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Atractyloside inhibition of adenine nucleotide transport in mitochondria from plants

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SUMMARY

Atractyloside completely inhibits the 2,4-dinitrophenol-stimulated ATPase activity of respiratory-primed cauliflower mitochondria, but has no effect on sonicated preparations or on the endogenous activity associated with intact mitochondria. Atractyloside also blocked the shrinkage and State 3 respiration initiated by ADP in intact mitochondria. The data indicate the presence in cauliflower mitochondria of an adenine nucleotide translocator similar to that described for animal mitochondria.

Passam *et al.*[1] recently reported that atractyloside failed to inhibit adenine nucleotide-dependent reactions in Jerusalem artichoke mitochondria. State 3 respiration and ATPase activity were, however, inhibited by bongkrekic acid. They suggested the absence of an adenine nucleotide translocator similar to that of animal mitochondria. We wish to point out that other plant mitochondria do possess an atractyloside-sensitive adenine nucleotide translocator, but that it may not be apparent unless adenine nucleotide transport is activated.

Isolated cauliflower mitochondria ordinarily do not exhibit an uncoupler-stimulated oligomycin-inhibited ATPase[2–5]. This is due to a lesion in the ATP transport system which can be overcome by (1) inverting the inner membrane *via* sonication, or (2) by making the membrane permeable with high pH treatment, or (3) by respiratory "priming"[2], similar to that reported by Takeuchi *et al.*[6] for castor bean mitochondria and by Carmeli and Biale[7] for sweet potato mitochondria. Respiratory priming involves a short burst of respiration in the presence of Mg^{2+} and phosphate, following which the ATP transport is activated for about 2 min[2].

Atractyloside has no effect on the endogenous or unprimed ATPase activity of

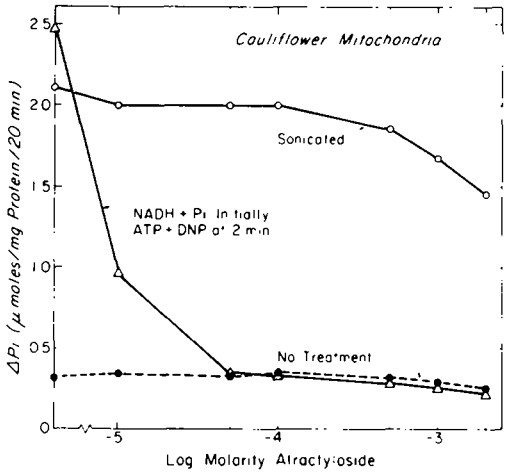

Fig. 1. Effect of atractyloside on cauliflower mitochondria ATPase activity. Reaction mixture contained 3 mM ATP, 200 mM sucrose, 20 mM KCl, 2 mM $MgCl_2$, 1 mg/ml bovine serum albumin and 20 mM *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.5. The procedures for mitochondria preparation and ATPase assay were as previously described[2]. For activation of the 2,4-dinitrophenol-stimulated ATPase, 0.25 μmole NADH and 0.5 mM KH_2PO_4 were added initially, with 3 mM ATP added at 2 min (when the NADH was exhausted) followed within 5 s by 0.1 mM 2,4-dinitrophenol (DNP).

cauliflower mitochondria (Fig. 1). This activity is not oligomycin sensitive[2]. If the mitochondria are primed by adding sufficient NADH to provide 2 min of respiration, followed by sequential addition of ATP and 2,4-dinitrophenol (the priming is collapsed if the 2,4-dinitrophenol is added first[2]), the ATPase is stimulated 7-fold. This activated ATPase is completely inhibited by $5 \cdot 10^{-5}$ atractyloside. Sonicated mitochondria have a high level of oligomycin-sensitive ATPase which requires Mg^{2+} and is not affected by 2,4-dinitrophenol[2]. This activity is insensitive to atractyloside until millimolar concentrations are approached. Similarly, atractyloside inhibits the 2,4-dinitrophenol-stimulated ATPase in intact liver mitochondria[8] while the ATPase activity of disrupted mitochondria is largely insensitive to atractyloside[9-11].

