Crystalline Structure of Sarcoplasmic Reticulum from Scallop

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ABSTRACT  Negatively stained sarcoplasmic reticulum from the scallop Placopecten magellanicus presented a variety of crystalline forms, the most common being tubular structures. These were characterized by paired rows of morphological units, spaced at ~120 Å, running diagonally across the tubules. The orthogonal unit cell (120 × 55 Å) contained two units, related by a twofold axis, which probably represented the part of the Ca2+-ATPase molecule projecting from the outer surface of the membrane.

Fragmented sarcoplasmic reticulum (FSR)1 prepared from rabbit skeletal muscle and membranes reconstituted in vitro from SR Ca2+-ATPase have been studied extensively by electron microscopy and x-ray diffraction. Negatively stained rabbit FSR shows vesicles of average diameter 0.15 µm (1) covered on their outer surface with projections ~60 Å long and 30–40 Å diam which represent part of the Ca2+-ATPase covered on their outer surface with projections ~60 Å long and 30–40 Å diam which represent part of the Ca2+-ATPase presented a variety of crystalline forms, the most common being tubular structures. These were characterized by paired rows of morphological units, spaced at ~120 Å, running diagonally across the tubules. The orthogonal unit cell (120 × 55 Å) contained two units, related by a twofold axis, which probably represented the part of the Ca2+-ATPase molecule projecting from the outer surface of the membrane.

MATERIALS AND METHODS

Preparation of Sarcoplasmic Reticulum Vesicles: Live sea scallops (Placopecten magellanicus) were obtained from the Marine Biological Laboratory, Woods Hole, MA. Strips of the striated adductor muscle were excised from the animal and chemically skinned in a buffered solution containing 0.05–0.1% wt/vol saponin, 100 mM NaCl, 8 mM MgSO4, 5 mM EGTA, 5 mM ATP, and 10 mM sodium phosphate, pH 7.0, for 3 h. The muscle bundles were then thoroughly washed with the same buffer without saponin (relaxing medium) and minced finely to allow a detailed structure analysis, although treatment with vanadate has recently been reported to cause an ordered surface array of subunits (11). Scallop SR consists of cisternae connected by tubular elements, and is closely associated with the sarcolemma (12). We have prepared FSR from the cross-striated adductor muscle of Placopecten magellanicus and examined it by electron microscopy. Negatively stained vesicles showed a crystalline appearance, and images of one class of tubular SR have been analyzed by two-dimensional reconstruction techniques.

1 Abbreviation used in this paper: FSR, fragmented sarcoplasmic reticulum.
by Castellani et al. (20), in an evaporator (Edwards High Vacuum, Grand Island, NY) at a 45° angle.

Electron micrographs were recorded in a Philips 301 electron microscope, fitted with an anticontamination device and calibrated using tropomyosin Mg2+-paracrystals. Micrographs were screened with a surveying optical diffractometer (21). Images giving the clearest diffraction patterns were selected (22) and digitized on a raster corresponding to ~6 Å in the image. Two-dimensional processing was carried out as described by Baker and Amos (22).

RESULTS

Membrane Composition and Activities

Polyacrylamide SDS gels of purified scallop FSR showed a major component with Mr = 106,000, the same as rabbit SR Ca2+-ATPase (e.g., reference 7). This accounted for ~85% of the protein in the membrane preparations (Fig. 1). The other proteins present in significant amounts had molecular weights of 59,000, 26,000, and 20,000, although none of these accounted for >5% of the total protein. A small contamination of actin (Mr = 42,000; <1% total protein) was sometimes present. These preparations had a Ca2+-ATPase activity 80–90% insensitive to oligomycin, with half-maximal activation at 0.1–0.2 μM free Ca2+. Typical enzyme activities are shown in Table I. Ca2+-ATPase activities of scallop FSR were high compared with those of rabbit. This may have been due to leakiness of the vesicles since calcium uptake, although present, was poor.

Electron Microscopy

Negatively stained preparations of FSR showed a variety of crystalline forms in the electron microscope, the most common being tubular structures (Fig. 2). These sometimes branched and often originated in sacs or cisternae. Spherical vesicles were also observed showing projections ~60 Å long emerging from the outer surface. Uncollapsed tubules were filled with stain and appeared to have a fringe of projections at the edges, similar to that of spherical vesicles. Their diameters ranged between 450 and 700 Å. Tubules collapsed onto the grid were uniformly stained and varied in width between 750 and 1,200 Å. This variability of diameters was probably due to intrinsic differences in tubule sizes as well as to a variable degree of flattening when they were laid on a grid. The superimposed image of the front and back half of the tubule, however, was similar in both uncollapsed and collapsed forms. All of them were characterized by a pattern of striations running diagonally across the tubules with a repeat of ~120 Å. There were two apparently identical rows of morphological units, 60 Å apart with different amounts of stain between alternate rows. Their size was comparable to that of the projections observed more easily on the surface of spherical vesicles and on negatively stained rabbit SR.

FSR unidirectionally shadowed with platinum showed features from only the front half (away from the support film) of the tubules (Fig. 3). Pronounced striations running diagonally across the tubules, seen more clearly in the collapsed forms, repeated at ~120 Å and wound in a right-handed manner. Under favorable orientation of the tubules to the direction of the metal stream, a finer midway striation was observed.

