Membranifibrils on Cristae and Grana Membranes[1]

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Abstract

Mitochondria cristae show a linear arrangement of the 90Å headpieces on the membrane surface when observed by negative staining. Treatment with Triton releases fibrous structures of 70Å diameter to which the 90Å headpieces are attached. (J. W. Stiles, Ph.D. thesis, Purdue University, 1969) These fibers have been purified and contain 15% lipid and very little cytochrome. Extraction of the membranifibril with acetone leaves a 30Å diameter fibrous, insoluble protein which may be the basis for the fibrous structure.

Similar linear arrangements of particles variously referred to as quantosomes or chloroplast F_1 have been observed on chloroplast grana membranes. Extraction of the grana with triton releases fibrous structures of 70Å diameter to which 100Å particles are attached. We propose that the basis of linear organization of particles on the surface of cristae and grana membranes is based on their attachment to a fibrillar structure in the membrane called the membranifibril.

Introduction

Globular particles have been demonstrated on the surfaces of various biological membranes. The membranes of mitochondrial cristae and of chloroplast grana are two such examples. Spherical 90Å particles are seen projecting on stalks from the surface of inner mitochondrial membranes when observed by negative staining (3). Oligomycin-insensitive ATPase activity has been shown to be associated with these particles (15). Numerous particles, 70-140Å in diameter, are found associated with the membranes of chloroplast lamellae when viewed by negative staining. Some of these particles have been identified as carboxydismutase (8, 21). Others have been variously associated with chloroplast fraction I protein, ca^{++}-dependent ATPase, and quantasomes (1, 9, 14).

The regular orientation of particles on the surfaces of their respective membranes suggests that a discrete substructure may be responsible for holding particles in place. Evidence for linear substructure in mitochondrial cristae (18, 19) and chloroplast grana (1, 14, 16) has been previously reported. Evidence has also been presented for fibers in plasma membrane (5). Linear elements have been observed in cholate extracts of mitochondria, but have not been interpreted as fibers (10, 13). A fibrillar structure which may be the basis of linear organization of particles in chloroplast grana and mitochondrial cristae will be described in this paper.

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Materials and Methods

Beef heart mitochondria prepared according to the method of Löw and Vallin (12) were homogenized in 0.25 M sucrose and stored at —20°C for at least 4 days prior to preparation of electron transport particles (ETP). Following thawing, the mitochondria were homogenized in 0.001 M Tris-HCl, pH 7.4, and centrifuged at 11,700 x g. The pellet was washed 4 times in this manner then resuspended in the buffer. The suspension was sonicated for two 5-min. periods in a brine bath using a Branson probe sonifier at maximal output. Heavy beef heart mitochondria were removed by centrifugation, at 11,700 x g. The supernatant was then centrifuged at 78,000 x g for 30 min. The pellet was washed and resuspended in 0.001 M Tris-HCl, pH 7.4. If the ETP were to be stored, the preserving medium of Hansen and Smith (7) was used.

To ETP suspended in buffer at a protein concentration of 30 mg/ml was added aqueous 10% Triton X-114 to give a final concentration of Triton of 3%. Following mixing, the solution was cooled in ice for 15 min. then solid KCl was added to a final concentration of 0.2 M. The mixture was incubated for 30 min. with stirring. Centrifugation at 78,000 x g for 30 min. yielded a yellow supernatant which was further purified by dilution with an equal volume of 0.001 M Tris-HCl, pH 7.4, which was 0.2 M in KCl, followed by centrifugation. Ammonium sulfate fractionation of the supernatant was achieved by the addition of the solid salt to 25% saturation. Centrifugation at 27,000 x g for 30 min. resulted in a sinking precipitate, a floating precipitate, and a straw-colored supernatant.

Chloroplasts prepared from spinach leaves were obtained by the method of Crane (2). After 2 washings with 0.05 M Tricine, pH 7.4, followed by centrifugation at 8,720 x g, the pellet was resuspended in buffer which was 4% in Triton X-100 and incubated for 30 min. with occasional stirring. The mixture was then centrifuged at 144,000 x g for 1 hr.

