REMOVAL OF STRUCTURAL PROTEIN FROM MITOCHONDRIA

W. L. Zahler, Akitsugu Saito and Sidney Fleischer
Department of Molecular Biology, Vanderbilt University
Nashville, Tennessee 37203

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Structural protein is a term originally used by Green et al.(1961) to designate the non-catalytic protein associated with mitochondrial membranes. This preparation, isolated utilizing detergents (Green,et al., 1961; Criddle, et al., 1962; Richardson, Hultin and Fleischer, 1964), is a colorless protein fraction which is insoluble at physiological pH. We wish to report the removal of a similar fraction from whole mitochondria using acid extraction. The extract, upon neutralization forms a precipitate which has similar properties to structural protein as defined above. The non-extractable residue retains the typical "unit membrane" appearance.

Materials and Methods

Mitochondria (about 25 mg protein) are extracted with 1.4% acetic acid for 30 minutes in a volume of 5 ml at 0-4° C. The insoluble residue is removed by centrifugation at 81,000 x g (average) for 1 hour. After washing once with 5 ml of 1.4% acetic acid the residues are resuspended and analyzed for phosphate (Chen, et al., 1965) and protein (Lowry, et al., 1961). A structural protein fraction is prepared from the acetic acid extract by titration to pH 6.5. At this pH, a flocculant precipitate rapidly develops which is sedimented to separate it from soluble proteins.

The fractions obtained are then characterized by electrophoresis using the method of Takayama, et al.(1966), with the following modifications: a) a pre-electrophoresis step is added as suggested by Mitchell (1967); b) five-inch gel tubes are used; c) Coomassie Blue is used for staining; d) ribonuclease is used as an internal standard.

Samples for electron microscopy were prepared as described previously (Fleischer, Fleischer and Stoeckenius, 1967). Araldite was used as the embedding medium instead of Epon. Sections were viewed in a Hitachi 11C electron microscope.

Mitochondria were prepared as described by Fleischer, et al. (1967) except that a Potter-Elvehjem apparatus was used in place of a Waring blender in the preparation of liver and kidney mitochondria[1].

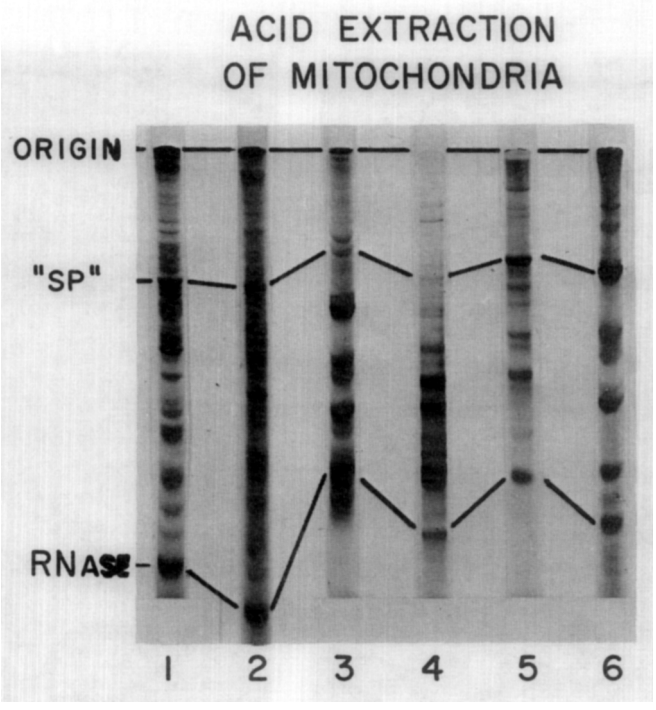

Fig. 1. Polyacrylamide gel electrophoresis of beef heart mitochondria and fractions obtained by the acid extraction procedure. There are: 1 -whole mitochondria; 2 -acid extract; 3 -residue; 4 -soluble fraction; 5 -structural protein; and 6 -structural protein prepared according to Richardson, Hultin and Fleischer (1964). Electrophoresis was carried out for 3 1/2 hours at 2.5 milliamps per tube.

