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INHIBITION OF SUCCINATE OXIDATION BY THE HERBICIDE UKJ72J

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Key Word Index—Plant mitochondria; herbicide; thiopyrimidine; UKJ72J; succinate oxidation; inhibitor.

Abstract—The inhibitory activity of the herbicide UKJ72J on succinate oxidation in mitochondria from various plant species was studied. In monocotyledons (Gramineae: wheat, oat, maize; Liliaceae: onion, leek) succinate oxidation was affected only at high concentrations. Among dicotyledons widely differing sensitivities were found: in Solanaceae (tomato, potato, tobacco), Leguminosae (mung bean, soybean) and Compositae (sunflower) I_{50} concentrations for UKJ72J inhibition were below 55 μM. In Cruciferae (turnip, cauliflower), Chenopodiaceae (lambsquarter, beetroot) and Compositae (endive) I_{50} were between 100 and 250 μM, whereas in Rosaceae (apple, pear) and Umbelliferae (carrot, fennel) I_{50} were near (apple) or higher than 500 μM. No correlation could be found between the sensitivity to UKJ72J of mitochondrial succinate oxidation in these families and their location in the presently accepted flowering plant classification.

INTRODUCTION

In a recent paper we showed that the herbicide UKJ72J (2-ethylamino, 4-amino, 5-thiomethyl, 6-chloropyrimidine; French Patent 2.398.737, 23/02/1977) is a potent inhibitor of succinate oxidation in potato mitochondria [1]. In rat liver and yeast (*Saccharomyces cerevisiae*) mitochondria, succinate oxidation is far less sensitive to UKJ72J [1]. Other chemicals display similar inhibitory action, namely TTFA (thenoyl-trifluoroacetone) and carboxin. TTFA inhibits succinate oxidation in the mitochondria of plants [2–4], fungi [5] and mammals [6–8]. Carboxin displays the same inhibitory action as TTFA (although at lower concentrations) in fungi [9, 10] and mammal [11] mitochondria; however, it is far less effective on plant mitochondria [12]. Hence, the action spectrum of UKJ72J is unique. In order to see whether plant mitochondria from various species were equally affected by UKJ72J, I studied its action on mitochondria prepared from 20 species from 9 families (2 monocotyledons and 7 dicotyledons).

RESULTS AND DISCUSSION

Mitochondria prepared from potato, turnip, onion, maize, sunflower, endive and cauliflower were of good quality: respiratory control ratios (RCR) were above 3.0 with succinate as substrate (RCR is the ratio between the rates of excess-ADP and ADP-limiting oxygen consumption). Mitochondria prepared from apple, carrot, lambsquarter, leek, pear, fennel and mung bean were of average quality: RCR were between 2.0 and 3.0 with succinate as substrate. Mitochondria prepared from other plants were of poor quality: despite numerous attempts, RCR with succinate as substrate could not be raised above 2.0 (oat: 1.9; tomato: 1.6; wheat: 1.6; beetroot: 1.8; tobacco: 1.6; soybean: 1.8).

Sensitivity of mitochondrial succinate oxidation to UKJ72J was found to differ widely among the plant species studied. An example is given in Fig. 1 which shows

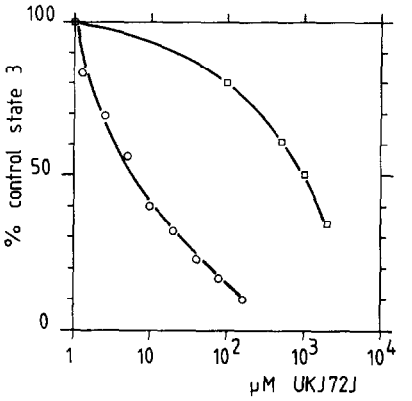

Fig. 1. Inhibition of succinate oxidation by UKJ72J in potato (○) and oat (□) mitochondria. Mitochondrial protein concentration was 0.64 mg/ml (potato) and 1.5 mg/ml (oat). Control oxygen consumptions were: potato, 183 nmol O_2/min/mg protein.; oat, 28 nmol O_2/min/mg protein.

that inhibition took place at far higher concentrations (two orders of magnitude) in oat mitochondria as compared to potato mitochondria. Table 1 shows the I_{50} values (concentrations at which mitochondrial succinate oxidation was 50% inhibited) for plant species. Standard deviations for I_{50} when expressed in μM were lower than 25% of the mean except for maize (35%), tobacco (29%), soybean (29%), beetroot (39%) and endive (42%). However, when I_{50} are expressed in nmol UKJ72J/mg mitochondrial protein, standard deviations are all lower than 26%, indicating that the scattering of I_{50} expressed in μM originates from the use of differing mitochondrial concentrations. Moreover, in some cases the titration curve of succinate oxidation as a function of UKJ72J concentrations has a low slope in the vicinity of 50% inhibition; hence, a slight displacement of the curve results in a significant variation in I_{50}.

