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Identification of polypeptides associated with sarcolemmal vesicles enriched in orthogonal arrays

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Electron microscopy of freeze-fracture replicas from the sarcolemmas of fast-twitch muscle fibers reveals orthogonal arrays of particles. The biochemical nature of macromolecules associated with the sarcolemmal orthogonal array was investigated using muscle fragments and isolated sarcolemmal vesicles. Muscle fragments incubated in vitro with the lectin concanavalin A exhibited a clustering of orthogonal arrays into local patches. Treatment with other lectins did not result in the clustering of arrays. Clustering was inhibited by the addition of α-methyl-D-mannoside, a ligand which also binds concanavalin A. These results suggest that the orthogonal arrays (or associated components) specifically bind concanavalin A. Sarcolemmal vesicles from rabbit sacrospinalis (SAC) and rat extensor digitorum longus (EDL) (both primarily fast-twitch) and rat soleus (SOL) (primarily slow-twitch) were obtained by a combination of low-salt fractionation and sucrose density gradient centrifugation. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins and glycoproteins solubilized from these vesicles revealed several bands. Four of these bands were present in gels from both the rabbit and rat fast-twitch muscle sarcolemmal preparations (that contained arrays), yet were absent in gels from rat slow-twitch muscle sarcolemmal preparations (not bearing arrays). An enrichment in vesicles containing arrays was achieved by binding SAC sarcolemmal vesicles to Con A-Sepharose 4B beads. SDS-PAGE analysis of array-enriched vesicles from the concanavalin A beads revealed enrichment of three major bands at M_r 93000, 54000 and 49000. These enriched bands correlate with three of the four bands common to fast-twitch EDL and SAC, yet absent in slow-twitch SOL sarcolemmal preparations. We conclude that at least one macromolecular component associated with the sarcolemmal orthogonal array is a concanavalin A binding glycoprotein. We further conclude that three candidates for this component co-purify with the morphological array, and have approximate molecular weights of 93000, 54000 and 49000.

Introduction

The plasma membrane of skeletal muscle fibers (sarcolemma) exhibits a specific membrane specialization when examined with freeze-fracture techniques. This specialization has the form of orthogonally-aggregated 6–7 nm particles clustered with a square lattice spacing of 7 nm (orthogonal arrays) [1].

The function of these arrays is unclear, and preliminary investigations have yielded only limited information concerning the biochemical

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and molecular nature of the constituent particles Orthogonal arrays of particles are known to be plentiful in the sarcolemma of normal fast-twitch skeletal muscle (such as rat extensor digitorium longus [2] and rabbit sacrospinalis [3], but scarce in normal slow-twitch muscle such as rat soleus [2]. The number of arrays is reduced in fast-twitch membranes in human Duchenne muscular dystrophy [4] and in the dystrophic mouse [5]. Similarly structured orthogonal arrays of particles have been observed in many other cell types The individual freeze-fracture particles comprising these arrays appear to be proteins [6] that span the lipid bilayer [7]. The arrays clump together in the membranes of cultured astrocytes when exposed to protein denaturants, colchicine, cytochalasin B and to CO_2 atmosphere [6]. Similarly, the arrays clump together in the plasma membranes of minced muscle fragments when incubated in solutions containing high concentrations of divalent cations [8]. These arrays have been reported to disappear rapidly (on the order of seconds) from the membranes of astrocytes subjected to N_2 atmosphere or to the metabolic inhibitor dinitrophenol [9]. Likewise, they disappear from muscle membranes when incubated with dinitrophenol [10] or in mildly acidic environments [8].

