Uncoupler-induced ATPase activity of plant mitochondria.

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The ATPase activity of mammalian mitochondria can be readily stimulated by a variety of protonophoric uncoupling agents, the extent being dependent on the assay conditions and the isolation medium used [1]. The situation in plant mitochondria is less straightforward. Jung and Laties [2] classified plant mitochondria into three types: (i) those where uncoupler readily stimulates activity, as in cauliflower [4,9], (ii) those where an 'ATP transport defect' exists and ATPase is induced only after a short burst of respiration in the presence of Mg2+ and Pi, as in cauliflower [4,9], (iii) those where a putative inhibitor protein limits activity, this latter class was subdivided into (a) those where electron transport is sufficient to cause inhibitor dissociation, as in castor bean endosperm [5], and (b) those where only sonication or trypsin treatment releases the inhibitor, as in potato [2].

Using isolated intact mung bean (Phaseolus aureus) mitochondria we decided to investigate further the factors controlling uncoupler-induced ATPase activity.

In agreement with previous findings [6], osmotic support markedly affected activity, with iso-osmolar sucrose completely abolishing activity. Removal of sucrose led to the appearance of uncoupler-induced activity, but had no effect on endogenous activity. These results are consistent with the existence of an ATP transport defect; removal of osmotic support would lead to swelling, resulting in greater permeability to molecules such as ATP. Cereijo-Santalo [7] argued that inhibition of ATPase activity in rat liver mitochondria by sucrose was due to depression of the water content of the matrix, i.e., an osmotic effect, rather than one involving ATP/ADP transport [8]. Furthermore, this 'sucrose effect' could be relieved by addition of a permeant salt. However, potassium acetate plus valinomycin did not significantly release the inhibition of activity by iso-osmolar sucrose in mung bean mitochondria.

Further evidence for an ATP transport defect was obtained from experiments involving 'respiratory priming': A short burst of respiration resulted in a significant increase in activity, this effect being most marked in a medium containing iso-osmolar sucrose. The effect could be inhibited 82% by the electron transport inhibitor, antimycin A. Furthermore, in agreement with results for cauliflower mitochondria [4], when 1mM succinate was present, 75% of the priming-enhanced activity was inhibited by the ATP/ADP translocase inhibitor, atractyloside (0.1 mM). This effect was not due to inhibition of ATPase activity per se, as 0.1 mM atractyloside caused only 18% inhibition under conditions where swelling would have abrogated any transport defect.

In contrast to castor-bean endosperm mitochondria, active state 2 respiration with 10 mM succinate did not increase uncoupler-stimulated activity. However, due to marked depression of basal activity by active respiration, the % stimulation of this activity was higher. Antimycin A partially restored this succinate inhibited activity.

Results presented here provide evidence that an ATP/ADP transport defect exists in isolated intact mung-bean mitochondria and is partially responsible for the suppression of uncoupler-induced ATPase activity. This transport defect can be overcome either by swelling due to removal of osmotic support, or by a short burst of respiration, before addition of uncoupler and ATP, which establishes a potential for the electrogenic accumulation of ATP. The transport defect is probably an artifact caused by the isolation conditions required to obtain intact plant mitochondria (see [1]). However, the maximum activity obtained under respiratory priming or swelling conditions was much lower (35%) than the rate of ATP synthesis (or ADP utilization) during state 4 respiration. Therefore, another factor(s) appears to be involved in suppressing uncoupler-induced ATPase activity in mung bean mitochondria, e.g., the inhibitor protein.

Mung-bean mitochondria appear therefore to represent an additional class to those described by Jung and Laties [2], with both inhibitor protein and ATP transport effects limiting uncoupler induced ATPase activity.

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