11], the ryanodine receptor Ca^{2+}-release channel tetramer [12–14] and its regulatory FKBP12 subunit [15], the Ca^{2+}-binding proteins calsequestrin of the terminal cisternae [16,17] and sarcalumenin of the

* Corresponding author. Fax: +353-1-269-2749; E-mail: kay.ohlendieck@ucd.ie

0005-2736/99/$ – see front matter
PII: S0005-2736(99)00024-3

longitudinal tubules [18], as well as the Ca^{2+}-ATPase isoforms of the sarcoplasmic reticulum [19,20]. However, it is not fully understood how signal transduction is mediated by direct triad receptor coupling and what auxiliary components provide the overall structural integrity of junctional couplings. Possible candidates which might be involved in the prevention of passive disintegration of junctional proteins and/or the functional coupling of triad protein complexes is the 90 kDa JSR-protein [21] and a 95 kDa integral glycoprotein of the sarcoplasmic reticulum named triadin [22,23].

Skeletal and cardiac muscle triadins have been cloned and sequenced [24–26] and shown to exist as a population of multimeric structures which co-localize with the ryanodine receptor in the junctional sarcoplasmic reticulum [23,27]. It is not clear whether triadin provides functional coupling between the voltage-sensing dihydropyridine receptor and the ryanodine receptor Ca^{2+}-release channel [28,29] or forms a linkage between the Ca^{2+}-binding protein calsequestrin and the ryanodine receptor [30,31]. Recently, Zhang et al. [32] described a quaternary complex consisting of triadin, the ryanodine receptor, calsequestrin and the calsequestrin-binding protein junction in cardiac junctional sarcoplasmic reticulum. The supposition that triadin primarily mediates intraluminal interactions between calsequestrin and the ryanodine receptor is supported by its deduced primary structure [24], as well as domain binding studies [30]. The analysis of the membrane topology of triadin monomers indicates that they contain a single transmembrane domain and that the bulk of this junctional protein is intraluminal [24,33]. Binding experiments with fusion proteins representing various triadin domains showed a Ca^{2+}-dependent interaction between triadin and calsequestrin [30]. Furthermore, interactions between the ryanodine receptor and triadin domains were found to be restricted to the luminal portion of triadin [30]. On the other hand, triadin was reported to bind to both the dihydropyridine receptor and the Ca^{2+}-release channel in blot overlay assays, suggesting a potential role in triad receptor coupling via cytosolic domains [34]. It was also shown that antibodies to triadin affect Ca^{2+}-release from the sarcoplasmic reticulum [35]. Based on the analysis of disulfide bonding, glycosylation and proteolytic degradation, Fan et al. [36] propose that triadin exhibits more than one transmembrane domain and mediates interactions between the II–III loop domain of the α_1-dihydropyridine receptor and the ryanodine receptor. In spite of the above described contradictory findings, it is clearly established that triadin is functionally important for junctional couplings in skeletal muscle fibers [37,38].

Comprehensive chemical crosslinking analyses of triads have shown that tetramers of the Ca^{2+}-release channel and the α_1-subunit of the dihydropyridine receptor exist in a very close neighborhood relationship [39,40], confirming the physical coupling hypothesis of excitation–contraction coupling in skeletal muscle [2–4]. Calsequestrin was also shown to form high-molecular-mass complexes above 3000 kDa with the ryanodine receptor in both slow- and fast-twitch skeletal muscle fibers [40,41]. In control experiments it was demonstrated that the abundant Ca^{2+}-ATPase tetramers [42] are not present in the above described triad complexes [39,41] illustrating the specificity of chemical crosslinking analysis employing the hydrophobic 1.2-nm probe dithiobis-succinimdyl-propionate. Although triadin is a relatively abundant component of the junctional sarcoplasmic reticulum [21–24], it was not detectable in chemically crosslinked triad complexes containing the dihydropyridine receptor, the ryanodine receptor and calsequestrin [41]. Thus, to further elucidate the membrane topology of native triadin and its potential interactions with other proteins, we investigated the configuration of disulfide-bonded triadin polymers and their neighborhood relationship with the Ca^{2+}-release channel in sarcoplasmic reticulum vesicles. The immunoblot analysis of high-molecular-mass triadin aggregates was performed using comparative one- and two-dimensional gel electrophoresis under reducing and non-reducing conditions, as well as following chemical crosslinking.

2. Materials and methods

2.1. Materials

Chemical crosslinker dithiobis-succinimidyl-propionate and chemiluminescence substrates were purchased from Pierce and Warriner (Chester, UK). Per-

oxidase-conjugated secondary antibodies, acrylamide stock solutions and protease inhibitors were obtained from Boehringer–Mannheim (Lewes, UK). Immobilon NC nitrocellulose membranes were from Millipore Corp. (Bedford, MA, USA). Monoclonal antibodies XIIC4 against sarcalumenin and 20A against the α_2-subunit of the dihydropyridine receptor were from Affinity Bioreagents (Golden, CO, USA). Polyclonal antibodies to the ryanodine receptor were purchased from Upstate Biotechnology (Lake Placid, NY, USA). Monoclonal antibodies IIG12 to triadin, VIIID1$_2$ to calsequestrin and IIID5 to the α_1-subunit of the dihydropyridine receptor were a generous gift from Dr. Kevin P. Campbell (Howard Hughes Medical Institute, University of Iowa, Iowa City, IA, USA). All other chemicals were of analytical grade and purchased from Sigma Chemical Co. (Dorset, UK).

