Ultrastructural Transformations in Bean Inner Mitochondrial Membranes

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ABSTRACT

The ultrastructure of inner membrane-matrix mitochondria isolated from bean (Phaseolus vulgaris) shoots was examined in different metabolic states. Gross ultrastructural transformations analogous to the condensed-to-orthodox configurational changes reported in mammalian mitochondria are observed on transition from nonrespiring to respiring metabolism. With the induction of oxidative phosphorylation, the particles remain in the orthodox configurational state. The reverse orthodox-to-condensed configurational changes observed in mammalian preparations do not occur. Optically monitored absorbancy studies with bean particles show a substrate-supported Pi-induced swelling under the same conditions that induce the condensed-to-orthodox ultrastructural transformation. The swelling is associated with the net uptake of K+ and Pi as well as a small Pi-induced respiratory stimulation. When phosphorylation is initiated with these swollen particles, the optically monitored volume remains unchanged. Thus a positive correlation exists between the ultrastructural configuration and the osmotic volume changes, which supports the conclusion that configurational changes reflect internal osmotic adjustments.

Ultrastructural configurational changes in mitochondrial membranes from animal tissues have been interpreted as reflections of energy-linked reactions associated with oxidative phosphorylation (7, 8, 19, 23). Hackenbrock (7) observed two basic ultrastructural configurations in isolated mouse and rat liver mitochondria as well as in vivo with ascites tumor cells (9) and correlated these configurational states with different energy events of oxidative phosphorylation. The orthodox configuration (7) is associated with the respiratory state IV and appears to resemble most closely the ultrastructure of mitochondria observed in situ. In comparison, the condensed configuration is associated with mitochondria poised in metabolic state I, in which substrate oxidation is rate-limiting, and state III, in which the electron transport chain is rate-limiting. The observed reversible configurational changes from one ultrastructural form to the other are attributed to mechanochemical coupling events associated with oxidative phosphorylation.

Alternatively, Izzard and Tedeschi (13) have suggested that ultrastructural changes are induced by energy-dependent transport of ions into the mitochondria matrix and concomitant osmotic swelling. The presence of Pi, during state IV respiration induces mitochondrial swelling, 80% of which can be accounted for by the active uptake of Pi and accompanying ions. The Pi-induced swelling is reversed by the addition of ADP, which results in contraction as measured by light-scattering changes as well as an efflux of ions (2, 13). Stoner and Sirak (21) reported qualitatively similar appearing orthodox and condensed ultrastructural states by incubating rat liver mitochondria in solutions of different KCl and sucrose osmolalities. In studies with isolated beef heart mitochondria, the generation of the high energy ultrastructural form, the energized-twisted configuration (19) has been found to be inhibited by p-chloromercuriphenyl sulphonate (12) a compound which specifically blocks the transport of Pi. Thus, an alternative interpretation of the configurational states may be that they represent metabolically triggered osmotic adjustments rather than mechanochemical coupling events associated with oxidative phosphorylation.

Studies with another energy-generating system, the rat liver inner membrane-matrix particles lacking outer membrane, demonstrated specific and dramatic ultrastructural transformations induced by oxidative phosphorylation (8). The transformations appear to be isovolumetric and were interpreted to be mediated by energized conformational activity on the inner membrane rather than energized ion-induced osmotic activity.

This study reports gross ultrastructural configurations of coupled inner membrane-matrix particles isolated from bean shoots. The particles show qualitatively similar condensed and orthodox configurational states to those reported in rat liver mitochondria. However, the transformations appear to be correlated with osmotically linked volume changes rather than attributed directly to metabolic events.

MATERIALS AND METHODS

Six-day-old etiolated bean (Phaseolus vulgaris) shoot mitochondria were isolated by differential centrifugation in a media of 0.4 M mannitol, 50 mM tris-Tricine, pH 7.8, 5 mM MgCl2, and 1 mg/ml bovine serum albumin as previously described (14). The osmolality of the medium was 440 to 460 milliosmoles as measured on an Osmometer (Advanced Instrument, Inc.). A harsh grinding procedure involving a mortar was used to strip off the outer membranes. Equivalent preparations showed succinate cytochrome c reductase (EC 1.3.99.1) activity recently reported to be associated with the lack of outer membranes (4). The incubation medium (6.4 ml) for ultrastructural examinations contained: 0.4 M mannitol, 10 mM K-PO4, pH 7.4, 5 mM MgCl2 and has an osmolality of 430 to 450 milliosmoles.