A major objective of the present study was to provide an assay for the orthogonal array in isolated membranes, thereby enabling the acquisition of more reliable data on the biochemical characteristics of array constituents or associated proteins. Previous sarcolemmal isolates from mammalian skeletal muscle have generally relied on a prolonged salt treatment to extract the contractile proteins [11–15]. Since the orthogonal array appears to be highly labile (see above), it was not likely that the arrays would remain clustered in the orthogonal conformation throughout the relatively harsh salt isolation procedures. Our assay for tracing the orthogonal array during isolation was a morphological one in which we determined the presence of arrays in the membrane using freeze-fracture electron microscopy. No information associating enzymatic activity or ligand binding characteristics with the array is yet available. Isolation of the component macromolecules is thus dependent on maintaining the array components in their aggregated state such that the assembly is recognizable in freeze-fracture replicas. Thus a more gentle isolation technique was desired. Techniques utilizing detergent solubilization of membrane proteins [16,17] would not be useful since membrane separation procedures, such as differential centrifugation, differential filtration and density gradient centrifugation [18–22] have proven useful given such constraints.

Materials and Methods

1. Sarcolemmal isolation

Rats (male Sprague-Dawley from Charles River, 150–300 g) were killed by decapitation, and the extensor digitorum longus (EDL) and soleus (SOL) muscles were rapidly placed into cold (4° C) 10 mM Tris-buffered 0.25 M sucrose (pH 7.4). Alternatively, rabbits (New Zealand White males, 2.0–3.0 kg) were anaesthetized with sodium pentobarbital (Nembutal, 60 mg/kg body weight), and the sacrospinalis (SAC) muscle was rapidly excised into cold Tris-sucrose. Sarcolemmas were isolated from all tissues by a modification of the methods of Barchi et al. [22]. The muscle was minced with scissors, washed with Tris-sucrose, and transferred to 10 volumes of fresh Tris-sucrose. Muscle was then homogenized, first in a Waring blender for 7 s and then in an Ultraturrax homogenizer for 15 s, and then passed through a 16 mesh sieve. The filtrate was centrifuged for 15 min at $100 \times g$ and the supernatant was saved. The pellet (P_1) was resuspended in 5 volumes of Tris-sucrose and recentrifuged. After two additional extractions of P_1, the four supernatants were combined and centrifuged for 30 min at $100\,000 \times g$. The resultant pellets (P_2) were resuspended in a small volume of Tris-sucrose, layered over a 28–68% continuous sucrose gradient, and centrifuged for 2 h at $100\,000 \times g$. The least dense band was collected and used in all further analyses.

2 Electron microscopy and freeze-fracture

Pellets and muscle tissues to be freeze-fractured were fixed for 12 h in cold cacodylate-buffered 2.5% glutaraldehyde-1% paraformaldehyde and washed in buffer. Specimens were cryoprotected by increasing the concentration of cold glycerols (10, 20 and 30% in cacodylate buffer for 0.5 h, 1.0 h and 2 h, respectively), and were then placed on

Balzers gold specimen supports. These samples were quickly frozen in Freon-22 maintained near its freezing point by liquid nitrogen.

Fracturing was performed in a Balzers BAF 300 freeze-etching device equipped with turbomolecular pump, a platinum-evaporating electron gun, a time and heat limiting electronic shutter [23], and an interlocked quartz crystal thin-film monitor (set at 180 Hz) to standardize replica thickness. Throughout fracturing, the vacuum was maintained below $6 \cdot 10^{-7}$ Torr, while the stage was maintained at $-115°C$. After fracturing, a platinum layer approx. 2 nm thick was deposited at an angle of 30°, and a carbon layer 40–80 nm thick was vertically evaporated to provide replica stability. Replicas were cleaned for 12–24 h in household bleach, and then for 0.5 h in 1% hydrogen peroxide. After careful rinsing in distilled water, replicas were collected on uncoated copper grids and viewed at 80 kV on a JEOL 100CX electron microscope.

3. Biochemical analyses

During the isolation procedure, the sarcolemmal membranes were followed by assaying the activity of the $(Na^+ + K^+)$-ATPase by the method of Kyte [24] except that phosphatidyl-L-serine was not included in the assay solution. Protein concentrations were determined by the method of Lowry et al. [25] using bovine serum albumin as standard. Sodium dodecyl sulfate (SDS)-polyacrylamide gels (SDS-PAGE) were electrophoresed according to the methods of Laemmli [26].

