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INHIBITION OF ELECTRON TRANSPORT IN *ARUM MACULATUM* MITOCHONDRIA BY POTASSIUM THIOCYANATE

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Key Word Index—*Arum maculatum*; Araceae; mitochondria; electron transport; inhibition; thiocyanate.

Abstract—Potassium thiocyanate (KSCN) has been shown to inhibit the oxidation of succinate in *Arum* mitochondria at concentrations which have little effect on the rate of oxidation of exogenous NADH, suggesting that the site of inhibition is on the succinic dehydrogenase. In whole mitochondria there is a strong synergistic interaction between KSCN and antimycin A or uncoupling agents. In sonically produced submitochondrial particles the oxidation of succinate was found to be more sensitive to KSCN and no synergistic interaction between KSCN and antimycin A or uncoupling agents was observed. The results have been discussed in relation to the ease with which KSCN can penetrate the mitochondria and reach its site of action.

INTRODUCTION

MITOCHONDRIA isolated from different species of plants exhibit varying degrees of sensitivity of electron transport to potassium cyanide; this can range from mitochondria isolated from *Helianthus tuberosus* tubers which are completely inhibited by cyanide[1] to those obtained from skunk cabbage (*Symplocarpus foetidus*) or *Arum maculatum* spadices which are completely insensitive to 10^{-3} M KCN.[2,3] Evidence is now available to suggest that the cyanide resistant component of respiration is mediated by a separate alternative oxidase,[1] which appears to branch from the normal cytochrome chain in the region of cytochrome *b*.[2] It is fairly certain that electrons transported by this cyanide insensitive oxidase are not coupled to the synthesis of ATP.[4,5]

Little positive data is available to define the characteristics of the functional components of the alternative oxidative chain. Spectrophotometric studies of isolated skunk cabbage mitochondria indicate that the alternative oxidase chain contains no functional cytochrome components.[1] Inhibitor studies have been a little more productive; the observation that iron chelating reagents such as KSCN, α,α'-dipyridyl and 8-hydroxyquinoline appear to selectively inhibit the alternative oxidase has led to the suggestion that it may contain a functional non-haem iron protein.[1,6]

Experiments described in this paper show that KSCN appeared to selectively inhibit the alternative, antimycin A resistant, oxidase in *Arum* mitochondria. This apparent selectivity disappeared when an uncoupling agent was added to whole mitochondria or when

[1] D. S. BENDALL and W. D. BONNER, *Plant Physiol.* **47,** 236 (1971).
[2] B. T. STOREY and J. W. BAHR, *Plant Physiol.* **44,** 115 (1969).
[3] D. S. BENDALL, *Biochem. J.* **70,** 381 (1958).
[4] B. T. STOREY and J. W. BAHR, *Plant Physiol.* **44,** 126 (1969).
[5] H. C. PASSAM and J. M. PALMER, *J. Exptl Bot.* (1972) in press.
[6] W. D. BONNER, D. S. BENDALL and M. PLESNICAR, *Federation Proc.* **26,** 731 (1967).

submitochondrial particles were used. Under these conditions the site of inhibition appeared to be at the level of succinic dehydrogenase rather than on the oxidase itself. The data are discussed with respect to the ease with which the inhibitor gains access to its site of action within the organelle.

RESULTS AND DISCUSSION

Bendall and Bonner[1] have reported that 0·1 mM KSCN will selectively inhibit the operation of the cyanide resistant oxidase in skunk cabbage mitochondria.* Data presented in Fig. 1 show that when using *Arum* mitochondria much higher concentrations of KSCN (50 mM) were necessary to inhibit electron transport and that the oxidation of succinate was much more sensitive to inhibition than was the oxidation of NADH or ascorbate plus *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD). Since it has been suggested that much of the oxidation of NADH is mediated by the cyanide resistant oxidase even in the absence of cyanide[5] it would appear that the inhibition of succinate oxidation cannot be the result of inhibition at a point on the electron transport chain traversed by electrons from both NADH or succinate, and is therefore likely to be located on the succinic dehydrogenase. The inhibition of oxidation of NADH and ascorbate + TMPD (Fig. 1) caused by the very high KSCN concentrations (0·5 M) probably results from a general disorganization of the membrane resulting from the chaotropic activity of KSCN.[7]

