The Induction of Energized Configurational Changes in Plant Mitochondria, *in vivo**

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Abstract

Configurational changes in the mitochondrial membranes of the salt gland of *Tamarix aphylla*, which are dependent on the biochemical state of the mitochondria, are demonstrated. In the energized state the cristae expand and become closely associated. There is also an increase in the density of the matrix and a formation of strands of material in the matrix and between the closely associated cristae membranes. The energized condition can be discharged by incubation in a medium containing 2,4-dinitrophenol and the mitochondria are comparable to those observed in glands fixed by typical methods for electron microscopy.

Introduction

Several investigators have clearly established a relationship between the ultrastructure and the metabolic states of mitochondria [1-17]. Green and his associates in a series of publications [5-13] have described configurational changes in mitochondrial cristae which are conditional on changes in the energy cycle of mitochondria. They have suggested that these configurational changes are related to energy-dependent, conformational changes in the tripartite repeating units of the cristae membrane [5, 8, 10]. Experimental evidence has led Green and his co-workers to postulate that the conformational

changes are intrinsic to the energy cycle of mitochondria and thus should be universal to mitochondria of all types [8, 9, 10, 11]. Harris et al. [7] and Williams et al. [9] have observed configurational changes in mitochondria both in situ and isolated from a number of animal tissues but there are no published reports of similar studies with plant mitochondria.

At the ultrastructural level, the membrane changes are to a great extent recognized because of the large number and close association of cristae membranes in animal mitochondria. Because of the fewer number and smaller size of cristae in most plant mitochondria the possibility of detecting these configurational states is much reduced. The mitochondria in the salt glands of Tamarix resemble the rat heart mitochondria in that they contain numerous cristae [18], and the present report concerns membrane changes in these mitochondria in relation to their functional states.

Experimental Methods

Cross sections, approximately 0.5 mm in size, were cut from small branches of Tamarix aphylla. These were immediately placed in an incubation medium containing sodium succinate (2 mM), potassium phosphate, pH 6.8 (10 mM), rotenone (20 µg/ml), oligomycin (60 µg/ml), or rutamycin (20 µg/ml), and iodoacetate (1 mM). The incubation mixture was continually aerated at 25°C throughout the experiment (15 min). This incubation medium was similar to that described by Harris et al. [7] for establishing energized conditions. Similar sections were incubated in the above medium which contained in addition, 5.0 mM 2,4-dinitrophenol (2,4-DNP). A high concentration of 2,4-DNP was used to overcome possible penetration problems and to assure uncoupling a condition which discharges the energized condition of mitochondria [7, 9].

After treatment the tissue was immediately placed in phosphate-buffered, pH 7.4, formaldehyde-glutaraldehyde fixative [19]. The tissue was fixed for about 60 min at room temperature and then post-fixed for 60 min at room temperature with 1% osmium in a phosphate buffer (pH 7.2). The tissue was dehydrated in acetone and embedded in Spurr's epoxy resin [20]. Thin sections were cut with a Porter-Blum MT-1 ultramicrotome and stained on the grid with uranyl acetate and lead citrate [21]. Observations and micrographs were made with a Philips EM 300 electron microscope. Some tissue sections were prepared for electron microscopy without prior incubations in either of the above incubation media.