Table 1. I_{50} values for UKJ72J inhibition of succinate oxidation in mitochondria from various plant species

	Families	Species	I_{50} (μM)	I_{50} (nmol/mg protein)
Monocotyledons	Gramineae	Oat	1100	4400
		Wheat	580	1300
		Maize	1000	3000
	Liliaceae	Onion	620	770
		Leek	2300*	2500*
Dicotyledons	Rosaceae	Apple	420	290
		Pear	580	455
	Umbelliferae	Carrot	990	2000
		Fennel	1570	4050
	Solanaceae	Potato	11	53
		Tomato	46	18
		Tobacco	55	25
	Cruciferae	Turnip	122	227
		Cauliflower	233	580
	Leguminosae	Mung bean	9	14
		Soybean	14	32
	Chenopodiaceae	Lambsquarter	179	99
		Beetroot	180	173
	Compositae	Endive	26	97
		Sunflower	155	68

*I_{50} values for leek were calculated from an extrapolation since 2 mM UKJ72J brought about only 42% inhibition.

Figure 2 pictures the results presented in Table 1. Solanaceae and Leguminosae were the most sensitive families (I_{50} below 55 μM). Cruciferae and Chenopodiaceae were of intermediate sensitity (I_{50} between 100 and 250 μM). Compositae displayed sensitivity with endive (26 μM) and moderate tolerance with sunflower (155 μM); this could be related to the distance between the two species within the Compositae. However I_{50} expressed as nmol UKJ72J/mg protein showed that sunflower mitochondria are equally sensitive to UKJ72J as those of endive. This may indicate that sunflower mitochondrial fractions contained a high proportion of non-mitochondrial protein. However, attempts to reduce it were without success. In the other families I_{50} were higher than 500 μM (except apple, 420 μM). The monocotyledonous species studied showed a great tolerance to UKJ72J (with respect to succinate oxidation), whereas dicotyledonous species were found all over the range of sensitivity.

Since a striking homogeneity was found within the families, sensitivity of mitochondrial succinate oxidation to UKJ72J might have some phylogenetic basis. It can be

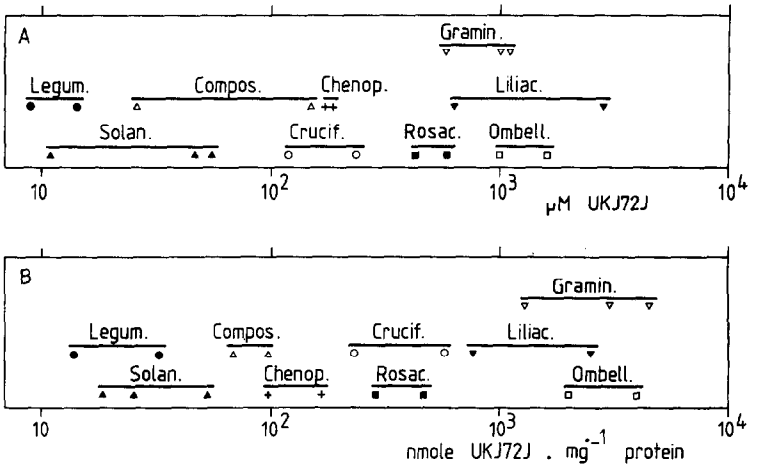

Fig. 2. Diagrammatic representation of UKJ72J inhibition of succinate oxidation in mitochondria from various plant species. A: I_{50} values were expressed in μM UKJ72J. B: I_{50} values were expressed in nmol UKJ72J/mg protein.

hypothesized that inhibition of succinate oxidase by UKJ72J results from binding of the herbicide onto an active site in a protein of the mitochondrial electron transfer chain. Differences in UKJ72J potency among plant species might result from modifications of the amino acid sequence at the active site and could thus reflect phylogenetic relationships between plant families. However, sensitivity to a chemical is bound to have less phylogenetic relevance than the actual amino acid sequence. This can be further altered in the case of a low selective pressure on the active site or by similarities between UKJ72J and naturally-occurring inhibitors. This would allow parallel, back and convergent substitutions. Hence, one must not expect to draw from this study such firm conclusions as those that can be derived from amino acid sequences in plastocyanin and cytochrome *c* [13, 14]. Indeed, the results presented here oppose families such as Leguminosae and Solanaceae on the one hand and Rosaceae and Umbelliferae on the other hand; in Hutchinson's and Thorne's classifications of plant species [15, 16], Leguminosae and Rosaceae are closely related and so are Solanaceae and Umbelliferae. Moreover, from the plastocyanin amino acid sequence analysis performed by Boulter *et al.* [14], Leguminosae and Rosaceae are closely related. Hence, UKJ72J sensitivity of mitochondrial succinate oxidation cannot be considered as a reliable phylogenetic marker. The principal conclusion to be drawn from this study is that one must be careful not to accept the common assumption that plant mitochondria from differing species have similar sensitivities to inhibitors.

