A MITOCHONDRIAL INNER MEMBRANE PREPARATION THAT SEDIMENTS AT 100 g

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INTRODUCTION

During the course of studies on the morphological changes that occur in rat liver mitochondria during certain ion movements (1), it was found that some extremely large structures formed when mitochondria were incubated in a reaction mixture that contained glutamate, phosphate, EDTA, and sucrose. Since the initial electron microscope studies revealed the presence of an unusual membranous structure, further studies were conducted so as to analyze its morphological and enzymatic characteristics. This structure in its morphology differs from other previously described inner and outer mitochondrial membrane preparations (2-6) and appears to be formed from the inner membrane of the mitochondria.

MATERIALS AND METHODS

Rat liver mitochondria were isolated according to Schneider and Hogeboom (7) in 0.25 M sucrose and 1 mM EDTA adjusted to pH 7.3 with Tris base. By an alternate method, the mitochondria were prepared according to Schnaitman et al. (8). Similar results were obtained (see Results) with either method. Preparations with respiratory control of less than four were discarded. Mitoplasts were prepared as described by Schnaitman et al. (8), using digitonin to remove the outer membrane of the mitochondria.

Formation of the 100 × g Inner Membrane Preparation

To 50 ml of a mixture that contained 10 mM glutamate, 10 mM phosphate, 1 mM EDTA, 10 mM Tris-HCl (pH 7.3), and 50 mM sucrose (this mixture will be referred to as basic incubation mixture), 2 or 3 ml of a mitochondria or mitoplast suspension (300-400 mg of protein) were added. The mixture was shaken manually at room temperature for 15 min and thereafter centrifuged at 100 g for 4 min. The brownish pellet was suspended in sucrose-EDTA and centrifuged again at 100 g. The resulting sediment, referred to as the 100 g fraction, was suspended in a minimal volume of sucrose-EDTA for further studies.

Glucose-6-phosphatase, glutamic dehydrogenase, monoamino oxidase, and cytochrome oxidase were assayed as described elsewhere (8). Protein was determined by the biuret method (9) and by the spectral difference between 215 and 225 nm (10).

RESULTS

In the initial experiments, when rat liver mitochondria that were incubated in the basic incubation mixture were examined in the electron microscope the structure shown in Fig. 1b was observed. A large number of mitochondria were in both the condensed and orthodox configurations (11); some mitochondria were swollen and devoid of outer membrane. However, a remarkable feature of this preparation was the presence of numerous threadlike structures to which a large number of small vesicles were attached. A preparation from the same batch of mitochondria, but in which the incubation in the basic mixture was omitted, revealed the absence of the threadlike structures (Fig. 1a), and the mitochondria were intact and mainly in the condensed configuration (11).

The findings of Fig. 1a and b largely ruled out the possibility of an extramitochondrial contamination and indicated that the incubation procedure induced the formation of the threadlike structures. Thus it was considered interesting to separate and
FIGURE 1. (a) Electron micrograph of freshly isolated mitochondria fixed with glutaraldehyde, postfixed with OsO₄, and stained with uranyl acetate and lead citrate. × 18,000. (b) Section of 8,000 g pellet after incubation in the basic medium for 7 min. × 25,000. (c) Light micrograph of a smear of the 100 g fraction stained with toluidine blue. × 1,450. (d) Electron micrograph of the preparation in Fig. 1 c. × 10,600.
TABLE I
Distribution of Marker Enzymes in the Fractions Obtained after Incubation of Mitochondria in the Basic Incubation Media

| Fraction       | Total activity of marker enzymes | Specific activity $\dagger$ |
|----------------|----------------------------------|-----------------------------|
|                | G-6-Pase* | MAO | Cyt. Ox.§ | GHD|| G-6-Pase | MAO | Cyt. Ox. | GDH|
| Mitochondria   | 59.85     | 4,622 | 483,895 | 395,010 | 315 | 0.19 | 14.8 | 1,533 | 1,254 |
| Sediment I     | 14.2      | 724  | 147,181 | 24,850 | 71  | 0.20 | 10.2 | 2,073 | 350  |

