The Effects of Drought Stress on Respiration of Isolated Corn Mitochondria

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ABSTRACT

Mitochondria were isolated from etiolated corn shoots (Zea mays L.) that were stressed to a measured water potential. The rates of mitochondrial respiration in state III, state IV, and without phosphate or ADP on a milligram protein basis decreased as water stress increased with succinate, malate-pyruvate, or reduced nicotinamide adenine dinucleotide as substrates. Coupling (as determined by respiratory control and ADP/O ratios) did not decrease with increasing water stress. At water potentials greater than −35 bars all respiration had ceased.

All experiments were carried out in a filled 4.0-ml glass temperature controlled reaction cell (27 ± 0.2 C) fitted with a Clark oxygen electrode (Yellow Springs Instrument Co.). Oxygen concentration was measured polarographically.

The reaction media for all experiments included 200 mM KCl, 20 mM tris-HCl (pH 7.5), 1 mg/ml bovine serum albumin, 4 mM KH2PO4, and about 1.0 mg of mitochondrial protein. Other reaction conditions are given in the figure legends. Mitochondrial protein was determined by the method of Lowry et al. (9).

RESULTS

Isolated mitochondria oxidizing succinate or malate-pyruvate showed reduced rates of O2 uptake as water stress increased (Figs. 1 and 2). Decreases in the rate of O2 uptake with increasing water deficit were observed when mitochondria were in either state III or IV until no respiratory activity was measured at a water potential of approximately −35 bars. No

MATERIALS AND METHODS

Corn seedlings (Zea mays L., Wf9 × M14) were grown on paper towelizing saturated with a 0.1 mM CaCl2 solution in glass trays covered with Saran Wrap slit at several places for aeration. Following 3 days of incubation in the dark at 30 ± 0.5 C, drought stress was induced by placing the seedlings on dry towelizing in uncovered trays for varying lengths of time before harvest. The water status of the etiolated shoot tissue was monitored with a Peltier type thermocouple psychrometer. Mitochondria were isolated from tissues of varying water potentials by the procedure of Miller et al. (12).

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FIG. 1. The effects of water stress on the rates of succinate oxidation by isolated mitochondria. Water status is presented as the difference from nonstressed corn in bars. The average water potential of nonstressed corn was 5.0 bars. Additions of 40 μmoles of succinate and 300 nmoles of ADP were made at 3 and 4 min, respectively, after the addition of the mitochondria to the reaction media (see "Materials and Methods").
stimulation in the rates of O₂ uptake was observed with either substrate in lightly stressed tissue.

The rates of oxidation of exogenous NADH by isolated mitochondria were also reduced as water deficits were increased (Fig. 3). This reduction was observed when isolated mitochondria were oxidizing NADH in state IV, state III, or without phosphate or ADP. Mitochondrial suspensions from lightly stressed tissue did not show a stimulation, but did maintain a rate comparable to the mitochondria isolated from nonstressed corn tissue.

Mitochondria exhibited normal respiratory control ratios for all water potentials up to the point where respiration was severely curtailed (Fig. 4). For all experiments respiratory control ratios for succinate, malate-pyruvate, and NADH averaged 1.91, 3.31, and 2.27. For the same respective substrates ADP/O ratios averaged 1.08, 1.70 and 1.04. These ratios are similar to values reported for normal mitochondria (11, 16). Maintenance of ADP/O levels indicates that phosphorylation is not uncoupled from respiratory activity.

**DISCUSSION**

It is evident that drought has a marked effect on the respiratory capacity of mitochondria isolated from water-stressed corn shoots. However, phosphorylation remains coupled to respiration even under severe water stress, a result contrary to the conclusions of Zholkevich and co-workers who studied drought effects on isolated pumpkin and pea root mitochondria.
The rates of oxidation of succinate, malate-pyruvate, and exogenous NADH were drastically inhibited at decreased water potentials. It has been suggested that as the free water content of the tissue is decreased and only bound water remains, the rate of respiration is reduced (7). Other work, notably that of Flowers and Hanson (5), indicates that the effects of water as a reactant in cellular processes are not the primary cause of respiratory inhibition in tissue of decreased water potentials.

We have shown in previous work (10) that alterations of mitochondrial membrane function occur with increasing water stress. Since membranes are the site of mitochondrial respiration and a structure-function relationship exists between membranes and respiration, changes in the phospholipid or protein structure of the mitochondrial membrane may cause the suppression of oxidation rates observed in water-stressed tissue. Membrane structure altered by depletion of water could, therefore, be the ultimate cause of the inhibited oxidation of respiratory substrates measured in mitochondria isolated from drought-stressed corn.

Our results show that mitochondria are affected by water stress and that these effects persist even following their isolation from stressed tissue. Although these results were not correlated with whole plant respiration, they do suggest a relationship between whole plant respiration as reported in the literature and in vitro mitochondrial respiration.

**LITERATURE CITED**