Indirect Enzyme-Linked Immunosorbant Assays (ELISAs) were used to demonstrate the specificity of two antisera for their respective antigens ($(Na^+ + K^+)$-ATPase and whole 'array prep' mixture). The method used was that of Engvall and Perlmann [27] as modified by Voller et al. [28], using horseradish peroxidase conjugated to goat anti-rabbit IgG. *o*-Phenylenediamine was used as the substrate to visualize primary antibody binding.

4. Minced muscle incubations

Minced muscle incubations were similar to those used previously [8]. In these studies, rabbit sacrospinalis samples were excised under sodium pentobarbital anaesthesia, plunged into cold, (4°C) 10 mM Tris-buffered 0.25 M sucrose (pH 7.4), minced with scissors to approx. 1 mm^3 cubes, and incubated for 3 days at 4°C. In experimental conditions, the buffered sucrose solutions also contained one of the following: (1) 2,5, 10, 20, or 50 $\mu g/ml$ concanavalin A; (2) 2, 5, 10, 20, or 50 $\mu g/ml$ wheat germ agglutinin; (3) 2, 5, 10, 20, or 50 $\mu g/ml$ soy bean agglutinin; (4) 2, 5, 10, 20, or 50 $\mu g/ml$ ricin type I; (5) 5 $\mu g/ml$ concanavalin A plus 0.1 M α-methyl-D-mannoside.

All lectins were purchased from Vector Labs, and all lectin-supplemented incubation solutions contained 1 mM $CaCl_2$ and 1 mM $MgCl_2$. All incubations contained 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) to inhibit proteolysis.

5. Batch incubation with Con A-Sepharose beads

Sacrospinalis vesicles were dialyzed against 1000 volumes of buffer containing 10 mM Tris-HCl (at either pH 7.4 or pH 6.0), 140 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.02% NaN_3. Two ml of dialyzed vesicles were added to an equal volume of Con A-Sepharose 4B beads equilibrated in the same buffer. The suspension was gently mixed for 30 min and then subjected to gentle centrifugation ($100 \times g$ for 2 min). The supernatant fluid was removed and the pellet of beads resuspended in 2 ml of 0.5 M α-methyl-D-mannoside in the same Tris-cation buffer to elute the concanavalin A bound vesicles. The suspension was again mixed for 30 min and gently spun as above. The supernatant fluid was removed, and a second α-methyl-D-mannoside elutation was performed as above. The first supernatant (concanavalin A non-binding) and the two α-methyl-D-mannoside supernatant fluids (concanavalin A binding) were analyzed by freeze-fracture electron microscopy and SDS-gel electrophoresis.

Results

1 Isolation procedure retaining arrays

As reported by Barchi et al. [22], the sucrose density gradients produced three main bands at about 27%, 33% and 36% sucrose. Assay of the $(Na^+ + K^+)$-ATPase activity showed that the highest specific activity was present in the least

dense band (see Table I). Freeze-fracture replicas of this band exhibited orthogonal arrays of particles in some of the vesicle membranes (Figs. 1 and 2).

2 SDS-gel electrophoresis of array-containing fractions

When the material in the least dense fraction of the gradients (crude membranes) was solubilized and electrophoresed on SDS-polyacrylamide gels, many distinct bands were visible by Coomassie blue staining (Fig. 3 – molecular weight standards in lane 1, rat EDL vesicles in lane 2, rat SOL vesicles in lane 3, rabbit SAC vesicles in lane 4 and SAC vesicles that had been bound and eluted from Con A-Sepharose in lane 5). We were primarily interested in the darker staining bands since particle density analysis from freeze-fracture replicas of normal intact sarcolemma suggested that the orthogonal array subunit is an abundant membrane protein [2]. Of particular interest were darker staining bands in preparations from fast-twitch rabbit SAC (lane 4) and rat EDL (lane 2) sarcolemma that appeared faint or non-existent in preparations from slow-twitch rat SOL (lane 3) sarcolemma. These criteria were met by three distinct bands with approximate molecular weights of 93000, 54000 and 31000, and a more diffuse band at about 49000

From these observations we concluded that the array-positive and array-negative sarcolemma preparations differ in polypeptide composition. We thought it possible that some bands that were reduced or absent in gels of slow-twitch sarcolemmal preparations (those from rat SOL) might correlate with the morphological orthogonal array. Furthermore, the polypeptide composition of the two fast-twitch sarcolemmal preparations (those from rat EDL and rabbit SAC) were very similar. Thus, it was possible to use the SAC preparation as a source of those polypeptides reduced or absent from SOL preparations. This was important for preparative purposes, as SAC muscle from rabbit is plentiful and homogeneous, while preparations from EDL and SOL muscles from rat are tedious and necessitate isolation on a much smaller scale.