Images of negatively stained collapsed tubules and their computed diffraction patterns are shown in Fig. 4. The pattern of reflections (Fig. 4, b and d) could be indexed by two orthogonal lattices (1/120 × 1/55 Å2) related by a vertical mirror line. The two lattices were produced by the superimposed front and back halves of the tubules. The variation in width of the tubules led to different relative orientations of the front and back lattices, and this sometimes caused some of their reflections to overlap. An example of a diffraction pattern where reflections from the two lattices overlap is shown in Fig. 4b. The reflections from the two lattices are well resolved in Fig. 4d. Diffraction patterns from images of

![Figure 1](image-url)  
**Figure 1** Densitometry trace of SDS polyacrylamide gel (7.5%) of scallop adductor FSR. The peaks corresponding to Mr = 106,000, 59,000, 26,000, and 20,000 are indicated (arrows). Double arrows mark dye front. Tail-less arrow indicates direction of migration in the gel.

![Table I](table-url)  
**Table I** Typical Enzyme Activities of Scallop FSR

| Activity                  | Value (μmol/min/mg) |
|---------------------------|---------------------|
| Ca2+-ATPase               |                     |
| +1 mM EGTA                | 0.163               |
| +0.3 μM free Ca2+         | 5.287               |
| Oligomycin-in sensitive   | 4.042               |
| Ca2+ ATPase               |                     |
| +1 mM ouabain             | 0.124               |
| +5 mM ATP                 | 4.166               |
| Na+,K+-ATPase             |                     |
| No ouabain                | 0.118               |
| +1 mM ouabain             | 0.023               |
| Succinic dehydrogenase    | 0.095               |
| (nmol/min/mg)             | 2.21                |
| Ca2+ uptake               |                     |
| No ATP                    | 2.4 (av. 6)         |
| +5 mM ATP                 | 60.7 (av. 6)        |
| Active uptake             | 58.3                |

* Determined in 0.1 M KCl, 5 mM potassium oxalate, 5 mM MgCl2, ± 5 mM ATP, 0.1 mM 4CaCl2, 40 mM Tes, pH 7.0, at 25°C in a volume of 1.1 ml containing 100 μg of membrane protein.
Figure 2: Electron micrographs of negatively stained scallop FSR. The top three images show tubules filled with stain. The bottom row shows tubules collapsed onto the grid. Dark striations (stain) alternate with rows of morphological units (white) running diagonally across the tubules. Bar, 0.5 μm. x 82,000.

The scallop muscle was initially disrupted in a relaxing medium containing EGTA in order to minimize the amount of homogenization that may disrupt the membranes. Duggan and Martonosi (23) showed that treatment of rabbit SR with EGTA increases the Ca\(^{2+}\)-ion permeability of the vesicles, and this may explain the high ATPase activities and low Ca\(^{2+}\) uptake shown by scallop FSR preparations. The crystalline appearance of these negatively stained membranes did not seem to be induced by the staining since thin sections of fixed preparations showed features similar to those of negatively stained images (data not shown). In addition, unidirectionally shadowed vesicles showed a highly ordered surface structure very similar to that in negatively contrasted images.

The question arises as to the relationship of the morphological units seen in the reconstructed image and the Ca\(^{2+}\)-ATPase. The 106,000-mol-wt protein seen on SDS gels of purified scallop FSR represented ~85% of the membrane protein and appeared to correspond to the Ca\(^{2+}\)-ATPase. Like rabbit SR, scallop SR had a fringe of projections 30–40 Å wide and ~60 Å long: only the 106,000-mol-wt protein was present in sufficient quantity to account for these. The units seen in the reconstructed image had an approximate diameter of 36 Å. If it were assumed that they corresponded to the 60-Å-long projections and penetrate across the membrane, they would have an approximate cylindrical shape of 36 × 100 Å with a volume of 1.02 × 10^5 Å\(^3\), quite close to the anhydrous volume of a protein with molecular weight 106,000 and partial specific volume 0.73 ml/gm, 1.28 × 10^5 Å\(^3\). Thus, the units seen in the reconstructed image corresponded to single ATPase molecules, rather than a domain of the ATPase. The arrangement of staggered pairs of units related by a twofold axis might suggest a possible dimeric functional grouping of ATPase molecules; however, the relationship of the strong and weak contacts between the morphological units to a possible oligomeric organization of the ATPase molecules will require further studies, now in progress, using low-dose electron microscopy.

Scallop FSR membranes strongly resemble vanadate-

unidirectionally shadowed tubules determined the assignment of reflections to the front or back side.

Negatively stained tubules that showed nonoverlapping diffraction patterns from front and back sides have been used for two-dimensional reconstruction (an example is shown in Fig. 5). The two sides, analyzed separately, appeared very similar. The back side, however, seemed consistently better preserved, probably owing to the support provided by the stain. The reconstructed image (Fig. 5c) shows the unit cell oriented with the short axis at an angle of ~55° to the tubule axis. It contained two morphological units ~36 Å diam related by a twofold axis. The strongest contact between units gave rise to rows in a direction parallel to that of the short unit-cell axis. Adjacent rows were staggered by about half a unit. Pairing of the rows appeared to be due to a weaker contact between staggered units.

Discussion

The membranes described in this paper can be identified as SR on the basis of enzyme activity, composition, and their substantial morphological similarity to rabbit SR.

Figure 3: Electron micrographs of SR tubules undirectionally shadowed with platinum. Prominent 120-Å striations are seen. The image on the far right also shows finer striations lying between the major striations, caused by exclusion of the metal. Bar, 0.5 μm. x 82,000.
treated rabbit SR (11), although the scallop preparation is enzymatically active and is not exposed to vanadate at any point. The same highly ordered membranes are seen in homogenates of the muscle made in the absence of ATP (e.g. 1 mM EGTA, 45 mM Tes, pH 7.5), so that vanadate contamination of the ATP is not a factor. Interestingly, the scallop SR also shows resemblances to two-dimensional crystalline arrays of Na⁺, K⁺-ATPase induced by vanadate (24, 25), suggesting that this type of ordered array of subunits may be a general feature of membrane transport enzymes.

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