TABLE 1. *Chemical Composition of Mitochondrial Membranifibril Fractions.*

Fraction	Cytochromes[1]				%
	c	c_1	b	$a+a_3$	Lipid[2]
Triton-prepared membranifibrils	0.058	0.028	0.020	0.078	14.4
DOC-prepared membranifibrils	0	0	0	0	9.6

[1] μmoles/g protein.

[2] Determined as % lipid $= \left(\frac{\text{mg lipid}}{\text{mg lipid} + \text{mg protein}} \right) \times 100$

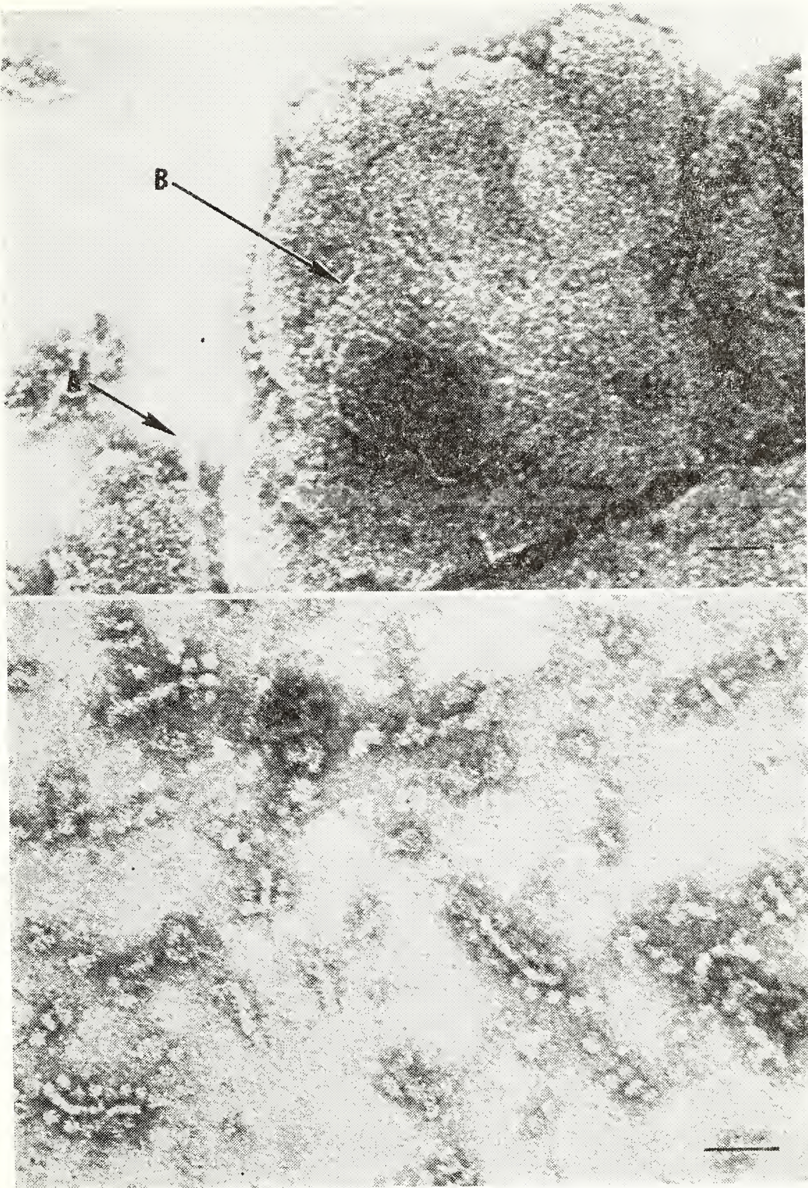

Figure 1. Mitochondrial membrane fragments showing 90Å particles and linear arrangement of these particles on the surface of the membrane. Phosphotungstate. X 164,000. Marker 500Å.