TABLE I

SPECIFIC ATPase ACTIVITIES

Specific ATPase activity of each of three bands appearing in the sucrose density gradients Activities are expressed in pmol P_i liberated/mg protein per hour. The highest specific activity was found in the least dense band (band 1), suggesting that a majority of sarcolemmal vesicles were found here Significant activity was also found in band 3, suggesting that some aggregation of vesicles had occurred and caused these sarcolemmal fragments to migrate to a denser region of the gradient

Band	% sucrose	Total activity	μg/ml protein	ATPase activity
1	27%	3 5	0 015	233.3
2	33%	0 4	0 081	4 9
3	36%	2 8	0 047	59 6

3. Effects of lectins on array distribution in muscle fragments

We previously described an in vitro muscle fragment system [8] useful for testing the effects of various reagents on orthogonal array distribution and stability. As reported previously, control incubations of rabbit SAC in Tris-sucrose for 3 days at 4°C produced no observable changes in array density, distribution or morphology However, the addition of 2, 5, 10 or 20 μg/ml concanavalin A to the incubation produced a distinct clustering of orthogonal arrays into local patches (Fig. 4). This effect was not noted with higher concentrations of concanavalin A (e.g., 50 μg/ml), nor was there any alteration in array distribution noted after incubation with either wheat germ agglutinin, soy bean agglutinin or ricin type I in any concentration tested. The clustering effect of low concentrations of concanavalin A was inhibited by concurrent incubation with 0.1 M α-methyl-D-mannoside (Fig. 5).

4. Further purification of array-containing sarcolemmal vesicles

Since concanavalin A appeared to bind at least one of the macromolecules associated with the orthogonal array, we suspected that we could obtain a sub-population of sarcolemmal vesicles enriched in arrays based on differing concanavalin A affinities. Freeze-fracture analysis of those SAC membrane vesicles bound to and eluted from the Con A-Sepharose beads showed intact arrays on many vesicles, estimated to be 10–100-fold in-