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[1] We wish to thank Mr. Niels Nielsen for providing the liver and kidney mitochondria and Mr. Kenneth Walton for the rat heart mitochondria.

Results

Typical results for the electrophoresis of the fractions obtained by acid extraction of whole beef heart mitochondria are presented in Figure 1. The residue of the acid extract has a simplified electrophoresis pattern when compared to that of whole mitochondria while the extract has a complex pattern. The structural protein fraction contains a major band which migrates the same as the major component of structural protein prepared by the method of Richardson, Hultin and Fleischer (1964). For this reason, and because the two fractions show simi-

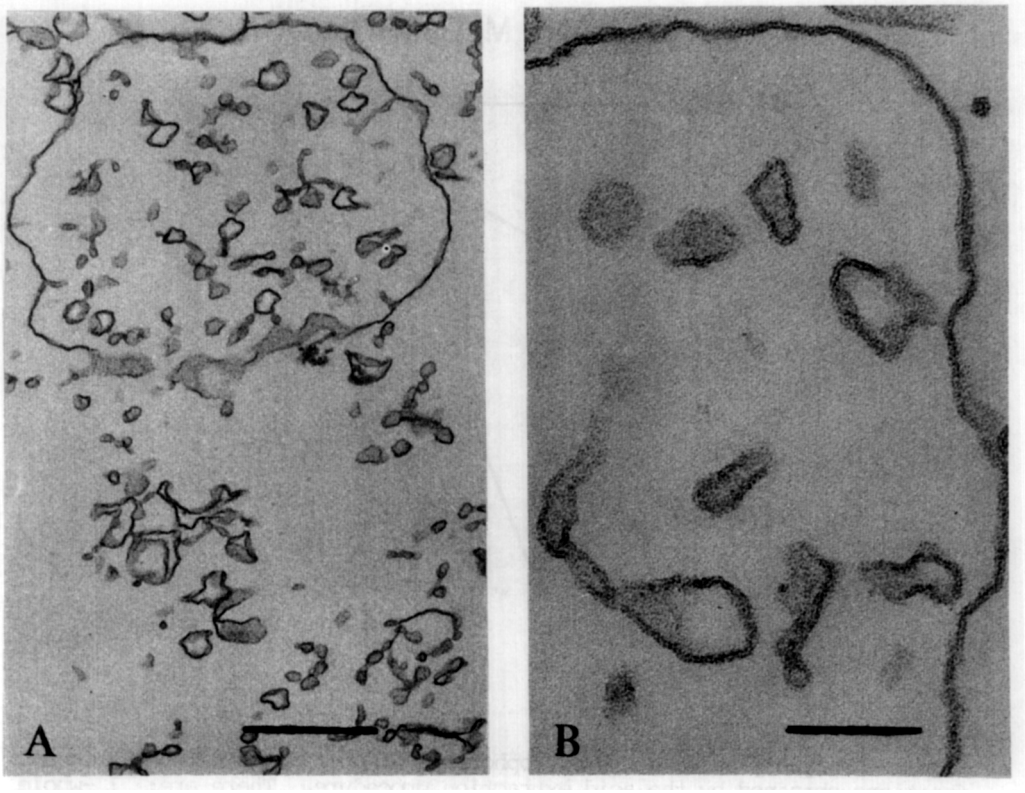

Fig. 2. Electron micrographs of the residue fraction obtained from beef heart mitochondria by the acid extraction procedure (cf Methods). The acid treatment has a disruptive influence on the mitochondrial structure (cf Fig. 2a). The trilaminar arrangement of the membrane is clearly visible (cf Fig. 2b). The optical magnification in Figs. 2a and 2b are 10,000 and 50,000 respectively; the bar represents 0.5 μ and 0.1 μ respectively.

lar properties and account for a large portion of the protein (approx. 45%), the major electrophoretic band is designated structural protein. It should be noted that the structural protein band has been essentially removed from the residue.