* Glucose-6-phosphatase (G-6-Pase), micromoles Pi formed in 10-min incubation.
§ Monoamino oxidase (MAO), nanomoles of benzaldehyde produced in 1 min.
§ Cytochrome oxidase (Cyt. Ox.), nanoatoms O consumed in 1 min.
¶ Glutamic dehydrogenase (GHD), nanomoles NADH produced in 1 min.
¶¶ The specific activity is expressed as micromoles or nanomoles per minute per milligram of protein of the respective marker enzymes.

analyze the structures so as to gain insight into their characteristics.

The separation of this fraction was achieved as described in Materials and Methods. No such fraction was formed from mitochondria in which the incubation step was not carried out. Fig. 1 c and d show the appearance of the 100 g fraction as observed under the light and electron microscopes, respectively. Light microscopy revealed a large membranous structure. The structure as observed with the electron microscope was very similar to the membranous structure shown in Fig. 1 b, except that the 100 g fraction was essentially free of mitochondria. The central filamentous membrane extended for approximately 30-50 $\mu$m (Fig. 1 d) and was surrounded by small vesicles. Peripheral to the small vesicles, large apparently empty vesicles were also detected. It is to be noted that the small vesicles that were attached to the central filamentous membranes very frequently contained dark granules.

The enzymatic characteristics of the 100 g fraction in comparison to those of control mitochondria are shown in Table I. Generally, about 20% of the mitochondrial protein sediments in the 100 g fraction. About 30% of the original cytochrome oxidase activity also appears in the 100 g fraction. The monoamino oxidase and glutamic dehydrogenase specific activities in the fraction are lower than in intact mitochondria. The absence of a significant number of contaminating microsomes is revealed by the low activity of glucose-6-phosphatase.

The data presented suggest that the structure is formed mainly at the expense of the inner membrane of the mitochondria. In order to test this idea the possible formation of the 100 g fraction from an outer membrane-free mitochondrial fraction, the mitoplast (8), was studied. Fig. 2 a shows that this preparation is virtually free of outer membrane; moreover, the monoamino oxidase activity of this preparation was almost nil (data not shown). The incubation of the mitoplasts in the basic incubation mixture induced the formation of structures strikingly similar to those obtained from intact mitochondria (Fig. 2 b) except that the large vesicles (Fig. 1 d) were not present.

DISCUSSION

The purpose of this communication is to describe some of the morphological and enzymatic characteristics of a membrane fraction that sediments at 100 g and is formed during the incubation of mitochondria with glutamate, phosphate, EDTA, and sucrose. Since this structure is also formed from outer membrane-free mitochondria, it is valid to conclude that this fraction is derived from the inner membrane of the mitochondria. Moreover, we would like to mention that this fraction possesses an intact electron-transport chain and an oligomycin-sensitive ATPase activity (unpublished data).

Obviously, the question arises as to what induces this peculiar arrangement of the membrane. In this sense, it is important to note that both the incubation medium and the mechanical factor of shaking are two critical factors. In experiments not
FIGURE 2  (a) Inner membrane-matrix (mitoplasts) preparation obtained after treatment of the mitochondria with digitonin (8). × 16,200. (b) Threadlike 100 g structure obtained after incubation of the preparation shown in Fig. 2 a in the basic incubation medium for 7 min. × 26,200.
shown, it has been found that incubation of mitochondria without shaking does not induce the formation of the 100 g structure. Thus these two factors probably induce the rupture of the membrane and its rearrangement.

Our present working hypothesis is that during the rupture of the inner membrane certain molecular groups that allow for the interaction between the various membranous structures are exposed. However, further experimentation is required to prove the validity of this hypothesis.

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