ULTRASTRUCTURAL STUDIES OF BEEF HEART MITOCHONDRIA

I. EFFECTS OF ADENOSINE DIPHOSPHATE ON MITOCHONDRIAL MORPHOLOGY

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SUMMARY. A study of the morphology of isolated beef heart mitochondria in various metabolic states has revealed that adenosine diphosphate alone promotes the formation of a highly condensed morphology which remains unchanged when oxidizable substrates and phosphate are introduced. Oxidation rates are substantially altered and adenosine triphosphate is formed while the mitochondrial morphology stays constant. This observation suggests that the morphological appearance of the inner mitochondrial membrane may depend primarily on osmotic changes created by experimental procedures and metabolism under steady state conditions.

Mitochondria have been shown to undergo changes in ultrastructure under a variety of conditions (1-10). Hackenbrock (1-3) examined isolated liver mitochondria in the electron microscope and demonstrated an ultrastructural transformation from the "high-energy" orthodox state to the "low-energy" condensed state when steady state metabolism was altered. Packer and his colleagues (4,5) carried this study a step further in showing that morphological changes occurred during oscillatory mitochondrial metabolism.

Green and his co-workers (6-8) have described three configurations (non-energized, energized and energized-twisted) of the inner mitochondrial membrane and have associated these configurations with metabolic states in the mitochondrion. The "non-energized" configurations were generated by uncouplers, adenosine diphosphate (ADP), translocation of ions and the absence of oxidizable substrate. The "energized" configurations appeared when oxidizable substrate or adenosine triphosphate (ATP) was added to "non-energized" mitochondria. The "energized-twisted" configuration was observed in the presence of oxidizable

substrate and inorganic phosphate (P_i). Both Hackenbrock and Green have assigned the change in morphology (configuration) to a conformational alteration within the membrane components. They have concluded that the morphological changes observed under the electron beam reflect molecular conformational energy capture within the membranes which may be used to drive energy-linked processes (e.g. ATP formation).

Recently, Stoner and Sirak (9) have proposed that these morphological states may be artifacts of fixation. Their results confirm (5) that glutaraldehyde interaction with mitochondrial enzymes occurs rapidly but glutaraldehyde treated mitochondria will still respond to the osmotic forces of the suspending medium. The "energized-twisted" configuration described by Green et al. (6-8) could be "passively" induced by varying the osmolarity of the medium in which the fixed mitochondria were suspended. Pfaff et al. (10) have also demonstrated that ultrastructural transitions occur when liver and kidney mitochondria are exposed to solutions of varying tonicity.

Our studies of structure-function relationships in beef heart mitochondria demonstrate the stability of a morphologically distinct membrane arrangement even though metabolism is altered from one steady state to another.

METHODS AND MATERIALS. Beef heart mitochondria prepared by a modification of the method of Crane, Glenn and Green (11) were further purified according to the method of Hatefi and Lester (12). Only mitochondria with high coupling efficiency and respiratory control ratios were utilized in these experiments. The experimental details including the preparation for electron microscopic examination are stated in the legends to the figures and table. Protein was determined using the biuret method of Gornall et al. (13).

RESULTS. The morphological configurations presented by Green et al. have been confirmed (Fig. 1). In the absence of added substrate (endogenous substrate present), a mixture of configurational states with predominately "non-energized" and "energized" cristae are observed (1A). A mitochondrion in the "energized-twisted" configuration may be seen occasionally. Although pyruvate