After incubation in the various metabolic states, the particles

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were harvested by centrifugation in a Sorval Model RC2-B at 27,000g for 5 min. In a few studies mitochondria were harvested by rapid centrifugation with a Beckman/Spinco 152 microfuge. After centrifugation the mitochondria were fixed as pellets for 3 hr at 4°C in 2.5 ml of 2% OsO4 containing 0.32 M mannitol, 4 mM MgCl2, 4 mM KPO4, pH 7.4, and allowed to stand 2 to 3 hr. The osmolarity of the fixative ranged from 430 to 450 milliosmoles.

Following fixation the pellets were dehydrated in a graded ethanol series, passed through two changes of acetone and embedded in Araldite-Epon (18). The pellets were transferred to Beem capsules and polymerization was carried out at 60°C for 48 hr. Ultrathin sections were made using an LKB-III ultratome. Sections were poststained on uncoated grids for 10 min in 0.5% aqueous uranyl acetate and 5 min in 0.4% lead citrate (Reynolds). Electron micrographs were taken on a Hitachi HS-8 or HU-11E electron microscope.

Respiration was monitored in a 3.2-ml constant temperature glass cuvette with a Clark oxygen electrode attached to a Beckman Model 160 gas analyzer with a Sargent Model DSRG recorder. Protein was determined by the Lowry method using albumin as a standard (17). Phosphate was measured by the procedure of Fiske and Subbarow (5). Potassium levels were determined by atomic absorption after collecting the mitochondria on Millipore filters as previously described (14). It was necessary to eliminate the washing step after filtration to prevent loss of the net accumulated salt.

RESULTS

Ultrastructural Studies. The electron micrographs in Figure 1 show that most of the particles are free from intact outer membranes and thus correspond to the inner membrane-matrix fraction. The particles show respiratory control ratios of 2.5 to 3.5 and ADP/O values between 1.6 and 1.9 with succinate (Fig. 2) and may be compared to the rat-liver particles termed "mitoplasts" which lack outer membranes (8).

The points on the oxygraph trace in Figure 2, where samples were taken for electron microscopic examination in the different energy states, are designated by the letters a to c and the corresponding electron micrographs are shown in Figure 1, A to F. We have interpreted the results in Figure 1 to indicate the occurrence of ultrastructural states, analogous to the condensed (Fig. 1A), and orthodox (Fig. 1, C and E) configuration in mammalian systems. Freshly isolated nonrespiring particles exhibit a compacted matrix interior with large irregularly shaped intracistae spaces. The inner membrane is convoluted and tightly folded. The initiation of respiration with succinate transforms the particles into an orthodox-like ultrastructure (Fig. 1, C–D). The internal matrix has expanded and become more diffuse, and the cristae spaces are reduced, while the lumen of the cristae are protracted and tubular in geometry. The orthodox configuration was observed in respiring particles incubated for periods as brief as 4 min, although longer incubation times resulted in more complete transformation.

The addition of ADP to the suspension induced state III respiration rates (Fig. 2), but an examination of the ultrastructure of the particles showed they maintained an orthodox configuration, indistinguishable in appearance from those respiring in state IV (Fig. 1, E–F). The matrix remained diffuse and the inner membrane is expanded outward with the cristae space tubular and protracted. In other preparations, the incubation time with ADP was extended to 1.0, 1.5, and 2.0 min with no apparent difference in the ultrastructural configuration.

The internal ultrastructure of the orthodox configuration of the isolated particles appears to most closely resemble mitochon-
Fig. 1. Electron micrographs of bean particles exhibiting different ultrastructural states. The suspension media contained 0.4 M mannitol, 10 mM K-PO₄, pH 7.4, 5 mM MgCl₂. A and B: Freshly isolated bean shoot particles in the absence of respiration at × 19,000 and × 57,000 respectively. C and D: Particles incubated 12 min in the presence of 8 mM succinate at × 50,000 and × 55,000 respectively. E and F: Particles incubated 12 min with succinate followed by 45 sec with 1 μmole ADP at × 34,200 and × 58,500 respectively.
where the particles and 0.7 mM; temperature reaction media Figure in suspension The in transformation added of the mitochondria not essential for oxidative phosphorylation. These results are consistent with the ultrastructural studies with isolated mitochondria from several animal species (20), which fail to show a correlation between metabolic events and configurational states.

While a lack of correlation is observed between the metabolic events and the ultrastructural configurational states in bean particles, a positive correlation exists between the absorbance changes indicative of osmotic volume changes and the gross ultrastructural transformation. The transition from the condensed to orthodox configuration (Fig. 2, A–D) is paralleled by an absorbance decrease which corresponds to salt-induced (K+ and P_i) swelling (Fig. 3). Respiration is stimulated slightly and salt uptake occurs. The addition of ADP has no observable effect on the absorbance measurement, consistent with the lack of changes in ultrastructure. This conclusion appears in harmony with the osmotic volume interpretation for the configurational transformations observed with rat liver and beef heart mitochondria (12, 13, 21).