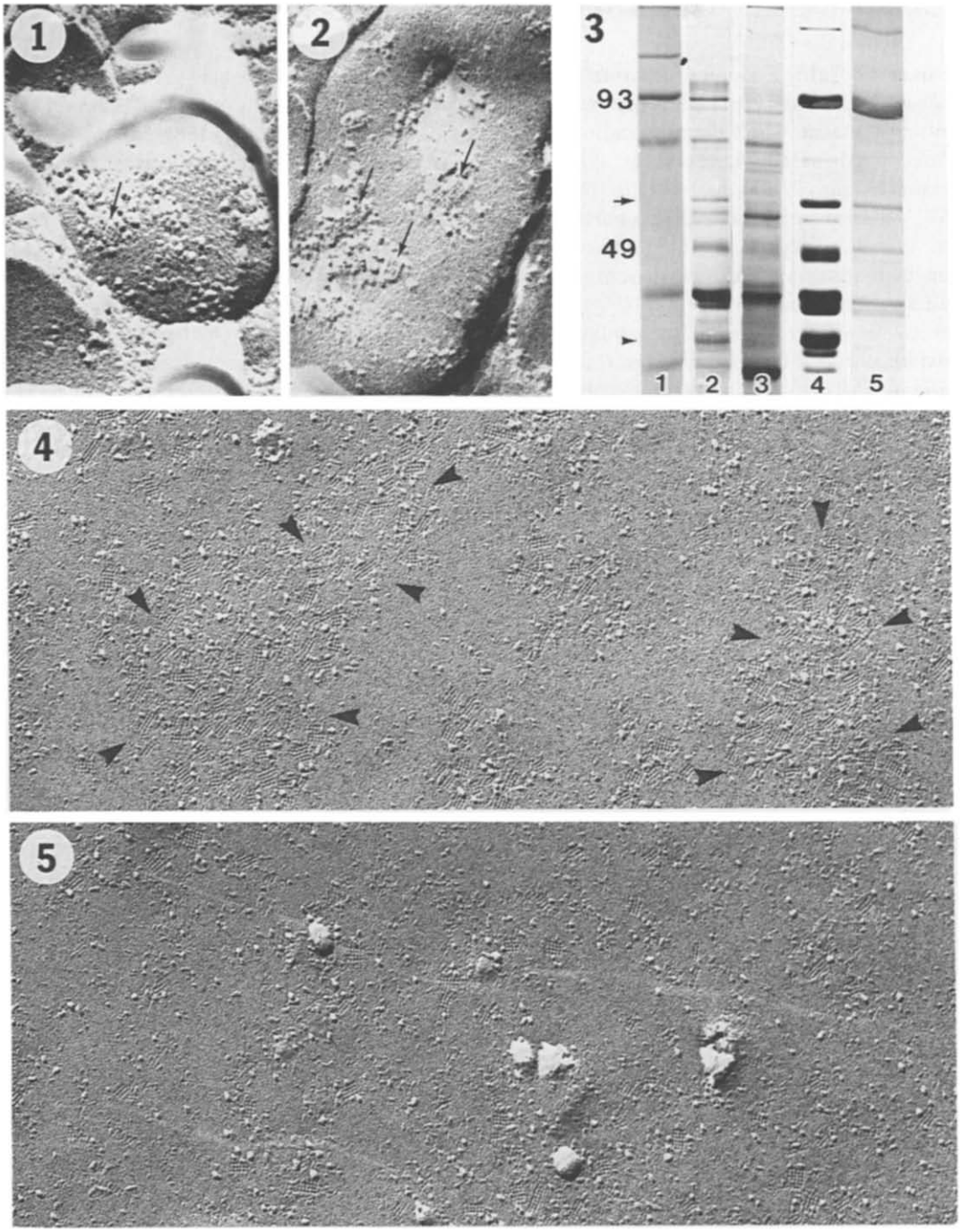

Fig. 1 Freeze-fracture replica of a sarcolemmal vesicle from the least dense band on the sucrose density gradient Note the presence of an intact orthogonal array (arrow) ×150000

Fig 2 Freeze-fracture replica of a vesicle eluted from Con A-Sepharose beads at pH 7 4 Note the presence of several intact orthogonal arrays (arrows) ×150000.

Fig 3. Tracks from SDS-gel preparations of sarcolemmal vesicles, comparing molecular weight standards (lane 1), rat EDL (lane 2), rat SOL (lane 3) and rabbit SAC (lane 4) Dense polypeptide bands common to rat EDL and rabbit SAC, yet absent or faint in rat SOL, are those at 93 and 49 kDa, as well as at 54 kDa (arrow) and 31 kDa (arrowhead) Lane 5 shows polypeptides which were bound and eluted from Con A-Sepharose beads at pH 7 4

Fig 4 Replica of sarcolemma of a musle fiber treated in vitro with 5 μg/ml concanavalin A Note the local clustering of orthogonal arrays (arrowheads) ×80000

Fig 5 Replica of sarcolemma of a muscle fiber treated in vitro with 5 μg/ml concanavalin A and 0 1 M α-methyl-D-mannoside simultaneously Note the absence of concanavalin A-induced clustering of orthogonal arrays ×80000

creased over unpurified membrane vesicles (Fig. 2). About one vesicle in 20 displayed at least one array after concanavalin A purification, and several displayed more than one array. Many vesicles also displayed single 6–7 nm particles, potentially representing array subunit particles.