Electron micrographs show the residue to be membranous; "unit membranes" can clearly be observed (cf Figure 2). Membranes are not observed in the structural protein fraction.

Mitochondria from several sources were extracted with acid. The residues from beef kidney and liver were also found to be membranous. The amount of protein in the residue fractions varies widely; it is highest in heart and lowest in liver (Table I). In contrast to the amount of protein, the lipid content sharply increases upon extraction, resulting in remarkably similar ratios of phosphorus to protein. This is especially significant in light of the variation observed in the starting material. In fact, practically all of the lipid phosphorus is retained in the residue upon extraction as indicated by the last two columns of Table I.

TABLE I

Comparison of Mitochondria From Different Sources

Source	Original	Residue		Lipid Phosphorus µg P/mg Protein	
	µg P / mg protein	µg P / mg protein	Percent	Calc.[a]	Obs.[b]
Beef Heart	18.0	31	41	13	11.3[c]
Beef Kidney	10.4	32	23	5.4	5.8
Beef Liver	8.7	35	15	7.3	7.6
Rat Heart	16.1	33	34	11	-
Rat Liver	11.6	36	21	-	-
Human Heart	18.2	32	47	15	13.4[c]

[a] Lipid phosphorus was calculated for the starting material by multiplying the amount of phosphorus per mg residue by the fraction of residue in the whole mitochondria

[b] See Fleischer et al. (1967)

[c] This value does not include 1.4 µg of bound, non-lipid phosphorus (Fleischer et al. (1962)

The structural protein fractions obtained from these different mitochondria all contained the structural protein band as a major component. Further, the percent protein in this fraction was greatest in liver and least in heart.

Discussion

These experiments show that structural protein can be removed from mitochondria with dilute acid leaving an insoluble residue which is membranous and retains a trilaminar arrangement. Practically all of the phospholipid remains with this fraction. Residues from several different mitochondria have similar phosphorus to protein ratios even though the percentage of lipid in the original mitochondria varies widely. These results suggest that the residue fraction is a basic component of the membrane. This is further substantiated by the correlation of the percent residue with the density of membranes observed morphologically in the different mitochondria, i.e., it is greatest in heart and least in liver. Further, there is an inverse correlation between the percent of protein in the structural protein fraction and in the residue fraction. These data raise an important question. What is the localization and function of structural protein?

Structural protein fractions as isolated by acid extraction contain little, if any, lipid. Yet, Richardson, Hultin and Fleischer (1964) found that structural protein as isolated by the procedure using bile salts spontaneously binds phospholipid. It is possible that the acid treatment affects the lipid distribution in these studies and therefore it remains an open question whether structural protein is in fact associated with phospholipid in the mitochondrion.

Structural protein also forms complexes with nucleotides (Richardson, Hultin and Green, 1963; Munkres and Woodward, 1967), and other proteins (Criddle, et al., 1962; Edwards and Criddle, 1966a, 1966b; Munkres and Woodward, 1967). The significance of these observations also remains to be evaluated.

One helpful approach to this question might be to consider several levels of organization in mitochondria: a) the primary level or the core

of the membrane, morphologically equated with the trilaminar arrangement; b) the secondary level consisting of components associated with the primary level but which are not essential to the trilaminar arrangement; and c) the tertiary level containing soluble proteins.

The residue from the acid extraction belongs to the primary level. At the present time we cannot evaluate whether acid extracted proteins, such as structural protein, also form part of this level. It is also unknown whether the primary level is sufficient to maintain the compartmentalization of the mitochondrion. A possible role of the secondary level would be to restrain soluble components, thereby extending the influence of the membrane beyond the width of the trilaminar arrangement. As discussed above, structural protein may fulfill this role through its broad complexing capabilities. The soluble proteins would then be part of the tertiary level.

Acknowledgements

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