EXPERIMENTAL

Plant material. Onion (*Allium cepa* L.), leek (*Allium porum* L.), pear (*Pyrus communis* L.), carrot (*Daucus carota* L.), fennel (*Foeniculum vulgare* L.), tomato (*Lycopersicon esculentum* L.), turnip (*Brassica napus* L.), cauliflower (*Brassica oleracea* L.) and endive (*Cichcorium endivia* L.) were bought on a local market and were of unknown cultivars. Cultivars of potato (*Solanum tuberosum* L.), beetroot (*Beta vulgaris* L.), apple (*Malus domestica* L.), oat (*Avena sativa* L.), wheat (*Triticum sativum* L.), maize (*Zea mays* L.), soybean (*Glycine max* M.) and sunflower (*Helianthus annuus* L.) were respectively: Bintge, B 4553, Golden delicious, Gambo, Clément, INRA 258, Hogdson and HA 89. Mung bean (*Vigna radiata* L.) was a gift of Dr. Tissut. For tobacco (*Nicotiana tabacum* L.) and lambsquarter (*Chenopodium album* L.), calli were used.

Mitochondria preparation. For onion, leek, carrot, fennel, potato, mung bean, turnip, cauliflower, beetroot and endive the grinding medium was 0.3 M mannitol, 0.1% BSA (bovine serum albumin), 5 mM cysteine, 2 mM EDTA (ethylenediaminotetraacetic acid), 10 mM MOPS (morpholinopropanesulphonate buffer) adjusted after grinding to pH 7.2. For the other species the grinding medium was: 0.5 M mannitol, 0.1% BSA, 1 mM EDTA, 5% (w/v) polyvinylpyrrolidone (40 000), 4 mM metabisulphite and 30 mM MOPS, pH 7. The washing medium was either 0.3 or 0.5 M mannitol, 0.1% BSA, 10 mM MOPS, pH 7.2.

Onion, leek, apple, pear, carrot, fennel, potato, tomato, turnip, cauliflower, beetroot and endive tissues were disrupted with a Moulinex mixer (Alençon, France). Oat, wheat, maize, mung bean, soybean and sunflower were used as 7-day-old etiolated shoots and ground in a mortar and pestle. So were ground tobacco and lambsquarter calli. The homogenate was squeezed through 4 layers of cheesecloth and the suspension was centrifuged for 5 min at 2000 *g*. The supernatant was centrifuged for 5 min at 10 000 *g* and the resulting pellet was resuspended in the washing medium, and centrifuged for 5 min at 10 000 *g*. The final pellet was the mitochondrial fraction used in the study.

Respiratory studies were performed in a 2 ml thermostated (25°) cell where oxygen concentration was monitored by means of a Rank Brothers (Cambridge, U.K.) oxygen electrode. For mitochondria prepared in 0.3 M mannitol media, the respiratory medium was: 0.3 M mannitol, 10 mM KCl, 5 mM $MgCl_2$ (except for potato: 0.1 mM), 10 mM NaPi, 10 mM MOPS, pH 7.2. For mitochondria prepared in a 0.5 M mannitol medium, mannitol concn in the respiratory medium was 0.5 M. Because of the high lipophilicity of UKJ72J, BSA was omitted from respiratory media to avoid binding of the herbicide onto it.

Mitochondria were incubated for 2 min with 0.3 mM ATP in order to activate succinate dehydrogenase and 5 mM succinate was then added. Effects on state 3 respiration were measured by addition of the desired UKJ72J concn *ca* 1 min after introduction of excess ADP (1 mM) and establishment of linearity. When state 3 oxygen consumption was not linear (tomato, tobacco, lambsquarter) the relevant corrections were made. For each species at least 3 independent mitochondrial preparations were tested for sensitivity to UKJ72J. UKJ72J was used as a DMSO (dimethylsulfoxide) soln. DMSO concn in the medium was always below 2%; the lack of effect of 2% DMSO on state 3 oxygen consumption was checked.

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