When a solubilized fraction of these concanavalin A-binding vesicles were examined on SDS gels, five major bands appeared (Fig. 3, lane 5). Three of these corresponded to bands present in fast-twitch sarcolemmal preparations (lanes 2,4) yet absent in a slow-twitch sarcolemmal preparation (lane 3) (with M_r 93000, 54000 and 49000). A higher molecular weight band (M_r 150000) did not appear on our gels of rat EDL sarcolemma, and a lower molecular weight band (M_r 45000) appeared dense on our gels of slow-twitch SOL in addition to fast-twitch EDL and SAC. Thus we thought it unlikely that either of these two bands correlated with macromolecules associated with the orthogonal array. We concluded that at least one of three polypeptides which are present in fast-twitch sarcolemma yet absent from slow-twitch sarcolemma (i.e., at M_r 93000, 54000 and 49000) is Con A binding and that these three co-purify with the morphological sarcolemmal orthogonal array.

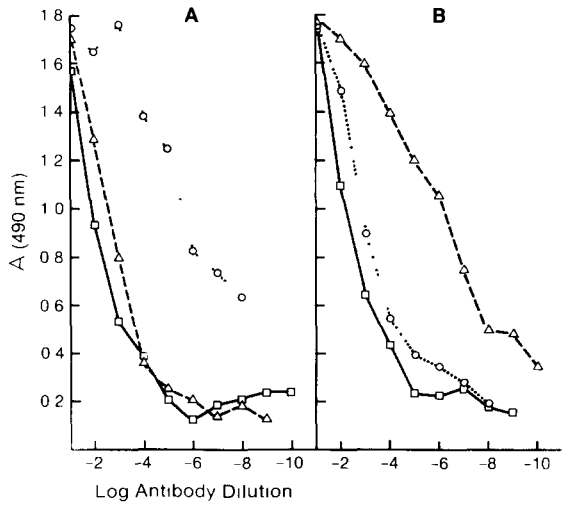

Fig. 6 ELISAs showing cross reactivity between anti-ATPase and anti-'array-prep' antibodies These results were obtained with polyclonal antisera (from guinea pigs) against rabbit $(Na^+ + K^+)$-ATPase (△– – –△), rabbit 'array prep' (○ ○) and normal guinea pig serum (□———□) Each was titered against rabbit 'array prep' antigens (A) and rabbit $(Na^+ + K^+)$-ATPase antigen (B) Compared to normal guinea pig serum controls, or to crossed antigen binding, antibodies were quite specific for their respective antigens Anti-ATPase binding to 'array prep' antigens was minimal, suggesting that the array prep was not contaminated with this antigen

5. Tests for the presence of $(Na^+ + K^+)$-ATPase in the array-enriched preparation

We produced antibodies in guinea pigs against purified $(Na^+ + K^+)$-ATPase from rabbit kidney [29], as well as against our post-concanavalin A 'array-prep'. We used these antisera to check the immuno-crossreactivity of the two preparations using 'crossed ELISAs' (Fig. 6) (see also Ref. 29). No $(Na^+ + K^+)$-ATPase immunoreactivity appears to be associated with the preparation enriched in orthogonal arrays.

Discussion

Several lines of evidence suggest that three proteins of approx. 49, 54 and 93 kDa are candidate components of the sarcolemmal orthogonal array. First, these three proteins are among those present in preparations of two types of fast-twitch sarcolemmas yet absent from slow-twitch sarcolemmal preparations. Second, concanavalin A treatment of skeletal muscle is known to cluster orthogonal arrays. These three proteins are among those preserved when sarcolemmal vesicles are sub-fractionated over Con A-Sepharose beads. Third, these three proteins are enriched in preparations which are also enriched in morphological orthogonal arrays.

The first step in obtaining this evidence was the isolation from fast-twitch skeletal muscle sarcolemma of vesicles which display orthogonal arrays when examined with freeze-fracture techniques. The low salt procedure described here does produce membrane vesicles which display intact orthogonal arrays. However, the yield of vesicles displaying arrays is much lower than might be expected based on the density of arrays in native muscle membranes. This is perhaps due to the rather harsh mechanical treatment used to separate the sarcolemma from the surrounding collagenous sheath and from the internal cytoskeletal proteins. Extraction with salt solutions and treatment with collagenase were tried previously and

rejected when the membrane vesicles produced were found to be devoid of orthogonal arrays. Milder chemical treatments designed to relax or dissociate muscle fibers may result in greater overall yield of sarcolemmal vesicles and also a greater percentage of vesicles bearing arrays.

