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Protoporphyrinogen oxidase inhibition by three peroxidizing herbicides: oxadiazon, LS 82-556 and M&B 39279

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Three chemically unrelated peroxidizing molecules, namely oxadiazon [5-(*t*-butyl)-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2-one], LS 82-556 [(*S*)3-*N*-(methylbenzyl)carbamoyl-5-propionyl-2,6-lutidine] and M&B 39279 [5-amino-4-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazol], are potent inhibitors of plant, yeast and mouse protoporphyrinogen oxidase.

Enzyme inhibition; Protoporphyrinogen oxidase; Herbicide; (Maize, Yeast, Mouse)

1. INTRODUCTION

We have recently found that diphenyl ether herbicides are powerful inhibitors of chloroplast and mitochondrial protoporphyrinogen oxidase [1], and are thus able to induce lethal accumulation of protoporphyrin IX in treated plants [2–4,6]. However, this last property is not restricted to diphenyl ethers, since comparable protoporphyrin IX accumulation can also be induced by three other different peroxidizing molecules (fig.1): oxadiazon [5,6], the pyridine derivative LS 82-556 [6,7], and M&B 39279 (in preparation and [8] regarding its peroxidative activity). Similarly to diphenyl ether herbicides, these molecules display light-dependent phytotoxicity, which is also explained by the photosensitizing properties of the accumulated protoporphyrin IX [9].

The aim of this study was therefore to examine the effects of oxadiazon, LS 82-556 and M&B 39279 on protoporphyrinogen oxidase activities from various origins, viz. chloroplasts, and plant, yeast and mouse liver mitochondria.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

LS 82-556 and oxadiazon were provided by Rhône Poulenc Agrochimie, France, and M&B 39279 by Rhône Poulenc Agriculture (formerly May & Baker Agrochemicals, England).

2.2. *Etiolated corn seedlings*

Etiolated corn seedlings were grown as in [1].

2.3. *Preparation of plant organelles*

Corn etioplasts were isolated as described by Prado et al. [10]. Mitochondria were isolated from potato tuber (cv. *Bintje*) according to [11].

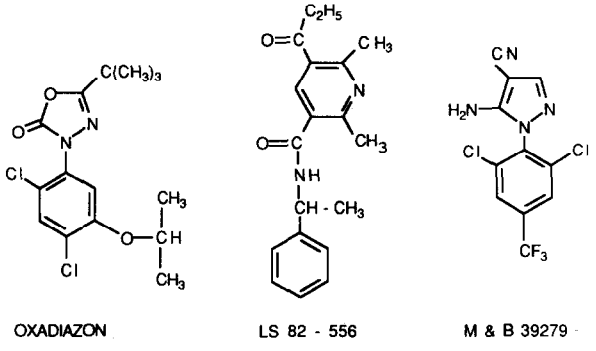

Fig.1. Structures of oxadiazon, LS 82-556 and M&B 39279.

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2.4. *Yeast mitochondrial membranes*

Laboratory strain FL 200 or commercially available baker's yeast (Fould Springer) were used to prepare a fraction enriched in mitochondrial membranes as described in [12].

2.5. *Mouse liver mitochondria*

Mouse liver mitochondria were isolated from DBA/2 strain livers by standard procedures [13].

2.6. *Protoporphyrinogen oxidase activity*

The enzyme was assayed spectrofluorometrically at 30°C by measuring under initial velocity conditions the rate of formation of protoporphyrin IX from chemically reduced protoporphyrinogen [14], as described by Labbe et al. [15].

2.7. *Statistics*

All experiments were performed at least twice, with not less than two replicates.