2.2. Skeletal muscle membrane preparations

Leg and back muscle from New Zealand white rabbits, which had been obtained from the Biomedical Facility of the National University of Ireland (Dublin), were homogenized in the presence of a protease inhibitor cocktail [43]. All preparative steps were performed at 0–4°C. Established subcellular fractionation methods were used to prepare the light sarcoplasmic reticulum fraction enriched in membranes derived from longitudinal tubules [44], the triad fraction [39], membranes highly enriched in extra-junctional transverse tubules [45], and the purified sarcolemma fraction using agglutination with wheat germ agglutinin [46]. For fiber-type specific immunoblots, microsomal membranes enriched in triads were prepared from isolated psoas and soleus muscles [41]. Protein concentration was determined according to Bradford [47] using myofibrillar proteins as a standard. For chemical crosslinking analysis, muscle membranes were incubated at room temperature for 30 min at pH 8 with the homobifunctional 1.2 nm probe dithiobis-succinimidyl-propionate at a concentration of 50 or 100 μg crosslinker per mg membrane protein [48]. The crosslinking reaction was terminated by the addition of ammonium acetate [39], and subsequently protein complexes were solubilized in SDS-containing sample buffer under non-reducing conditions [49].

2.3. One- and two-dimensional immunoblot analysis

Electrophoretic separation of membrane proteins was performed according to Laemmli [49]. Using the Bio-Rad Protean II xi cell (Bio-Rad Laboratories, Hemel Hempstead, UK), large 3–12% (w/v) gradient SDS–polyacrylamide gels were run for 5000 Vh at constant voltage with 60 μg protein per lane (see Fig. 1). For the comparative analysis of reduced, non-reduced and chemically crosslinked samples, a Bio-Rad Mini-Protean II gel system was employed. One-dimensional 7% (w/v) gels [41] (see Fig. 2) and diagonal 5% (w/v) non-reducing/6% (w/v) reducing two-dimensional mini-gels [42] (see Fig. 3) were run in the first dimension at a constant voltage for 440 Vh with 15 μg protein per lane. The second dimension of two-dimensional gels was electrophoresed at a constant voltage for varying Vh, optimized for proper detection of specific antigens in the second dimension slab gel. Electrophoretic transfer of gels onto Immobilon NC membranes was carried out according to Towbin et al. [50] using a Hoefer Transfor Cell TE-52X (Hoefer Scientific Instruments, San Francisco, CA, USA) for large standard gels and a Bio-Rad Mini-Protean II blotting system for mini-gels. Nitrocellulose sheets were blocked with 5% (w/v) fat-free milk and incubated with 1:1000 diluted primary antibodies, followed by a washing step and a final incubation with peroxidase-conjugated secondary antibodies as previously described in detail [39,43]. Visualization of immunodecorated protein bands was accomplished by enhanced chemiluminescence methodology [51].

3. Results

3.1. Subcellular expression of triadin in skeletal muscle

Prior to our one- and two-dimensional immunoblot analysis of the relative electrophoretic mobility of triadin and other key components of excitation–contraction coupling, we performed a standard subcellular fractionation study. Immunoblotting of subcellular membrane fractions from rabbit skeletal muscle clearly showed that triadin is highly enriched in vesicles derived from the triad region of the junc-

tional sarcoplasmic reticulum (Fig. 1a,b). Longitudinal tubules of the sarcoplasmic reticulum, non-junctional transverse tubules and the sarcolemma do not appear to contain significant amounts of triadin. Using monoclonal antibody IIG12 to triadin, this result was obtained under both reducing and non-reducing conditions. Chemical reduction with dithiothreitol, as illustrated in Fig. 1b, results in a markedly increased electrophoretic mobility of triadin as compared to non-reduced species of this junctional component (Fig. 1a). With respect to a fiber type-specific distribution, triadin appears to be present in higher abundance in predominantly fast-twitching psoas fibers as compared to the slow soleus muscle (Fig. 1c).

Since we were interested in studying triadin under non-reducing conditions, in which this protein appears as a broad band in large polyacrylamide gels (Fig. 1a), we performed the more detailed analysis of the spatial relationship between triadin and other triad proteins with a mini-gel system. In contrast to the diffuse immunodecorative band in large standard gels, non-reduced triadin protein, transferred from mini-gels, was visualized as a distinct band using enhanced chemiluminescence as a detection system (Fig. 2a). However, the intensity of this extremely high-molecular-mass triadin band was relatively weak. We therefore carried out a more refined immunoblot analysis using diagonal non-reducing/reducing two-dimensional gel electrophoresis (Fig. 3).