Another possibility considered was that endogenous proteolysis might occur during the membrane isolation steps and contribute to the low yield of orthogonal arrays. However, the addition of EDTA andPMSF (as proteolysis inhibitors) to our isolation buffer did not increase array number nor change the protein composition as evidence by SDS-gel electrophoresis or freeze-fracture techniques. Although some proteases may not have been affected by this treatment, we suspect that endogenous proteolysis was not the major cause of our low yields of arrays.

In vitro muscle incubation experiments indicated some type of interaction between the arrays and concanavalin A. The clustering of arrays noted in replicas of concanavalin A-treated muscle was not as pronounced (that is, clusters of arrays were not as tightly packed) as that obtained by treatment with cytoskeletal disruptors or protein denaturants [6]. However, the observed clustering of arrays was repeatable, dose-consistent, and blocked by high concentrations of concanavalin A (see Ref. 30). Clustering of arrays was not observed following concurrent incubation with concanavalin A and α-methyl-D-mannoside. These results suggest that a component of the orthogonal array contains one or more high mannose-type oligosaccharides.

A comparison of two fast-twitch (array-rich) muscle sarcolemmal preparations (rabbit SAC and rat EDL) with one slow-twitch (array-barren) muscle sarcolemmal preparation (rat SOL) was used to investigate which bands on our SDS gels were likely to be associated with the morphological array. This cross-species comparison may have eliminated some bona-fide array subunit candidates, since some differences in banding patterns may be due to interspecies protein heterogeneity within the same protein. However, there is evidence that at least one other membrane specialization represented by an aggregate of protein particles, the gap junction, is biochemically conserved across a wide range of species in any given organ (cf. Ref. 31). Interestingly, gap junctional proteins may not be conserved between various tissues [31]. If the same is true of orthogonal array protein(s), tissue specificity could make comparative biochemical investigations very difficult.

In a previous report [32] we mentioned that examination of partially purified sarcolemma of EDL and soleus revealed two proteins of 117 and 130 kDa, present in large amounts in EDL, but virtually absent in soleus. In that study lactoperoxidase iodination was used to label whole muscle with ^{125}I and then survey EDL vs. soleus for major differences in proteins labeled from outside before cell fractionation. There are many membrane proteins whose concentration is now known to differ between fast and slow twitch muscle but which do not correlate with the orthogonal arrays when purified preparations are freeze-fractured (e.g., the $(Na^+ + K^+)$-ATPase) The approach of these earlier studies was abandoned soon after it began in favor of the approach employed in the present work which we feel has a greater likelihood of yielding information about the orthogonal array. An antibody preparation against the proteins of the array enriched vesicles has been produced and interestingly has been shown to stain array rich regions of astrocytes at the light and electron microscopy levels [33]. We are presently pursuing this as a most hopeful approach for identifying and purifying the array components.

We cannot assume that other bands which do not bind concanavalin A are not associated with the orthogonal array. In particular, there is at least one band (31 kDa) which does not appear to be concanavalin A binding, but is present in SAC and EDL sarcolemma lpreparations yet absent in SOL sarcolemmal preparations. Furthermore, it would be unreasonable to conclude that each of these three bands represent components of the array. The sarcolemmal preparation is of course enriched in other proteins as well, at least before concanavalin A purification. For instance, we know that the crude preparation is enriched in $(Na^+ + K^+)$-ATPase. Vanadate-ATP is known to cluster the $(Na^+ + K^+)$-ATPase into hexagonal lattice [34]. We examined vanadate-ATP-treated $(Na^+ + K^+)$-ATPase with freeze-fracture and found no 'array-like' clusters in the cleared mem-

branes (data not shown). Furthermore, we found no hexagonal lattice structures in our purified sarcolemmal vesicle preparation. Also, our 'crossed-ELISA' test (Fig. 6) showed no immunoreactivity between the post concanavalin A array-preparation and antisera against ATPase. Thus, the $(Na^+ + K^+)$-ATPase is not a measurable component of the array-enriched preparation. Further work with the anti-array-preparation antibodies should define the relationships of the candidate peptides to the orthogonal array.