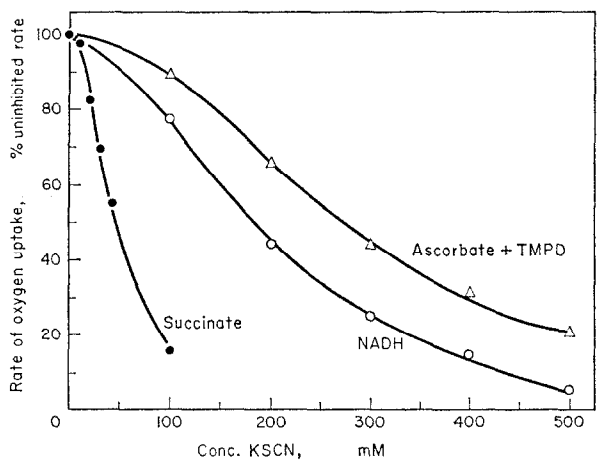

FIG. 1. THE INFLUENCE OF KSCN ON OXYGEN UPTAKE USING EITHER 20 mM SUCCINATE, 1 mM NADH OR 30 mM ASCORBATE + 0·5 mM TMPD AS ELECTRON DONORS.
Oxygen uptake was measured polarographically as described in the text in the presence of 1 mM FCCP. The uninhibited rates of oxygen uptake for each substrate expressed in nmol oxygen/min/mg protein were succinate 241, NADH 1599 and ascorbate + TMPD 538.

The oxygen electrode traces presented in Fig. 2 show that there is a synergistic interaction between KSCN and antimycin A. In the absence of antimycin A KSCN (50 mM) caused a 44% inhibition of succinic oxidase activity while in the presence of antimycin A

* *Editorial note.* It has been drawn to our attention that, due to a printer's error, the concentration of KSCN used by Bendall and Bonner was given incorrectly in their paper as 0·1 mM; the actual concentration used was 0·1 M.

[7] Y. HATEFI and W. G. HANSTEIN, *Proc. Natl. Acad. Sci.* **62**, 1129 (1969).

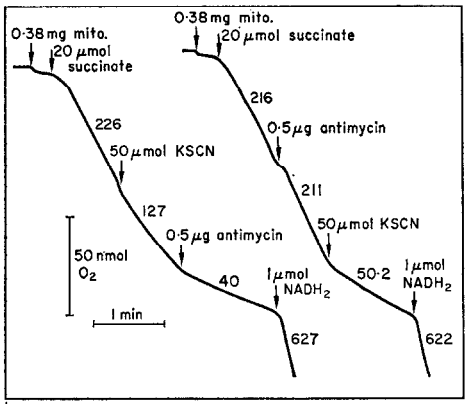

FIG. 2. OXYGEN ELECTRODE TRACES SHOWING THE INHIBITION OF SUCCINATE OXIDATION BY KSCN AND THE SYNERGISTIC INTERACTION WITH ANTIMYCIN A.

The experiment was carried out in the absence of ADP or uncoupling agent. Figures above the trace are the rates of oxygen uptake in nmol/min/mg protein.