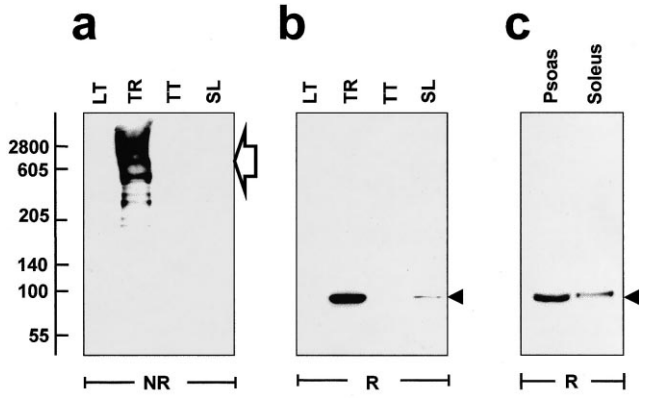

Fig. 1. Subcellular expression of triadin in skeletal muscle. Shown are immunoblots stained with monoclonal antibody IIG12 to triadin. Proteins were transferred from 3–12% (w/v) gradient gels, which had been electrophoresed under non-reducing conditions (NR) (a) or reducing conditions (R) (b,c). Individual lanes in a and b represent membrane preparations enriched in longitudinal tubules from the sarcoplasmic reticulum (LT), triad junctions (TR), extra-junctional transverse tubules (TT) and the sarcolemma (SL). In c are shown triad preparations from predominantly fast-twitching psoas muscle and slow-twitching soleus muscle. Arrowheads indicate the position of the reduced triadin monomer band of apparent 95 kDa. Disulfide-bridged clusters of triadin are marked by a large open arrow. Sizes of molecular mass standards (in kDa), as deduced from rat myofibrillar proteins, are indicated on the left.

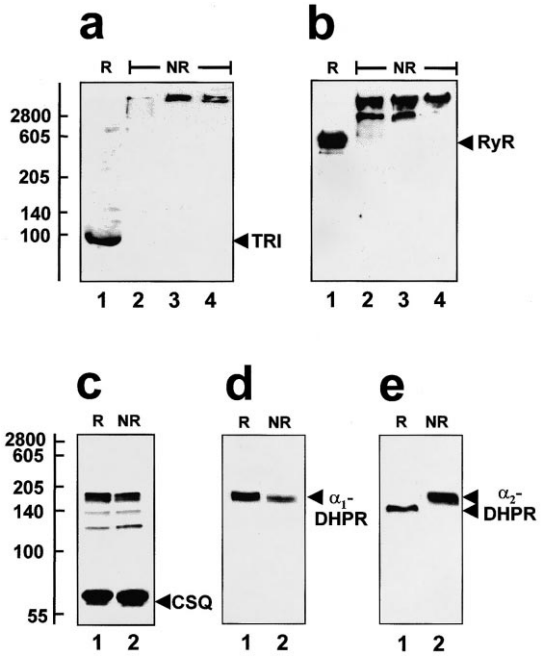

Fig. 2. Triad complexes in the junctional sarcoplasmic reticulum. Shown are immunoblots of triad couplings isolated from rabbit skeletal muscle stained with monoclonal antibody IIG12 to triadin (TRI) (a), polyclonal antiserum to the ryanodine receptor (RyR) (b), monoclonal antibody VIIID1$_2$ to calsequestrin (CSQ) (c), monoclonal antibody IIID5 to the α_1-subunit of the dihydropyridine receptor (α_1-DHPR) (d) and monoclonal antibody 20A to the α_2-subunit of the dihydropyridine receptor (α_2-DHPR) (e). Proteins were transferred from 7% (w/v) gels under reducing conditions (R) (lane 1), non-reducing conditions (lane 2) or non-reducing conditions following chemical cross-linking using 50 (lane 3) and 100 (lane 4) μg dithiobis-succinimidyl-propionate per mg membrane protein. The position of immunodecorated monomers is indicated by arrowheads. Sizes of molecular mass standards (in kDa), as deduced from rat myofibrillar proteins, are indicated on the left.

