The experimental herbicide UKJ72J is an inhibitor of succinate oxidation in plant mitochondria

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1. INTRODUCTION

Two inhibitors of mitochondrial succinate oxidation have been described so far, namely thenoyltrifluoroacetone (TTFA) [1–3] and carboxin [4–6].

TTFA inhibits succinate oxidation in mitochondria from mammals [1–3], fungi [7] and higher plants [8–10]. It is believed to have at least two sites of action in the succinate–ubiquininone oxidoreductase part of the electron transfer chain [3], and in addition to inhibit the alternate oxidase (CN^--insensitive) in plant mitochondria [9,10]. Moreover, it permeabilizes the mitochondrial membrane to H^+ [11] and to other ions such as Ca^{2+} and Mg^{2+} [12].

Carboxin displays the same inhibitory action as TTFA (although at lower concentrations) in fungi [4, 5] and mammalian [6] mitochondria. However, it is far less effective on plant mitochondria [13].

UKJ72J (2-ethylamino,4-amino,5-thiomethyl, 6-chloropyrimidine) is a new experimental herbicide from the thiopyrimidine family [14]. Here, we show that it is an inhibitor of succinate oxidation. Its inhibitory activity presents the following features: (i) it is specific for succinate oxidation; (ii) it is specific for plant mitochondria as compared to fungi and mammalian ones.

Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; CCCP, carbonylcyanide *M*-chlorophenylhydrazone; I_{50}, concentration at which an activity is 50% inhibited; MOPS, morpholinopropanesulfonate; NADH, nicotinamide adenine dinucleotide (reduced form); PMS, phenazinemethosulfate, TTFA, thenoyltrifluoroacetone; UKJ72J, 2-ethylamino, 4-amino, 5-thiomethyl, 6-chloropyrimidine

2. MATERIALS AND METHODS

Potato (*Solanum tuberosum*, cv. Bintge) tuber mitochondria were prepared by a conventional method and purified on a Percoll gradient as in [15]. Rat liver mitochondria were prepared by differential centrifugation as in [16]. Yeast (Saccharomyces cerevisiae) mitochondria were obtained as in [17].

Respiratory studies were performed in a 4-ml thermostated cell where the oxygen concentration was monitored with a Yellow Spring oxygen electrode. Substrate concentrations were as follows: 6 mM for succinate, 25 mM for malate, 10 mM for glutamate and 2 mM for NADH. Respiratory media were conventional.

Effects on state 3 respiration were measured by addition of the chemical under study about 1 min after introduction of excess ADP (1.25 mM) and the establishment of linearity.

Succinate–PMS oxido-reductase activity was measured polarographically in the basic reaction mixture with 5 mM succinate, 1 mM ADP, 1 mM PMS, 0.1 μM antimycin A and 1 mM KCN.

To determine the effect of UKJ72J on the

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mitochondrial membrane permeability to H^+, mitochondria were suspended in 150 mM NH_4NO_3, 10 mM MOPS (pH 7.2) and absorbance at 520 nm was monitored. To determine its effect on the K^+ permeability, mitochondria were suspended in 150 mM KNO_3, 10 mM MOPS (pH 7.2) and the same procedure was followed. The 2-ml reaction mixtures contained 0.2–0.25 mg protein/ml and had an initial absorbance of 0.6–0.7.

The results presented here are the means of at least 3 independent experiments.

3. RESULTS AND DISCUSSION

Table 1 shows that UKJ72J is a potent inhibitor of succinate oxidation in potato tuber mitochondria. I_{50} concentration was 60 nmol/mg mitochondrial protein. In our experimental conditions it corresponded to 6–9 μM UKJ72J. At 100 M UKJ72J, the inhibition amounted to 80–90%. TTFA was less potent with a I_{50} of 160 nmol/mg protein (standard deviation = 30). This result is in agreement with those in [8–10] where an I_{50} of 40–60 μM, corresponding to 120 nmol TTFA/mg protein, was found. Hence, UKJ72J is a more potent inhibitor than TTFA. In addition, although inhibiting to some extent malate oxidation, UKJ72J displayed a far greater activity towards succinate oxidation (table 1; fig. 1). In this respect, UKJ72J was not less specific than TTFA (see [9,11]. External NADH oxidation was slightly inhibited (fig. 1) with a I_{50} of 2100 nmol/mg protein. It can be thus concluded that UKJ72J is a specific inhibitor of succinate oxidation in potato tuber mitochondria. However, its action on malate and external NADH oxidations (though limited) suggests that this inhibitor possesses other site(s) of action.