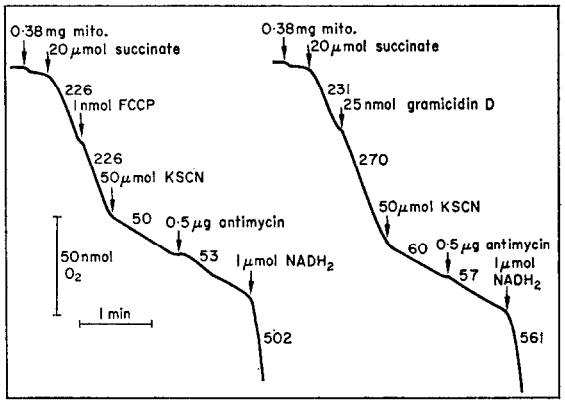

FIG. 3. OXYGEN ELECTRODE TRACES SHOWING THE ABSENCE OF ANY SYNERGISTIC INTERACTION BETWEEN KSCN AND ANTIMYCIN A ON THE INHIBITION OF SUCCINATE OXIDATION IN THE PRESENCE OF UNCOUPLING AGENTS.

the same concentration of KSCN resulted in a 76% inhibition of the electron transport rate. However, the site of inhibition does not appear to be located on the alternative oxidase because the addition of NADH resulted in the very rapid utilization of oxygen shown in Fig. 2. No energy acceptor (ADP) or uncoupling agent was added to the mitochondria in the experiment which yielded these traces because under normal conditions *Arum* mitochondria do not show any respiratory control and show high rates of respiration in the absence of ADP. When the experiment was repeated in the presence of either FCCP or gramicidin D the addition of KSCN alone resulted in a high level of inhibition which could not be enhanced by the addition of antimycin A, as can be seen from the

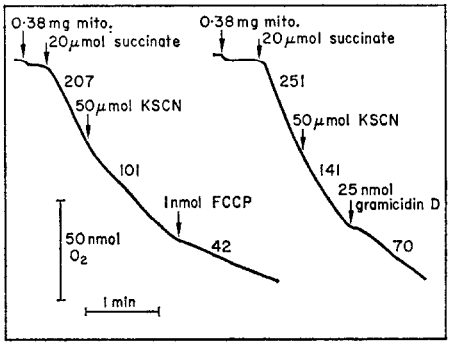

FIG. 4. OXYGEN ELECTRODE TRACE SHOWING A SYNERGISTIC INTERACTION BETWEEN KSCN AND UNCOUPLING AGENT.

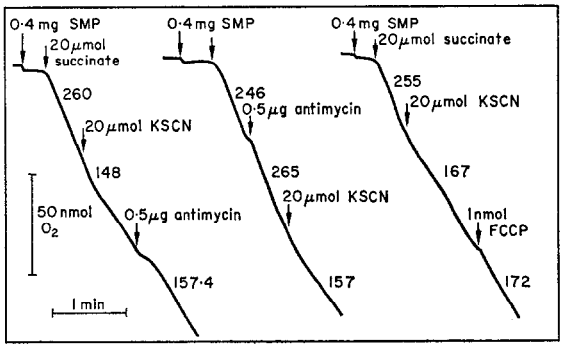

FIG. 5. OXYGEN ELECTRODE TRACES SHOWING THE INFLUENCE OF KSCN ON THE RATE OF SUCCINATE OXIDATION IN SUBMITOCHONDRIAL PARTICLES.

No synergistic interaction between KSCN and antimycin A or FCCP is apparent. Assayed in the absence of ADP or uncoupling agent except where indicated on the trace.

traces shown in Fig. 3. From the data shown in Fig. 4 it can be seen that if the uncoupling agent was added after the KSCN it resulted in a further inhibition. It is therefore apparent that there is a synergistic interaction between KSCN and uncoupling agents or antimycin A. In all cases it is also apparent that the oxidation of succinate is much more sensitive to inhibition than is the oxidation of NADH.