Figure 2. Membranifibrils purified by ammonium sulfate fractionation from Triton-treated ETP. Uranyl acetate. X 184,600. Marker 500Å.

Protein was assayed by the modified biuret procedure of Yonetani (25). Phospholipid was extracted using the standard Folch procedure (4) with aqueous 0.74% KCl as the salt. The organic layer was evaporated to dryness and the phospholipid taken up in 10 ml of chloroform-methanol, 2:1. One ml of this was combined with an equal amount of concentrated sulfuric acid and heated over a flame until the solution blackened. Then 2-3 drops of concentrated hydrogen peroxide were added and the mixture boiled again. The clarified solution was then tested for phosphate by the method of Lindberg and Ernster (11). Cytochromes were determined from difference spectra recorded with a Unicam SP 800 recording spectrophotometer by the method of Williams (24). The extinction coefficients of Vanneste (23) were used.

Samples were negatively stained with either 1% phosphotungstate at pH 6.9 or saturated uranyl acetate. Microscopy was done on a Philips EM 200 using Kodak Electron Image Plates.

Sonication to release membranifibrils was carried out with a high-power sonicator kindly supplied by Quigley-Rochester Scientific Inc.

Results and Discussion

Examination of untreated electron transport particles by negative staining reveals flattened vesicles from the surface of which project 90Å particles (Fig. 1). These particles are visible on the membrane surface as well as at the edges of the vesicles. In this latter profile view, particles can frequently be seen attaching to the membrane edge by a stalk. Closer examination of Figure 1 suggests that the white rim to which the particles attach at the edge of the vesicle might be a discrete structure rather than just a rolled edge. At arrow A this white rim is seen to extend out from the membrane surface as a linear structure. The parallel array of lines on the membrane surface (at arrow B) also suggests a linear substructure associated with these membranes.

Treatment of ETP with Triton X-114 releases linear structures to which the 90Å particles are attached. Following ammonium sulfate fractionation, a purified fraction of these structures was obtained (Fig. 2). The central strands have an average diameter of 70Å. The 90Å particles are arranged opposite one another in a regular fashion along the central strand. This linear structure has been called the membranifibril (17). Treatment of ETP with deoxycholate and KCl, according to the method of Tzagoloff et al. (22), releases linear elements which resemble the membranifibril. Similar structures have also been observed following sonication of whole beef heart mitochondria (Fig. 3).

Preliminary chemical analysis of the purified Triton-prepared membranifibrils revealed that they are composed mostly of protein. Hence the name fibril is applicable. Several bands were observed on gel electrophoresis. The membranifibrils also contain a small amount of lipid, about 15%, and very little cytochrome. The preparation shows ATPase activity. Initial flavin analysis indicates that there may be an acid-extractable