Table 1

Inhibitory activity of UKJ72J on mitochondria from various origins

Mitochondria	Succinate	Malate
Potato	60	4500
Yeast	2100	–
Rat liver	1300	1500

I_{50} values are expressed in nmol/mg mitochondrial protein. Standard deviations were 1–19% of the mean values, except for rat malate 45%

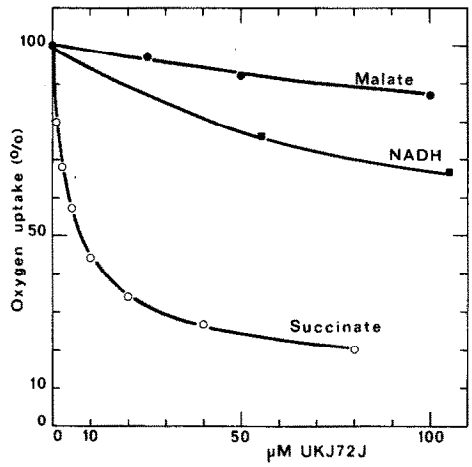

Fig. 1. Typical experiment showing inhibition of state 3 oxygen consumption in potato tuber mitochondria oxidizing various substrates and treated with UKJ72J. Mitochondrial protein was 0.15 mg/ml.

UKJ72J inhibition on succinate oxidation could result from either an inhibition of succinate transport into the mitochondria or an inhibition of electron transfer between succinate dehydrogenase and ubiquinone. To determine which of the two processes was inhibited by UKJ72J, we studied its effect on succinate–PMS oxido-reduction. The experiment showed that 10 μM UKJ72J had no effect on this reaction, allowing us to discard an inhibition of succinate transport as a basis for UKJ72J inhibition. However, at 20-times the I_{50} concentration, UKJ72J 35% inhibited succinate–PMS oxido-reduction. Similarly, at 8-times the I_{50} concentration, TTFA 48% inhibited this reaction, in agreement with the results on heart muscle mitochondria in [3].

UKJ72J 100 μM did not increase malate-driven state 4 oxygen consumption in tightly coupled potato tuber mitochondri; 10 μM UKJ72J did not induce any mitochondrial swelling in either NH_4NO_3 or KNO_3 (isoosmotic). At 200 μM (roughly 20-times the I_{50} concentration) UKJ72J induced only limited swellings in these salts. They amounted to less than 1% of the ones induced by 10 μM CCCP and 10 μM valinomycin respectively. From those experiments we can conclude that, at inhibitory concentrations, UKJ72J did not increase the permeability of the mitochondrial membrane to H^+ or K^+. TTFA was as ineffective as UKJ72J on K^+ permeability. Conversely, it induc-

ed a limited (2–4% of that induced by 10 μM CCCP) swelling in NH_4NO_3, demonstrating an increase in H^+ permeability, in agreement with the results in [11].

In spite of some similarities, the information so far available does not allow us to decide whether UKJ72J acts at the same site as TTFA and carboxin.

A striking feature of UKJ72J action is its far smaller inhibitory activity on yeast and rat liver mitochondria (table 1). It denotes a specificity towards plant mitochondria (soybean, mung bean and pea mitochondria were equally sensitive to UKJ72J; not shown). This specificity is opposite to that displayed by carboxin [4–6,13]. From the toxicological point of view, the low inhibitory activity of UKJ72J in mammalian mitochondria is very interesting and it demonstrates the feasibility of designing pesticides which, although acting at the mitochondrial level, are safe for man and animals.

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