Results

The mitochondria in the salt glands of Tamarix fixed directly without incubation have numerous cristae (Fig. 1). There is no
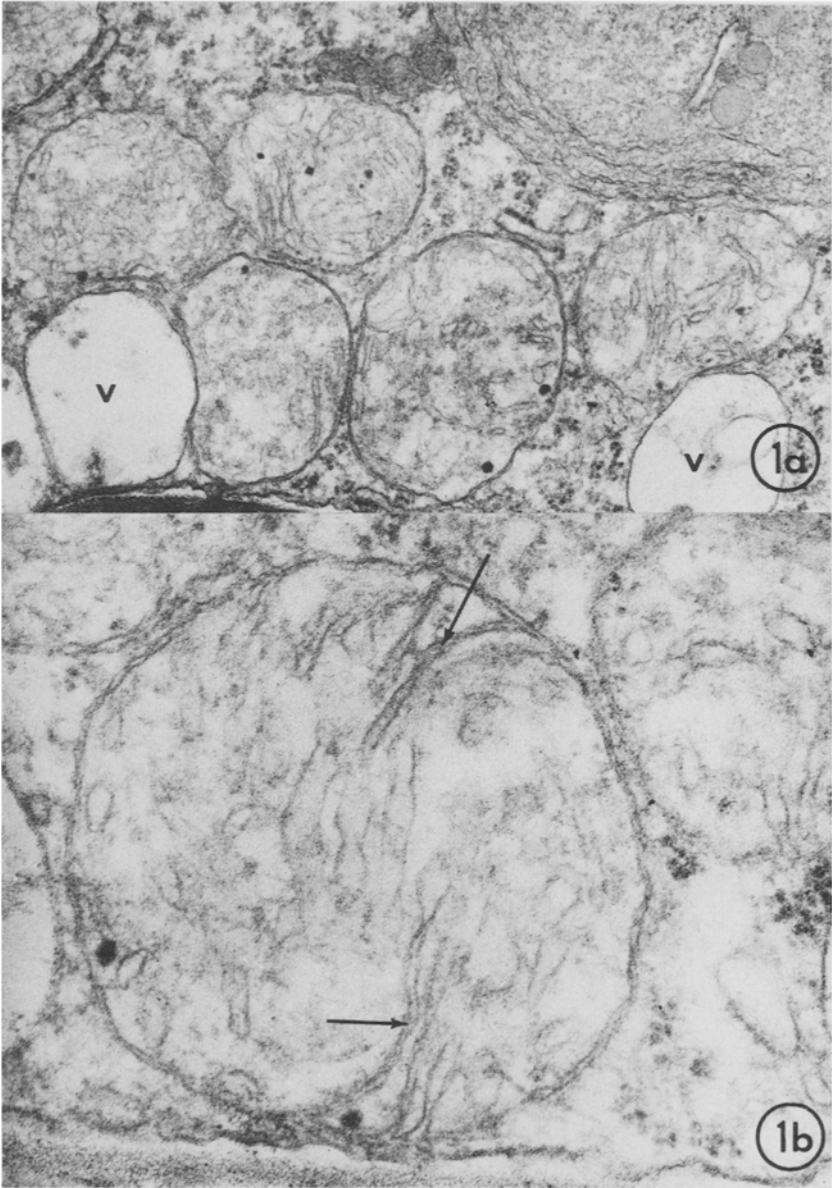
Figure 1. Mitochondria of salt glands of tissues that were prepared for electron microscopy without prior incubation. A. Low magnification view of several mitochondria and vacuoles, v, × 41,500. B. A high magnification view of a mitochondrion. Note: the relative translucency of the matrix, unexpanded cristae (arrows), and apparent absence of a direct relationship between the cristae. These characteristics establish the mitochondrion as in the orthodox, non-energized configuration. × 99,000.

apparent association between the membranes of the different cristae, the mitochondrial matrix has a slight but a relative uniform electron density, and frequently small electron dense granules are observed in the matrix. The intracristal space is reduced (Fig. 1B, arrows) and the cristae and mitochondria can be classified as being in the orthodox mode, non-energized configuration [6, 9-11]. It has been pointed out that the orthodox, non-energized state is the form in which *in situ* mitochondria are most commonly observed [7, 9].

Incubation of the tissue with succinate and inorganic phosphate in a well aerated medium established the energizing conditions and the presence of oligomycin in the incubation medium blocks the discharge of the energized state by oxidative phosphorylation [7]. When the tissue was incubated under these conditions the mito-chondria were obviously different from those in salt glands of untreated tissue (compare Fig. 1 to Fig. 2). The mitochondria are in the condensed configurational mode with a dense, contracted matrix (Fig. 2A, 2B), expanded intracristal space (Fig. 2A, 2B) and there is a close association of adjacent cristae membranes (Fig. 2A, B). The distance between the cristae averages about 125 Å. This close, parallel association of the cristae membranes and the expansion of the intracristal space identifies these mitochondria as in the aggre-gated, energized state.

One of the most distinguishing fixtures observed at high magnifica-tion of these energized, condensed mitochondria are electron dense strands of material extending across the region between two adjacent cristae membranes (Fig. 2B). These strands measure about 90 Å in width. An interconnecting network of strands is also observed in the matrix (Fig. 2b). The meshes between the strands are relatively electron transparent and generally circular in outline.

After incubating the tissue in a medium containing the uncoupler, 2,4-dinitrophenol the mitochondria are in the orthodox configuration (Fig. 3). The mitochondrial matrix or intercristal space is expanded, the cristae are flattened (Fig. 3) and the intracristal space is con-tracted. These mitochondria are virtually identical in ultrastructure to mitochondria fixed by standard methods (compare Fig. 3 to Fig. 1).