3.2. *Triad complexes in the junctional sarcoplasmic reticulum*

Comparison of the relative electrophoretic mobility of triadin under reducing versus non-reducing conditions strongly indicates that triadin behaves

like a disulfide-bridged protein cluster. While reduced triadin runs at a relative apparent molecular mass of 95 kDa, the estimated size of its non-reduced oligomeric structure is above 3000 kDa (Fig. 2a). Since in the top area of polyacrylamide gels the relationship between the electrophoretic mobility of a protein and the logarithm of its molecular mass is not linear [49], only a comparison to very high-molecular-mass proteins such as titin could give an indication of the enormous size of the triadin aggregates. Immunoblot analysis of the ryanodine receptor Ca^{2+}-release channel revealed a major band of apparent 565 kDa under reducing conditions and two main bands under non-reducing conditions, one of approximately 2300 kDa and the other one above 3000 kDa (Fig. 2b). The two non-reduced bands probably represent ryanodine tetramers and large heterogeneous ryanodine receptor-containing triad protein complexes, respectively. The top section of the highest molecular mass band recognized by the polyclonal antibody to the RyR1-isoform exhibited approximately the same position as the non-reduced triadin band. Following chemical crosslinking with the hydrophobic 1.2 nm probe dithiobis-succinimidyl propionate, the immunodecoration of triadin intensified and the triadin band also showed a slight shift to a complex of even higher relative molecular mass (Fig. 2a). Interestingly, at the lower crosslinker concentration of 50 μg/mg protein no change in the electrophoretic mobility of the ryanodine receptor was observed. Only a higher crosslinker concentration caused a shift of all detectable RyR1-species to the very high-molecular-mass species (Fig. 2b). Although the resolution of one-dimensional polyacrylamide gel systems is limited, these findings indicate that native triadin is mostly self-aggregated, but appears to exist in close proximity to the Ca^{2+}-release channel units.

Other abundant triad proteins involved in excitation–contraction coupling, such as the major Ca^{2+}-binding protein calsequestrin and the transverse-tubular dihydropyridine receptor, did not exhibit drastic changes in their electrophoretic mobility between reducing and non-reducing conditions. None of their non-reduced protein bands overlapped with the high-molecular-mass triadin band. Monoclonal antibody $VIIID1_2$ to calsequestrin recognized both the major calsequestrin monomer of apparent 63 kDa and high-molecular-mass proteins between 120 kDa and

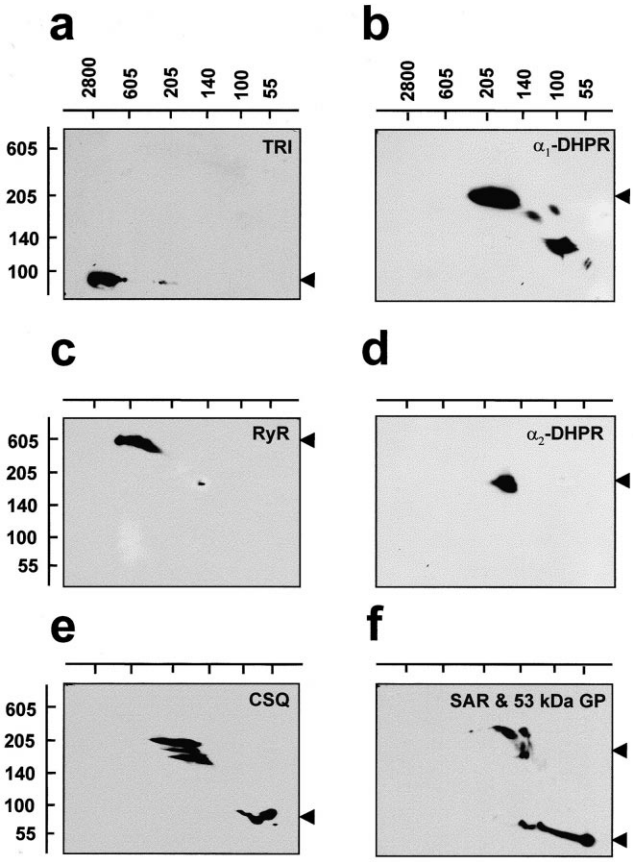

Fig. 3. Self-aggregation of triadin in the sarcoplasmic reticulum. Shown are immunoblots of rabbit muscle membrane proteins which had been separated by diagonal non-reducing/reducing two-dimensional gel electrophoresis. Immunodecoration was performed with monoclonal antibody IIG12 to triadin (TRI) (a), monoclonal antibody IIID5 to the α_1-subunit of the dihydropyridine receptor (α_1-DHPR) (b), polyclonal antiserum to the ryanodine receptor (RyR) (c), monoclonal antibody 20A against the α_2-subunit of the dihydropyridine receptor (d), monoclonal antibody $VIIID1_2$ to calsequestrin (CSQ) (e), as well as monoclonal antibody XIIC4 against sarcalumenin (SAR) (f), which also recognizes the sarcoplasmic reticulum glycoprotein of 53 kDa (53 kDa GP) (f). Monoclonal antibody $VIIID1_2$ also recognizes calsequestrin-like proteins of higher relative molecular mass than the 63 kDa calsequestrin monomer (e). The position of immunodecorated monomers in the second dimension is marked by arrowheads and sizes of molecular mass standards (in kDa), as deduced from rat myofibrillar proteins, are indicated on the left.