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References

1 Rash, J E, Ellisman, M H., Staehelin, L A and Porter, K R (1973) Excerpta Med Int Congr Ser 333, 271
2 Ellisman, M H, Rash, J.E, Staehelin, L A and Porter, K R (1976) J Cell. Biol. 68, 752
3 Smith, D S, Baerwald, R J and Hurt, M A (1975) Tissue Cell 7, 369
4 Schotland, D L, Bonilla, E and Wakayama, Y (1980) Muscle Nerve 3, 21
5 Ellisman, M.H. (1981) Brain Res. 214, 261
6 Anders, J J and Brightman, M W. (1982) J Neurocytol 11, 1009
7 Landis, D M D, Reese, T S, Ornberg, R L and Graham, W F (1981) Soc Neurosci. Abstr 7, 305
8 Hatton, J D and Ellisman, M H (1982) Soc Neurosci Abstr 8, 696
9 Landis, D M D and Reese, T S (1981) J Cell Biol 88, 660
10 Fischbeck, K H, Bonilla, E and Schotland, D L (1982) J Cell Biol 95, 362a
11 Peter, J B (1970) Biochem Biophys Res Commun 40, 1362
12 Andrew, C G and Appel, S H (1973) J Biol Chem 248, 5156
13 Vandenburgh, H H, Sheff, M F and Zacks, S I (1974) J Membrane Biol 17, 1
14 Smith, P B and Appel, S H (1977) Biochim Biophys Acta 466, 109
15 Barchi, R L, Weigele, J B, Chalikian, D.M and Murphy, L E (1979) Biochim Biophys Acta 550, 59
16 Samaha, F J and Gergely, J. (1965) Arch Biochem Biophys 109, 76
17 Kracke, G R, Beringer, T and Koenig, E. (1975) Exp Neurol 46, 32
18 Kidwai, A M, Radcliffe, M A, Lee, E A and Daniel, E.E (1973) Biochim Biophys Acta 298, 593
19 Kidwai, A M. (1974) Methods in Enzymology (Fleischer, S and Packer, L, eds), Vol. 31, p 134, New York
20 Boegman, R J (1974) Comp Biochem 48, 237
21 Caswell, A H, Lau, Y H. and Brunschwig, J-P (1976) Arch Biochem Biophys 176, 237
22 Barchi, L L, Bonilla, E and Wong, M (1977) Proc Natl Acad Sci USA 74, 34
23 Ellisman, M H and Staehelin, L A (1979) Freeze-fracture Methods, Artifacts and Interpretations (Rash, J E and Hudson, C S, eds), p 123, New York
24 Kyte, J (1971) J Biol Chem. 246, 4157
25 Lowry, O H, Rosebrough, N J, Farr, A L and Randall, R J (1951) J Biol Chem 193, 265
26 Laemmli, U K (1970) Nature 227, 680
27 Engvall, E and Perlmann, P (1972) J Immunol 109, 129
28 Voller, A, Bidwell, D E. and Bartlett, A (1979) The Enzyme-Linked Immunosorbent Assay (ELISA) A guide with abstracts of microplate applications
29 Ariyasu, R G., Nichol, J A. and Ellisman, M H (1985) J Neurosci 5, 2581
30 Bourguinon, L Y.W and Rozek, R J (1980) Cell Tissue Res 205, 77
31 Nicholson, B J, Hunkapiller, M W, Grim, L B, Hood, L E and Revel, J-P (1981) Proc Natl Acad Sci USA 78, 7594
32 Ellisman, M.H, Brooke, M.H, Kaiser, K K. and Rash, J E (1978) Exp Neurol 58, 59–67
33 Hatton, J D, Cox, G F, Miller, A L, Nichol J A and Ellisman, M H (1986) Soc Neurosci Abstr 12, 1461
34 Zampighi, G, Kyte, J and Freytag, W (1984) J Cell Biol 98, 1851