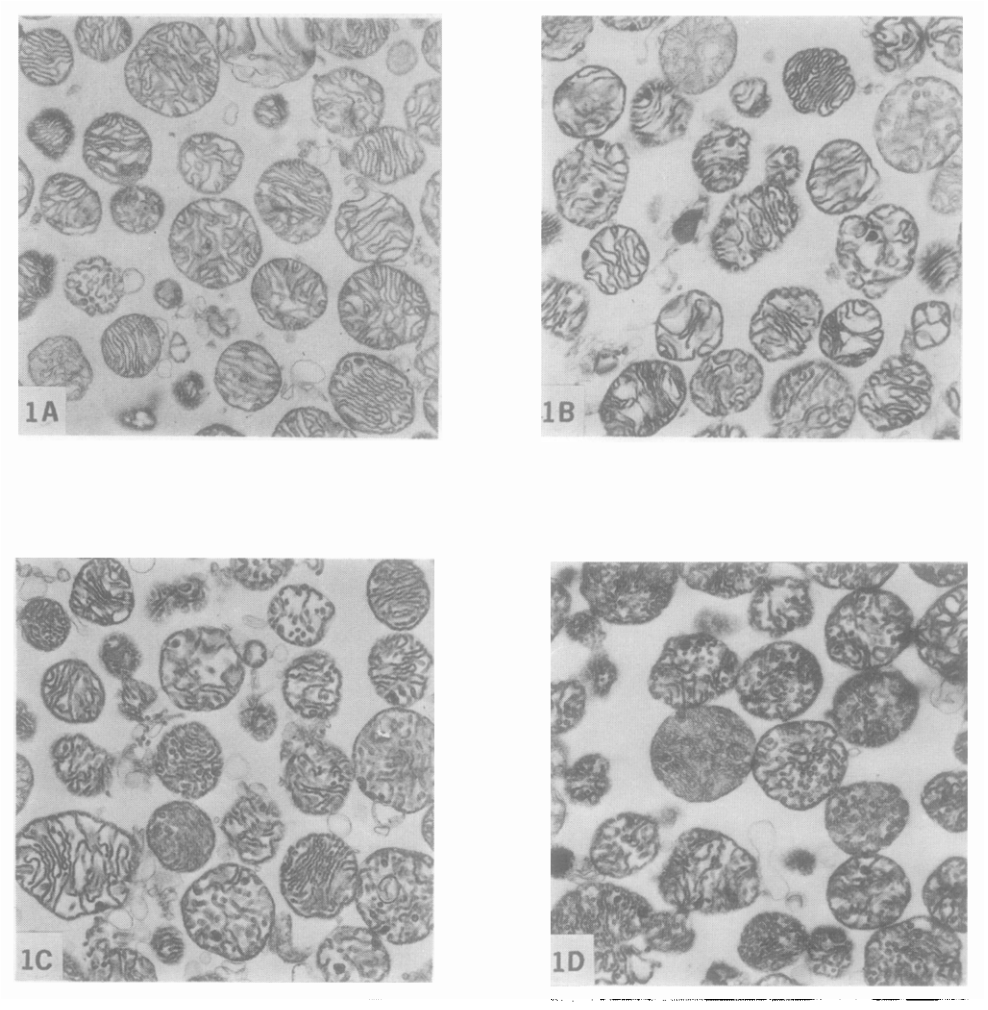

Figure 1. Heavy beef heart mitochondria were incubated (30°) in a buffered solution of 0.25 M sucrose containing 5 mM tris-HCl (pH 7.5) at a protein concentration of 1 mg/ml. Two ml samples of each treatment were removed after 1 minute incubations in media containing the following reagents: (A) No additions; (B) 30 µmoles pyruvate and 3 µmoles malate; (C) 20 µmoles P_i; (D) 30 µmoles pyruvate, 3 µmoles malate and 20 µmoles P_i. These aliquots were mixed immediately with 8 ml of a solution that was 0.25 M in sucrose, 0.05 M in the sodium salt of cacodylic acid, 2% in glutaraldehyde and 2% in acrolein at pH 7.5. Samples remained in this fixative at room temperature for 15 to 30 minutes. The remaining steps in the procedure for electron microscopy are those described by Green et al. (7). Specimens were examined with a modified RCA EMU-2 electron microscope. Magnification x 10,000.

plus malate (1B) generates the "energized" state, significant numbers of mitochondria in the "non-energized" (12 to 18 percent) and "energized-twisted" state (5 to 12 percent) are present. When P_i is added alone (1C) or together with