190 kDa representing calsequestrin-like proteins (Fig. 2c). Antibody labeling of the α_1-subunit of the dihydropyridine receptor did not reveal a difference in relative molecular mass between the reduced and non-reduced molecule (Fig. 2d), while immunodeco-

ration of the α_2-subunit showed a slight difference in molecular mass using these two conditions (Fig. 2e). The decrease in electrophoretic mobility of the reduced α_2-subunit is due to the loss of δ-subunits, otherwise bound via disulfide bridges to the larger subunit of this receptor.

3.3. Self-aggregation of triadin in the sarcoplasmic reticulum

Since monoclonal antibody IIG12 recognizes triadin much more reliably under reducing conditions and since two-dimensional electrophoretic techniques have a better resolving power than one-dimensional systems, we repeated the immunoblot analysis of Fig. 2 with a diagonal two-dimensional gel system as illustrated in Fig. 3. Diagonal non-reducing/reducing two-dimensional gel electrophoresis is an ideal analytical procedure for differentiating between protein species existing as monomers or predominantly as oligomeric structures in native membranes under non-reducing conditions. In contrast to chemical crosslinking techniques, which can give information about close neighborhood relationships of even weakly associated complexes, two-dimensional analysis in the presence of ionic detergent exclusively recognizes complexes covalently aggregated by disulfide bridges. The combination of these two biochemical techniques can give sufficient information to draw basic conclusions about the spatial configuration of large membrane protein complexes. If a protein does not change its electrophoretic mobility between non-reducing and reducing conditions, it runs on the diagonal of the two-dimensional slab gel. In stark contrast, if a component of a disulfide-bonded high-molecular-mass complex is electrophoresed under reducing conditions in the second dimension, a distinct shift off the diagonal is observed.

The comprehensive two-dimensional immunoblot analysis, as shown in Fig. 3, illustrates this analytical principle and confirms the existence of very large triadin complexes under non-reducing conditions. Most importantly, with respect to very high-molecular-mass complexes, no distinct overlap of immunoreactivity was observed between the ryanodine receptor, the α_1-dihydropyridine receptor, the α_2-dihydropyridine receptor, calsequestrin, calsequestrin-like proteins and triadin (Fig. 3a–e). Especially significant is the finding that the molecular species representing the largest complexes of the ryanodine receptor and triadin differ in their electrophoretic mobility (Fig. 1a,c). This result clarifies the potentially ambiguous data of one-dimensional immunoblot analysis (Fig. 2) and suggests that under non-reducing conditions both triad components do not exist as tightly associated supramolecular complexes.

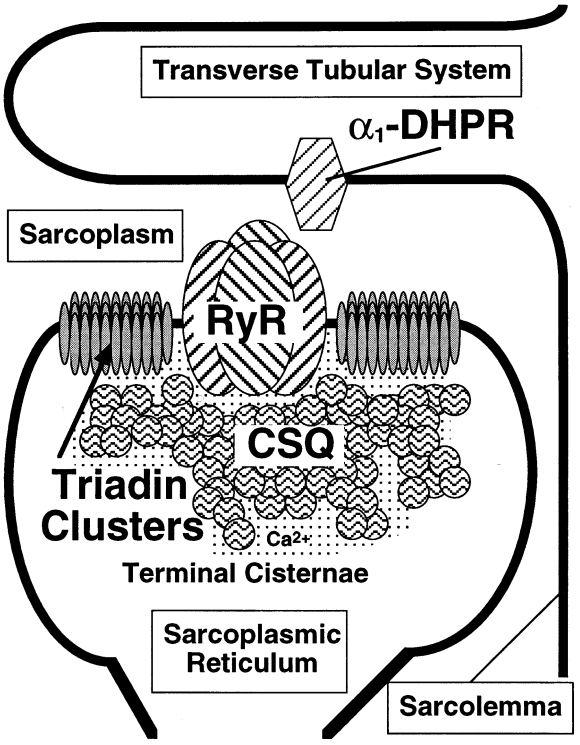

Fig. 4. Diagrammatic presentation of the spatial configuration of key triad components involved in excitation–contraction coupling at skeletal muscle junctional couplings. Shown is the terminal cisternae region, the junctional sarcoplasmic reticulum membrane and the surface membrane invaginations forming the transverse tubular system. While the voltage-sensing α_1-subunit of the dihydropyridine receptor (α_1-DHPR) is localized to the transverse tubules, the ryanodine receptor (RyR) Ca^{2+}-release channel is found to be enriched in the junctional sarcoplasmic reticulum membrane. The major Ca^{2+}-binding protein of the sarcoplasmic reticulum, calsequestrin, is present in the luminal region of the terminal cisternae. The results presented in this report suggest that triadin exists primarily as large self-aggregates in the junctional membrane of the sarcoplasmic reticulum. Although triadin clusters are located in close proximity to the Ca^{2+}-release channel tetramers, this abundant triad component does not appear to be tightly linked to the other major constituents of skeletal muscle junctional couplings believed to play a central role in the regulation of the excitation–contraction–relaxation cycle and Ca^{2+}-homeostasis.