One possible explanation of the observed interaction between KSCN and antimycin A or FCCP is to consider that the locus of inhibition is the internally located succinate dehydrogenase. In order to reach this site the KSCN would need to traverse the inner mitochondrial membrane which it normally does as the anion SCN^-.[8] In normal mitochondria lacking an energy acceptor the entry of SCN^- would be opposed by the membrane potential.[8] When an uncoupling agent such as FCCP or gramicidin D is added the membrane potential would be collapsed and it is then possible that SCN^- would enter and reach its site of inhibition more readily. The synergism between KSCN and antimycin A can be explained on the same basis. It has been shown both in skunk cabbage[4] and *Arum* mitochondria[5] that the conventional, antimycin A sensitive, cytochrome chain is coupled to the synthesis of ATP, its operation could therefore be expected to maintain a membrane potential across the inner membrane. In contrast, the alternative antimycin A resistant oxidase does not appear to be coupled to the synthesis of ATP;[4,5] therefore, it may not be able to generate a membrane potential across the coupling membrane. In the absence of antimycin A both oxidases would contribute to the total rate of oxygen consumption, thus the activity of the conventional electron transport chain would generate a membrane potential which would oppose the entry of SCN^- and result in a low level of inhibition. The addition of antimycin A would prevent the operation of the conventional oxidase, thus removing the source of the membrane potential allowing the more rapid penetration of SCN^- to its site of action.

When the experiments were repeated using sonically produced submitochondrial particles, in which the polarity of the membrane was reversed so that there was no permeability barrier between SCN^- and the succinic dehydrogenase, it was impossible to show any synergism between KSCN and antimycin A or FCCP (Fig. 5). 50 mM KSCN used in previous experiments with whole mitochondria caused a very high level of inhibition (over 80%) when added alone to submitochondrial particles, so a lower concentration of KSCN (20 mM) was used in this experiment to produce approximately a 50% inhibition. The oxidation of NADH was relatively unaffected by either 20 or 50 mM KSCN in these submitochondrial particles. This observation is in agreement with the explanation of the synergistic interactions outlined above.

The data presented in this paper confirm that KSCN and antimycin A do act synergistically to inhibit respiration in *Arum* mitochondria. There are, however, some very important differences between these *Arum* mitochondria and the skunk cabbage mitochondria in which Bendall and Bonner[1] found a synergistic interaction between KSCN and antimycin A both in the presence of FCCP and in submitochondrial particles using very much lower concentrations of KSCN.

EXPERIMENTAL

Arum maculatum inflorescences were collected at the stage just prior to the opening of the spathe (stages β and γ, see James and Beevers[9]). The sterile heads of the spadices were used in these experiments. The coloured cell contents of the epidermises were removed by wiping with damp cotton wool, the mitochondria

[8] P. MITCHELL and J. MOYLE, *Europ. J. Biochem.* **9**, 149 (1969).
[9] W. O. JAMES and H. BEEVERS, *New Phytol.* **49**, 535 (1950).

were then extracted using the method described by Storey and Bahr[2] except that 10 mM Tris hydroxymethyl-aminomethane (Tris) was used instead of 10 mM morpholinopropane sulphate as the buffer. Oxygen uptake was measured polarographically using a Clarke electrode fitted to a Gilson 'Oxygraph', in 1 ml of a medium containing 300 μmol sucrose, 5 μmol *N*-Tris (hydroxymethyl) methyl-2-aminoethane sulphonate (TES), 50 μmol $MgCl_2$, 25 μmol KH_2PO_4 at pH 7·2 and 25°. Other additions made during the experiment are stated in the figures. Mitochondria containing between 0·2 and 0·5 mg protein were added to start the reaction. Submitochondrial particles were prepared by exposing a mitochondrial suspension containing approximately 15 mg protein/ml to ultrasonic oscillations (20 Kc) using a Dawe Soniprobe operating at 75% of full power for 2–3 sec for every ml of suspension treated.[10] The unbroken mitochondria were removed by centrifugation at 40 000 *g* for 15 min, the particles were then sedimented at 140 000 *g* for 60 min in an MSE 50S centrifuge. The particles were finally suspended in the same medium as the mitochondria to yield a preparation containing 20–30 mg/ml of protein. The protein concentration was measured using the biuret reagent[11] after solubilization in 10% deoxycholate.

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[10] H. C. Passam and J. M. Palmer, *J. Exptl Bot.* **22**, 304 (1971).
[11] A. G. Gornall, C. J. Bardawill and M. M. David, *J. Biol. Chem.* **173**, 751 (1949).