Cauliflower mitochondria also show inhibition of the ADP-stimulated respiration by atractyloside (Fig. 2A) as reported for animal mitochondria[8,9,12,13]. Here respiratory priming of the AdN translocator is inherent. The inhibited State 3 respiration is gradually released by consecutive additions of ADP in agreement with the finding that atractyloside

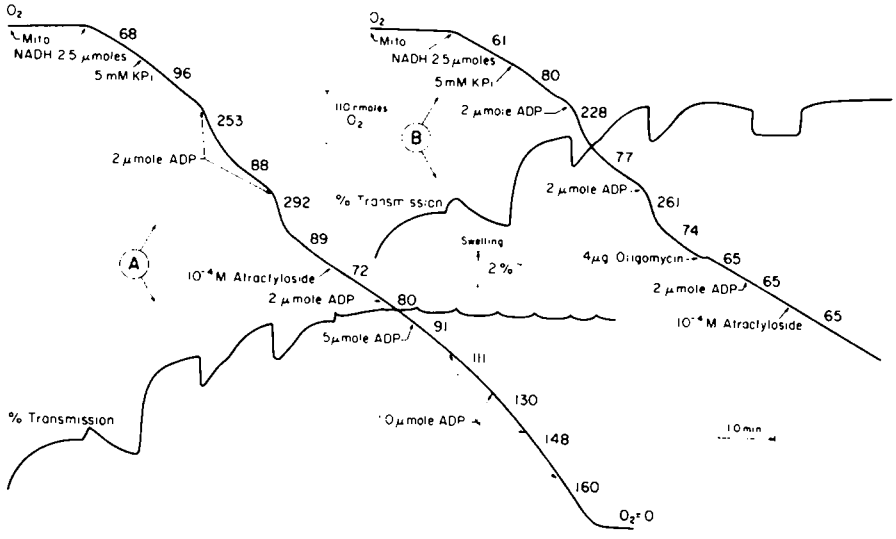

Fig. 2. Oxygen uptake and swelling-shrinkage traces for cauliflower mitochondria. Numbers are nanomoles O_2/min per mg protein. Percent transmission was measured at 520 nm. Reaction mixture as described in Fig. 1 *minus* the ATP. Additions as indicated.

is a competitive inhibitor of ADP[14]. The sharp contraction shown by the transmission trace on addition of ADP is eliminated when atractyloside is present. In oligomycin-blocked mitochondria atractyloside causes a sharp reversal of the ADP contraction (Fig. 2B). Stoner and Sirak[15] have shown these changes to be due to competitive binding of the ADP and atractyloside to the transporter.

Corn mitochondria exhibit an uncoupler-stimulated ATPase without respiratory priming[16] although it is enhanced by priming[2]. In these mitochondria atractyloside blocks the normal State 3 respiration and shrinkage initiated by ADP addition[17], and also the exit of ADP–As during arsenate uncoupling[18]. We have found that atractyloside inhibits the 2,4-dinitrophenol-stimulated ATPase activity of intact corn mitochondria, but has virtually no effect on sonicated preparations (Jung, D.W. and Hanson, J.B., unpublished).

Based on the above evidence we believe plant mitochondria have the same type of adenine nucleotide translocator as animal mitochondria. Plant mitochondria have been reported to differ from animal mitochondria in several aspects[19,20]. These differences may be inherent or due to damage suffered by the harsh grinding and release of vacuolar contents during isolation. It appears that some plant mitochondria preparations, particularly those which do not exhibit an uncoupler-stimulated oligomycin-inhibited ATPase activity, have an ATP transport lesion. In these cases, the effectiveness of atractyloside can only be assessed after the activity of the adenine nucleotide transporter is restored. The best assay for this purpose is the uncoupled ATPase. It has not yet been possible to demonstrate an uncoupler-stimulated ATPase in Jerusalem artichoke mitochondria[21].

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