While it is clear that the gross ultrastructural changes reported here do not correspond to mechanochemical events (4, 10, 11, 24). The absence of an ADP-induced ultrastructural transformation in coupled bean particles demonstrated that it is not essential for oxidative phosphorylation. These results are consistent with the ultrastructural studies with isolated mitochondria from several animal species (20), which fail to show

FIG. 2. Oxygraph traces of respiring bean shoot particles. The reaction media was identical to Figure 1. Protein was 0.8 mg in A and 0.7 mg in B. In B the letters a to c correspond to the points where the particles were harvested for electron microscopy as shown in Figure 1, A to F. Concentration of added Na-succinate was 8 mM; temperature was 26 C. Numbers refer to nmoles O_2/min.

FIG. 3. Effect of P_i and ADP on swelling of bean shoot particles. The suspension media is identical to Figure 1C, with the exception of the concentration of P_i which was varied as shown. The ADP added was 0.5 μmoles; 1.0 mg protein.

FIG. 4. Stimulation of respiration in bean particles by P_i. The suspension media contained: 0.4 M mannitol, 50 mM tris-Tricine, pH 7.8. Protein was 0.8 mg. A: Stimulation by the addition of 40 mM K-PO_4, pH 7.8; B: effect of increasing concentration of P_i on respiratory stimulation.

Table 1. Salt Uptake by Bean Particles
The results are an average of five experiments. The medium and procedures are given in "Materials and Methods." Net uptake of K+ and P_i is the difference between the levels measured with and without 8 mM succinate. Protein is 1.3 mg.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time of Incubation</th>
<th>K+</th>
<th>Net K+</th>
<th>P_i</th>
<th>Net P_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>− Substrate</td>
<td>30 sec</td>
<td>363 ± 10</td>
<td></td>
<td>108 ± 5</td>
<td></td>
</tr>
<tr>
<td>+ Substrate</td>
<td>3 min</td>
<td>422 ± 13</td>
<td>59</td>
<td>132 ± 9</td>
<td>24</td>
</tr>
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fundamental to oxidative phosphorylation, they do not exclude
the possible occurrence of primary energy-linked conforma-
tional events associated with energy conservation at a molecu-
lar level which might not be detectable by electron microscopy.

It is not possible from the present investigations to distin-
guish whether the anion, \( P_1 \), or the cation, \( K^+ \), is the actively
accumulated species. However, studies with corn mitochon-
dria have reported evidence for a \( P_1 \) pump (11). In addition,
corn mitochondria (10, 11) as well as bean mitochondria (un-
published data), do not swell in the presence of the CI anion
of \( K^+ \), but rather contract. This would support the importance
of \( P_1 \) as the critical ion necessary for the swelling and that it
is this ion which is actively transported.

LITERATURE CITED
1. ANAGNOSTE, E. and H. TEBESCHI. 1970. The mechanism of low amplitude
orthophosphate-induced swelling in isolated rat liver mitochondria. J.
2. AZZONI, F. G. and A. AZZI. 1966. Mechanisms for reversible and irreversible
volume changes induced by inorganic phosphate in liver mitochondria. In:
J. M. Tager, S. Papa, E. Quagliariello. E. C. Slater, eds., Regulation of
pp. 332-350.
mitochondria. II. Low amplitude swelling and shrinkage cycles. Biochim.
47: 610-624.
5. FISKE, C. H. and Y. SUBBAROW. 1925. The colorimetric determination
mechanical activity in mitochondria. I. Reversible ultrastructural changes
with change in metabolite steady state in isolated liver mitochondria. J.
Cell Biol. 30: 269-297.
mechanical activity in mitochondria. II. Electron transport-linked ultra-
8. HACKENBROCK, C. R. 1972. Energy-linked ultrastructural transformations in
isolated liver mitochondria and mitoplasts. Preservation of configurations
1971. Oxidative phosphorylation and ultrastructural transformation in
734.
of mitochondria of constant volume. Tissue Cell 1: 527-552.
Structure of Plant Cells. Springer-Verlag, New York.
Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193:
265-275.
conformational basis of energy transformations in membrane systems. I.
59: 624-631.
possible relationship between ultrastructure and biochemical state of
volume and ultrastructure of mitochondria isolated from rat liver and
activity, ion translocation and conformational changes. Arch.
swelling and acetate uptake in corn mitochondria. Biochemistry 8: 1293-
1213.