Discussion and Conclusions

In the studies reported in this paper, it has been possible to demonstrate in an *in vivo* plant system, configurational changes in the mitochondria which are related to their biochemical states. These results also clearly show a relationship between the energy states of the mitochondria and configuration of the mitochondrial membranes and support the universality of the concepts outlined by Green and his associates [8-13].
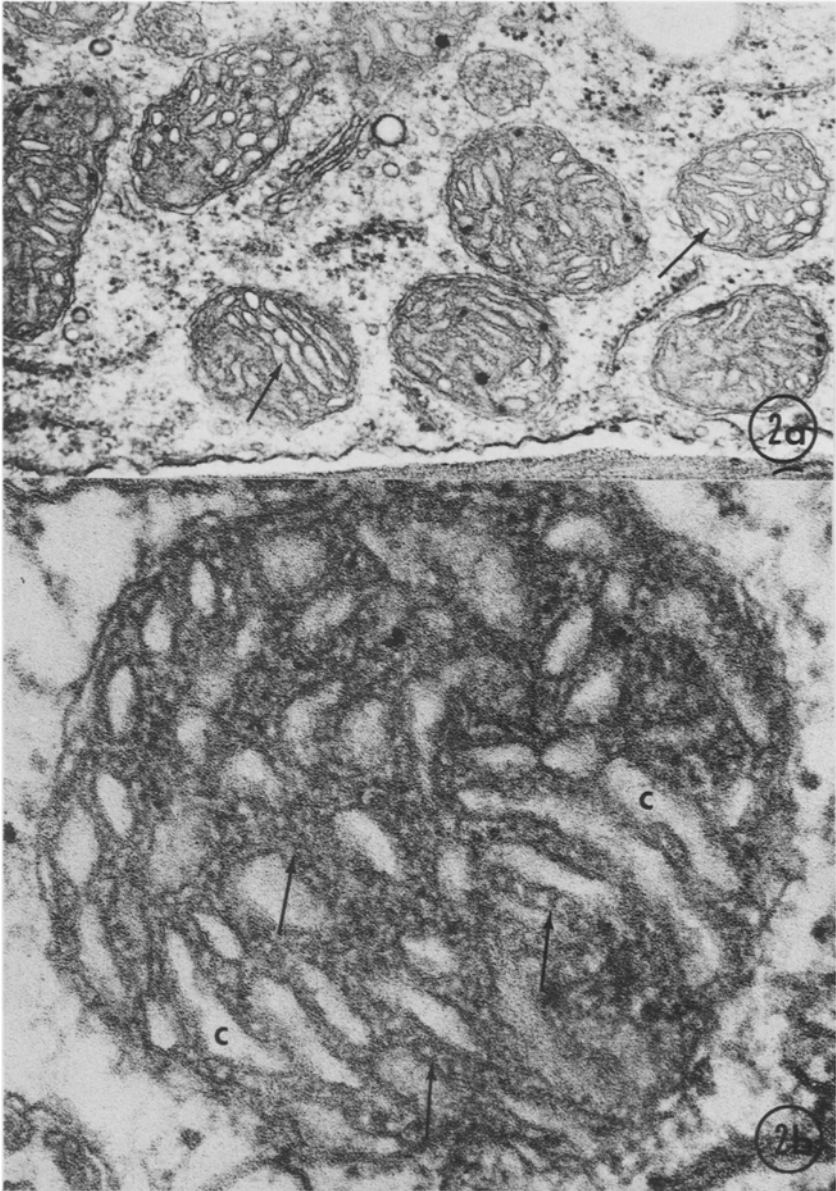
Figure 2. Electron micrographs of mitochondria of a salt gland in tissue after incubation in an energizing medium. A. Low magnification of several mitochondria. Note: the expanded condition of the cristae (arrows), and the density of the matrix. ,× 50,500. B. A high magnification view of an energized mitochondria. Note, the expansion, translucency, and close association of the cristae, c. Note, also the electron dense strands between the cristae and the network of strands in the matrix region, arrows. × 165,000.

Figure 3. Micrographs of mitochondria of salt glands in tissues incubated in the presence of 2,4-dinitrophenol. The cristae are considerably flattened (arrows); the intercristal space is expanded, s, and has a reduced electron density as compared to Fig. 2. Note how similar these mitochondria are to those in Fig. 1. ×96,500.

Although it can be demonstrated that configurational changes can be induced in the mitochondria of *Tamarix* by energizing conditions, as shown by comparing mitochondria in Fig. 1 and those in Fig. 2 the cristae were not observed in the energized twisted configuration as noted in the rat heart mitochondria by Harris *et al.* [7]. The absence of this configuration may possibly relate to an insufficient level of phosphate which induces the energized-twisted state [5]. Another possibility is the osmotic conditions of the medium and the relative quantity of intercristal matrix which from the results of Williams *et al.* [9] influence the modes of the energized configuration. Thirdly, the absence may be related to a general difference in cristae morphology and number in plant mitochondria as compared to animal mitochondria.

The occurrence of strands of material between cristae in the energized mitochondria (Fig. 2B) has been previously observed by Hackenbrock [22] in rat liver mitochondria in the orthodox conformation. The function and nature of these strands is unknown but it is possible these might represent an association of head-pieces [10] of repeating units of adjacent cristae membranes. The presence of the strands might be a useful criterion for identifying the energized state of mitochondria particularly in those mitochondria which have few and small cristae.

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