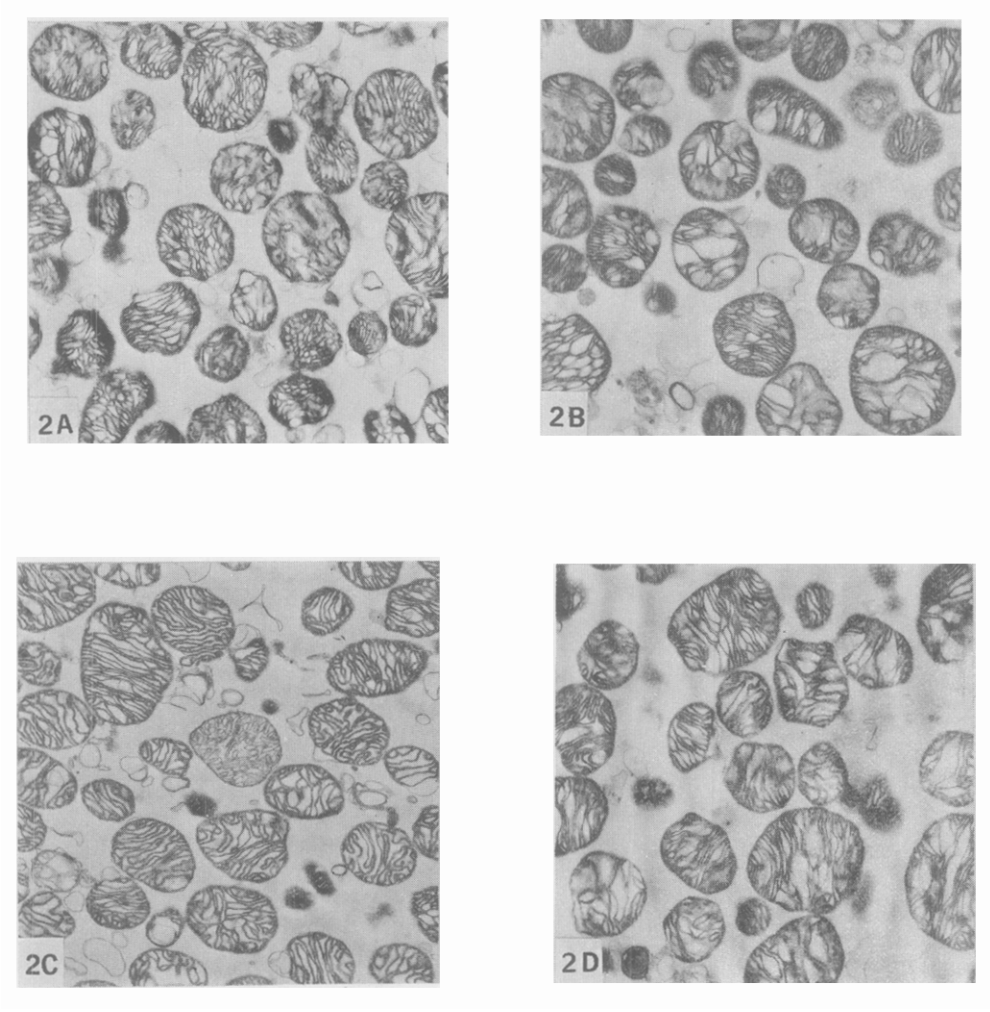

Figure 2. Heart mitochondria were suspended, fixed and examined under the conditions described in Figure 1. The following reagents were added prior to incubations of the suspensions for specified times: (A) 1 µmole ADP for 1 minute; (B) 30 µmoles pyruvate plus 3 µmoles malate for 1 minute, followed by 1 µmole ADP for 1 minute; (C) 1 µmole ADP for 1 minute followed by 20 µmoles P_i for 15 sec.; (D) 1 µmole ADP for 1 minute then 30 µmoles pyruvate plus 3 µmoles malate for another minute followed by 20 µmoles P_i for 15 seconds. Magnification x 10,000.

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substrate (1D) the population of mitochondria is more than 85 percent in the "energized-twisted" configuration.

The results involving ADP are shown in Fig. 2. The addition of ADP alone converts the morphologically mixed population into a relatively homogeneous population of the "non-energized" configuration (2A). The addition of ADP to pre-

TABLE I

Oxidation Rates of Mitochondria in Various Metabolic States.

Conditions: Oxidation rates were determined polarographically with a Gilson Medical Electronics Oxygraph. Pyruvate 15 mM and malate 1.5 mM-pH 7.4 were used as substrate. Other conditions were identical to those described in Figures 1 and 2.

Additions	mμ atoms oxygen/min/mg protein
none	30.2
P_i	53.5
ADP	29.6
P_i then ADP (state III)	338.8
ADP then P_i (state III)	289.9
*P_i then ADP (state IV)	67.3
*ADP then P_i (state IV)	74.2

* Oxidation rates after added ADP has been depleted.

dominantly "energized" mitochondria that are oxidizing pyruvate plus malate (Table I and Fig. 2B) alters the morphological appearance into the "non-energized" configuration while oxygen uptake remains constant. The addition of P_i to mitochondria that are exposed to substrate and ADP promotes a rapid increase in the oxygen consumption even though the cristae remain in an essentially "non-energized" configuration (2C). Some "energized" membrane configurations are visible when mitochondria are first exposed to ADP, then pyruvate plus malate and finally P_i which stimulates respiration (2D). The addition of ADP to mitochondria in any configuration or morphological state almost universally transforms the population into a condensed (non-energized) membrane network.

DISCUSSION. The "non-energized" configuration of the inner mitochondrial membrane may be generated by ADP alone. The addition of oxidizable substrate and phosphate does not change this morphological arrangement. Under these conditions the mitochondria form ATP but morphological transitions are not apparent.

The ability of ADP to uniformly transform all morphological configurations of the inner mitochondrial membrane into a condensed (non-energized) configuration, essentially devoid of matrix, leads us to challenge the postulated energy-capturing role assigned to gross configurational transformations in energy-linked mitochondrial processes (1-3, 6-8). The most damaging evidence against this interpretation is the discharge of the "energized" configuration generated by oxidizable substrates upon the addition of ADP when energy is not siphoned off. This does not imply that conformational changes at the molecular level are not involved in oxidative phosphorylation but only serves to point out that morphological changes of entire populations of mitochondria under steady state metabolism is unlikely to be a driving force for energy-linked functions. Rather, they may be a result of metabolic activity which creates osmotic transitions through translocation of ions. We conclude that although conformational changes may be taking place at the molecular level within the mitochondrial membrane, energy transducing processes including oxidative phosphorylation need not be expressed as gross morphological changes in the mitochondrial membrane.

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