Thus, although chemical crosslinking implies that both triadin clusters and ryanodine tetramers exist in a close neighborhood relationship in the junctional sarcoplasmic reticulum (Fig. 2a,b), triadin appears to predominantly form self-aggregated complexes under native conditions (Fig. 3a). For control purposes, nitrocellulose replicas of two-dimensional slab gels were also immunodecorated with monoclonal antibody XIIC4 which recognizes sarcalumenin of apparent 160 kDa and the immunologically related 53 kDa sarcoplasmic reticulum glycoprotein (Fig. 3f). Both proteins are abundant markers of the sarcoplasmic reticulum and their immunodecoration pattern did not overlap with the high-molecular-mass band of triadin, showing the specificity and resolving power of the two-dimensional gel system employed in this study.

4. Discussion

The glycoprotein triadin of the skeletal muscle sarcoplasmic reticulum is an abundant component of the junctional face membrane and is considered to play an important role in triad architecture and/or regulation of Ca^{2+}-homeostasis [22–37]. A recent report suggests a potential role for triadin is to act as a negative regulator of the ryanodine receptor, while calsequestrin would represent an activator of Ca^{2+}-release units[38]. Our comparative immunoblot analysis of skeletal muscle triadin employing one- and two-dimensional gel electrophoresis under reducing and non-reducing conditions and electrophoretic separation following chemical crosslinking revealed that the 95 kDa monomer exists under native conditions as an extremely high-molecular-mass polymer of above 3000 kDa. These triadin clusters appear to be closely located to the ryanodine receptor Ca^{2+}-release channel tetramer. However, a distinct immunoreactive overlap between protein bands representing the two protein complexes was only observed following chemical crosslinking. Two-dimensional gel electrophoresis with higher resolving and detection power than conventional one-dimensional electrophoresis did not reveal a direct coupling between both triad proteins under non-reducing and non-crosslinked conditions. This suggests that both protein species, although they exist in a close neighborhood relationship, are not tightly coupled to each other. Furthermore, in contrast to other triadic proteins, triadin appears to form mainly self-aggregates under non-reducing conditions. These results agree with previous findings on triad complex formation in native skeletal muscle vesicles [39–41] and are summarized diagrammatically in Fig. 4.

The transverse tubular α_1-dihydropyridine receptor and the ryanodine receptor of the sarcoplasmic reticulum form complexes as demonstrated by differential co-immunoprecipitation experiments [52], binding studies with receptor domains [53] and ultrastructural co-localization [54]. However, if these triad receptor complexes are chemically crosslinked, separated on a non-reducing gel, excised and then re-electrophoresed under reducing conditions, the abundant junctional protein triadin is not detectable [39,41]. Triadin appears to form disulfide-bonded homo-polymers which are in the close vicinity, but not directly linked, to the major triadic complex consisting of the ryanodine receptor, the α_1-dihydropyridine receptor and calsequestrin [40]. Consequently triadin clusters might be of central importance to the overall structural integrity of junctional couplings in the muscle periphery and thereby provide indirectly the functional linkage between the luminal Ca^{2+}-reservoir and the Ca^{2+}-release units. However, under physiological conditions direct coupling appears to be tighter between the two triad receptors and the major Ca^{2+}-binding protein, as compared to triadin clusters.

Dysgenic myotubes, which are lacking the α_1-dihydropyridine receptor, exhibit a reduction in the expression of triadin and the ryanodine receptor [55]. However, both junctional components are still capable of forming clusters and attain their mature distribution with respect to cross-striation [55]. This would agree with our hypothesis that triadin exists mainly in self-aggregates and that its association with the transverse tubules is largely independent of direct interactions with the α_1-subunit of the dihydropyridine receptor complex. Although physical coupling between the voltage sensor and the Ca^{2+}-release channel underlie excitation–contraction coupling in mature skeletal muscle fibers, triad receptor interactions do not appear to be responsible for the initial formation and maintenance of junctional couplings [56]. Other triadic components such as triadin clus-

ters or the 90 kDa JSR protein might be involved in the actual structural formation of membrane–membrane couplings in the muscle periphery. In the dyspedic mouse, which lacks detectable levels of the ryanodine receptor resulting in perinatal death, triadin, calsequestrin and the dihydropyridine receptor are present [57]. Furthermore, in a transgenic myogenic cell line lacking the ryanodine receptor, both the dihydropyridine receptor and triadin are detectable [58,59]. Hence, localization of the Ca^{2+}-release channel to the junctional sarcoplasmic reticulum is not a prerequisite for the targeting of triadin to the junctional face membrane in skeletal muscle fibers. This also confirms the above described finding that disulfide-bonded triadin clusters are not tightly associated with other components of the junctional couplings.