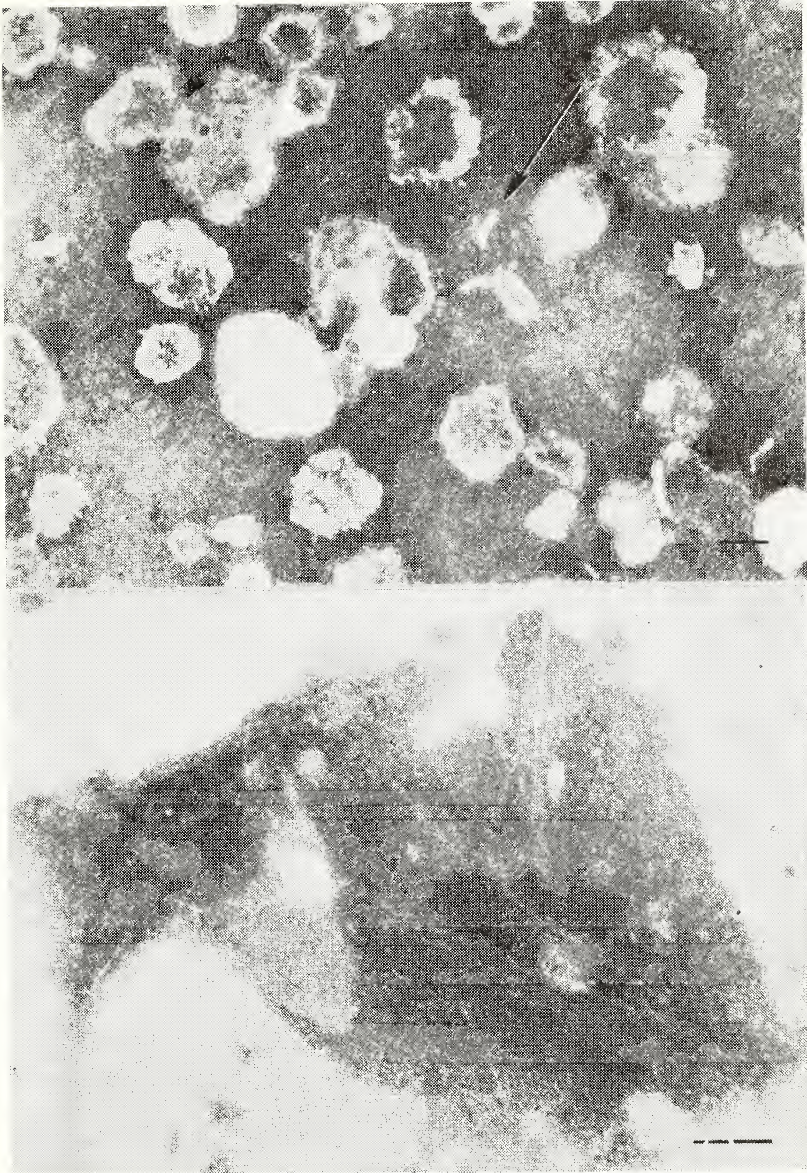

Figure 3. Sonicated mitochondria showing released membranifibrils. Phosphotungstate. X 123,000. Marker 500Å.
Figure 4. Chloroform-methanol extracted membranifibrils. Phosphotungstate. X 213,000. Marker 500Å.