It remains to be determined what role triadin oligomerization plays in the development of the excitation–contraction coupling apparatus [60] and whether triadin exclusively mediates interactions between the dihydropyridine receptor and the ryanodine receptor [34,36] or is involved in functional interactions between calsequestrin and the Ca^{2+}-release channel [30,31]. Elucidation of triadins role in excitation–contraction coupling of mature skeletal muscle fibers can probably only be achieved by refined methodological approaches such as future studies of triadin knock-out mice. Nevertheless, here we could clearly show that triadin forms large self-aggregates under non-reducing conditions in native membranes and thus appears not to be directly linked to the other main triad components implicated in the regulation of the excitation–contraction–relaxation cycle and Ca^{2+}-homeostasis. Homo-polymers of this 95 kDa junctional protein are therefore good potential candidates for forming the structural backbone responsible for triad maintenance. Thus in analogy to the dystrophin–glycoprotein complex, which stabilizes the sarcolemma during muscle contraction by providing a link between the actin membrane cytoskeleton and the extracellular matrix component merosin [61], triadin could be one of the major structural elements mediating the interaction between the terminal cisternae and the transverse tubules.

Acknowledgements

This work was supported by a TMR Network Grant from the European Community (FMRX-CT960032) to K.O. We are indebted to Dr. Kevin P. Campbell for his generous gift of monoclonal antibodies to muscle proteins used in this study.

References

[1] M.B. Cannell, C. Soeller, Trends Pharmacol. Sci. 19 (1998) 16–20.
[2] S. Fleischer, M. Inui, Annu. Rev. Biophys. Biophys. Chem. 18 (1989) 333–364.
[3] E. Rios, J. Ma, A. Gonzalez, J. Muscle Res. Cell Motil. 12 (1991) 127–135.
[4] G. Meissner, X. Lu, Biosci. Rep. 15 (1995) 399–408.
[5] C. Franzini-Armstrong, A.O. Jorgensen, Annu. Rev. Physiol. 56 (1994) 509–534.
[6] W. Melzer, A. Herrmann-Frank, H.C. Luettgau, Biochim. Biophys. Acta 1241 (1995) 59–116.
[7] B.E. Flucher, C. Franzini-Armstrong, Proc. Natl. Acad. Sci. USA 93 (1996) 8101–8106.
[8] T. Tanabe, in: C. Peracchia (Ed.), Handbook of Membrane Channels, Academic Press, New York, 1994, pp. 177–186.
[9] W.A. Catterall, Annu. Rev. Biochem. 64 (1995) 493–532.
[10] L.L. Isom, K.S. DeJong, W.A. Catterall, Neuron 12 (1994) 1183–1194.
[11] C.A. Gurnett, K.P. Campbell, J. Biol. Chem. 271 (1996) 27975–27978.
[12] C. Franzini-Armstrong, F. Protasi, Physiol. Rev. 77 (1997) 699–729.
[13] T. Wagenknecht, M. Radermacher, Curr. Opin. Struct. Biol. 7 (1997) 258–265.
[14] R. Zucchi, S. Ronca-Testoni, Pharmacol. Rev. 49 (1997) 1–51.
[15] A.B. Brilliantes, K. Ondrias, A. Scott, E. Kobrinsky, E. Ondriasova, M. Moschella, T. Jayaraman, M. Landers, B. Ehrlich, A.M. Marks, Cell 77 (1994) 513–523.
[16] L. Fliegel, M. Ohnishi, M.R. Carpenter, V.K. Khanna, R.A.F. Reithmeier, D.H. MacLennan, Proc. Natl. Acad. Sci. USA 84 (1987) 1167–1171.
[17] K. Yano, A. Zarain-Herzberg, Mol. Cell Biochem. 135 (1994) 61–70.
[18] E. Leberer, B.G. Timms, K.P. Campbell, D.H. MacLennan, J. Biol. Chem. 265 (1990) 10118–10124.
[19] A.N. Martonosi, Biosci. Rep. 15 (1995) 263–282.
[20] D.H. MacLennan, W.J. Rice, N.M. Green, J. Biol. Chem. 272 (1997) 28815–28818.
[21] W. Guo, A.O. Jorgensen, K.P. Campbell, J. Biol. Chem. 269 (1994) 28359–28365.