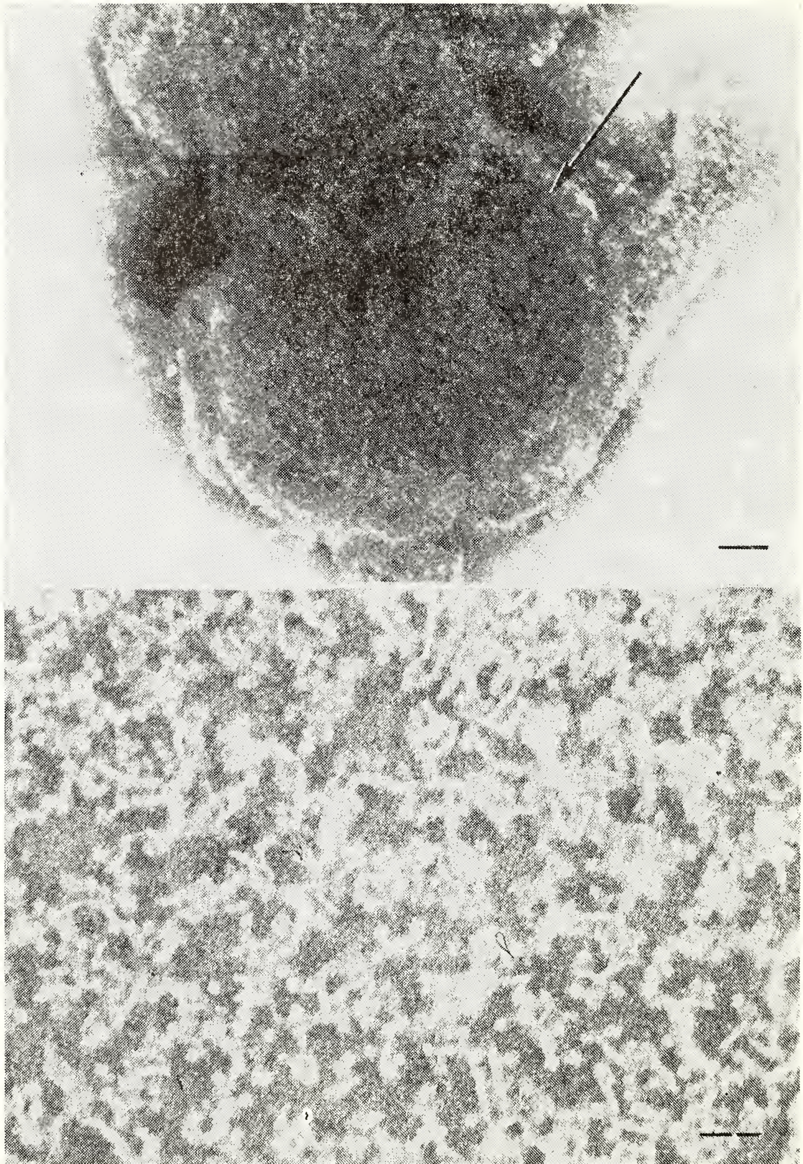

Figure 5. Chloroplast grana membrane showing linear arrangement of 100Å particles on the surface of the membrane. Phosphotungstate. X 136,600. Marker 500Å.

Figure 6. Membranifibrils isolated from chloroplasts by Triton treatment. Phosphotungstate. X 184,600. Marker 500Å.

flavin associated with the membranifibril fraction. The chemical composition of the mitochondrial membranifibrils is found in Table 1.

Kopaczyk et al. (10) have isolated a rutamycin-sensitive ATPase fraction (P_2) by cholate and ammonium sulfate treatment of inner mitochondrial membrane which resembles our membranifibril fraction both chemically and structurally. Their P_2 fraction showed no cytochrome aa_3 and low levels of the b and c cytochromes. The lipid content of their fraction was reported as 10.7%. The fraction showed minimal rutamycin-sensitive ATPase activity in the absence of added phospholipid. Maximal activity was achieved by the addition of 1 mg of phospholipid per mg of P_2 preparation to the assay medium.

Membranifibrils are clearly evident in their electron micrographs of the rutamycin-sensitive ATPase (P_2) fraction. However, they interpret these structures as headpiece-stalk sectors projecting exteriorly from phospholipid bilayers or coiled phospholipid micelles. A lipid-based structure hardly seems likely considering the low lipid content of the P_2 fraction and of our membranifibril preparation.

Extraction of the membranifibril with acetone or chloroform-methanol leaves a 30Å diameter fibrous, insoluble protein (Fig. 4). This fibrous protein may be the basis of membranifibril structure.

When untreated washed and lysed chloroplasts are observed by negative staining, the grana membranes are seen to have a particulate surface (Fig. 5). These particles have an average diameter of 100Å and appear to be aligned in rows (indicated by arrow). Treatment of the chloroplast fragments with Triton releases fibrous structures very similar to the mitochondrial membranifibrils (Fig. 6). These structures are collected in the 144,000 x g supernatant and have been called chloroplast membranifibrils (20). These structures consist of 70Å diameter strands to which the 100Å particles are attached. Chemical composition and enzymatic activity of the chloroplast membranifibrils is currently under investigation. Structures resembling chloroplast membranifibrils have been seen following sonication of intact chloroplasts.

It is proposed that the membranifibril is the basis of the linear arrangement of particles on the surface of mitochondrial cristae and chloroplast grana membranes by virtue of the attachment of these particles to the fibrillar structure. In mitochondria, such a structure would correspond to the basepieces which Green has proposed as the site of the electron transfer chain (6). However, since the membranifibril contains very little cytochrome it is unlikely that cytochrome dependent electron transport function is associated with the site of attachment of the 90Å particles.

Summary

A new structural element, the membranifibril, was isolated from the membranes of mitochondria and chloroplast grana by Triton treatment. Preliminary investigation of the chemical composition and the enzymatic

activity of these fibrils was conducted. It was proposed that the membranifibrils are the basis of the linear organization of the particles on these membranes.

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