[22] A.H. Caswell, N.R. Brandt, J.P. Brunschwig, S. Purkerson, Biochemistry 30 (1991) 7507–7513.
[23] C.M. Knudsen, K.K. Stang, A.O. Jorgensen, K.P. Campbell, J. Biol. Chem. 268 (1993) 12637–12645.
[24] C.M. Knudsen, K.K. Stang, C.R. Moomav, C.A. Slaughter, K.P. Campbell, J. Biol. Chem. 268 (1993) 12646–12654.
[25] N.L. Taske, H.J. Eyre, R.O. O'Brien, G.R. Sutherland, M.A. Denborough, P.S. Foster, Eur. J. Biochem. 233 (1995) 258–265.
[26] W. Guo, A.O. Jorgensen, L.R. Jones, K.P. Campbell, J. Biol. Chem. 271 (1996) 458–465.
[27] S.L. Carl, K. Felix, A.H. Caswell, N.R. Brandt, J.P. Brunschwig, G. Meissner, D.G. Ferguson, Muscle Nerve 18 (1995) 1232–1243.
[28] N.R. Brandt, A.H. Caswell, S.R. Wen, J.A. Talvenheimo, J. Membr. Biol. 113 (1990) 237–251.
[29] K.C. Kim, A.H. Caswell, J.A. Talvenheimo, N.R. Brandt, Biochemistry 29 (1990) 9281–9289.
[30] W. Guo, K.P. Campbell, J. Biol. Chem. 270 (1995) 9027–9030.
[31] W. Guo, A.O. Jorgensen, K.P. Campbell, Soc. Gen. Physiol. Ser. 51 (1996) 19–28.
[32] L. Zhang, J. Kelley, G. Schmeisser, Y.M. Kobayashi, L.R. Jones, J. Biol. Chem. 272 (1997) 23389–23397.
[33] I. Marty, M. Robert, M. Ronjat, I. Bally, G. Arlaud, M. Villaz, Biochem. J. 307 (1995) 769–774.
[34] A.H. Caswell, N.R. Brandt, J.P. Brunschwig, S. Purkerson, Biochemistry 30 (1991) 7505–7513.
[35] N.R. Brandt, A.H. Caswell, J.P. Brunschwig, J.J. Kang, B. Antoniu, N. Ikemoto, FEBS Lett. 299 (1992) 57–59.
[36] H. Fan, N.R. Brandt, A.H. Caswell, Biochemistry 34 (1995) 14902–14908.
[37] G. Liu, I.N. Pessah, J. Biol. Chem. 269 (1994) 33028–33034.
[38] M. Ohkura, K. Furukawa, H. Fujimori, A. Kuruma, S. Kawano, M. Hiraoka, A. Kuniyasu, H. Nakayama, Y. Ohizumi, Biochemistry 37 (1998) 12987–12993.
[39] B.E. Murray, K. Ohlendieck, Biochem. J. 324 (1997) 689–696.
[40] B.E. Murray, G.R. Froemming, P.B. Maguire, K. Ohlendieck, Int. J. Mol. Med. 1 (1998) 677–687.
[41] B.E. Murray, K. Ohlendieck, FEBS Lett. 429 (1998) 317–322.
[42] P.B. Maguire, K. Ohlendieck, FEBS Lett. 396 (1996) 115–118.
[43] K. Ohlendieck, F.N. Briggs, K.F. Lee, A.W. Wechsler, K.P. Campbell, Eur. J. Biochem. 202 (1991) 739–747.
[44] K.P. Campbell, C. Franzini-Armstrong, A.E. Shamoo, Biochim. Biophys. Acta 602 (1980) 97–116.
[45] M. Rosemblatt, C. Hidalgo, C. Vergara, N. Ikemoto, J. Biol. Chem. 256 (1981) 8140–8148.
[46] K. Ohlendieck, J.M. Ervasti, J.B. Snook, K.P. Campbell, J. Cell Biol. 112 (1991) 135–148.
[47] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[48] S.S. Wong, Chemistry of Protein Conjugation and Crosslinking, CRC Press, Boca Raton, FL, 1991.
[49] U.K. Laemmli, Nature 227 (1970) 680–685.
[50] H. Towbin, T. Staehelin, J. Gordon, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.
[51] S.J. Bradd, M.J. Dunn, Method. Mol. Biol. 19 (1993) 211–218.
[52] I. Marty, M. Robert, M. Villaz, K. DeJongh, L. Lai, W.A. Catterall, M. Ronjat, Proc. Natl. Acad. Sci. USA 91 (1994) 2270–2274.
[53] X. Lu, L. Xu, G. Meissner, J. Biol. Chem. 269 (1994) 6511–6516.
[54] B.A. Block, T. Imagawa, K.P. Campbell, C. Franzini-Armstrong, J. Cell Biol. 107 (1988) 2587–2600.
[55] B.E. Flucher, S.B. Andrews, S. Fleischer, A.R. Marks, A.H. Caswell, J.A. Powell, J. Cell Biol. 123 (1993) 1161–1174.
[56] J.A. Powell, L. Petherbridge, B.E. Flucher, J. Cell Biol. 134 (1996) 375–387.
[57] E.D. Buck, H.T. Nguyen, I.N. Pessah, P.D. Allen, J. Biol. Chem. 272 (1997) 7360–7367.
[58] R.A. Moore, H.T. Nguyen, J. Galceran, I.N. Pessah, P.D. Allen, J. Cell Biol. 140 (1998) 843–851.
[59] F. Protast, C. Franzini-Armstrong, P.D. Allen, J. Cell Biol. 140 (1998) 831–842.
[60] G.R. Froemming, K. Ohlendieck, Biochim. Biophys. Acta 1387 (1998) 226–238.
[61] K. Ohlendieck, Eur. J. Cell Biol. 69 (1996) 1–10.