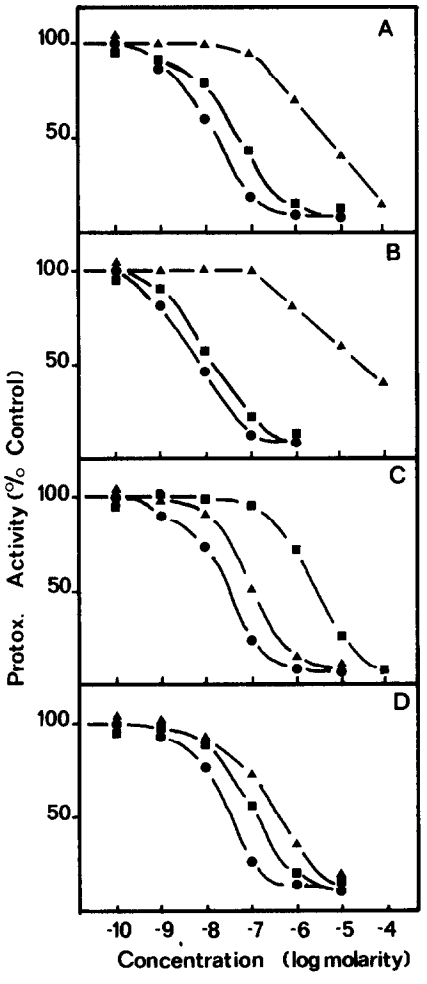

Fig.2. Effects of oxadiazon, LS 82-556 and M&B 39279 on protoporphyrinogen oxidase (Protox) activities of corn etioplasts (A), potato tuber mitochondria (B), yeast mitochondria (C) and mouse liver mitochondria (D). Activities were measured under initial velocity conditions and are expressed as percent of control values, which were 8.1–9.5, 2.1, 7.4 and 7.2 nmol·h^{-1}·mg protein^{-1} for corn etioplasts, potato tuber mitochondria, yeast mitochondria and mouse liver mitochondria, respectively. (●) Oxadiazon, (▲) LS 82-556, (■) M&B 39279.

3. RESULTS

3.1. *Effects of oxadiazon, LS 82-556 and M&B 39279 on protoporphyrinogen oxidase activity of chloroplasts, and plant, yeast, and mouse liver mitochondria*

We have previously shown that acifluorfen methyl, a typical diphenyl ether herbicide, inhibits protoporphyrinogen oxidase activities from chloroplasts, and from plant, yeast, and mouse liver mitochondria [1].

In the same way, oxadiazon, LS 82-556, and M&B 39279 appeared to be potent inhibitors of this enzyme, irrespective of the biological origin (fig.2).

Oxadiazon was the most active molecule, with I_{50} values of 9, 11.5, 30 and 40 nM for enzymes from potato mitochondria, corn etioplasts, yeast mitochondria and mouse liver mitochondria, respectively.

M&B 39279 was a strong inhibitor of potato mitochondrial and corn etioplast enzymes (I_{50} values, 15 and 80 nM, respectively); it was less efficient on mouse liver and yeast enzymes (I_{50} values, 0.5 and 3 μM, respectively).

LS 82-556 was much less inhibitory against the plant enzymes, with I_{50} values of 4 and 40 μM for corn etioplasts and potato mitochondria, respectively. However, it was more efficient in the case of yeast and mouse liver activities, with I_{50} values of 0.1 and 0.3 μM, respectively.

4. DISCUSSION

We have previously shown [1] that the ability of diphenyl ethers to cause protoporphyrin IX to accumulate in treated plants can be explained by their inhibitory effect on protoporphyrinogen oxidase, as also found by Witkowski and Halling (unpublished). Contrary to earlier speculations [4,5], these herbicides do not appreciably prevent the conversion of protoporphyrin IX to Mg-protoporphyrin IX [1], or to heme [1,18], and

there is no reason to postulate that their main effect is the direct stimulation of the synthesis of porphyrin precursors, as proposed by Kouji et al. [18]. However, one is struck by the speed at which protoporphyrin IX accumulates in treated mature tissues [4,5,7]. This suggests the possibility that a consequence of the effect of DPEs on protoporphyrinogen oxidase could be the indirect stimulation of δ-aminolevulinic acid synthesis.

The above results show that oxadiazon, LS 82-556 and M&B 39279 also inhibit protoporphyrinogen oxidase. In treated cells, the accumulating protoporphyrinogen molecules presumably diffuse out of their site of synthesis and are then subjected to non-enzymatic oxidation to protoporphyrin IX (fig.3). Because of their abnormal subcellular location, the resulting protoporphyrin IX molecules are beyond the reach of metal chelatases, and cannot be further metabolized to magnesium and iron porphyrins. As a consequence, they accumulate and give rise to lethal effects in the light, due to their photodynamic properties [9]. This sequence of events is well documented in the cases of the human inherited diseases protoporphyria and porphyria variegata [16].

Among the compounds examined, oxadiazon appears to be the most efficient, with I_{50} values always below 100 nM. M&B 39279 is also a potent inhibitor, especially on plant enzymes. By contrast, the relatively poor efficiency of LS 82-556 on plant protoporphyrinogen oxidases could explain the high concentration needed, in our previous work, to induce protoporphyrin IX accumulation in soybean cell cultures [17]. However, LS 82-556 is quite efficient in inducing protoporphyrin IX accumulation in whole plants [7], possibly because it is very efficiently absorbed and translocated.

The sensitivities of plant, yeast and mammalian protoporphyrinogen oxidases to herbicides pose two kinds of problems: (i) since tetrapyrroles can be synthesized in chloroplasts and mitochondria, the subcellular origin of the protoporphyrin IX which accumulates in treated plants remains to be determined; and (ii) the sensitivities of mammalian and yeast protoporphyrinogen oxidases require careful studies on the toxicological properties of these herbicides. In conclusion, the above results show that diphenyl ethers and other peroxidizing herbicides, oxadiazon, LS 82-556 and M&B 39279,

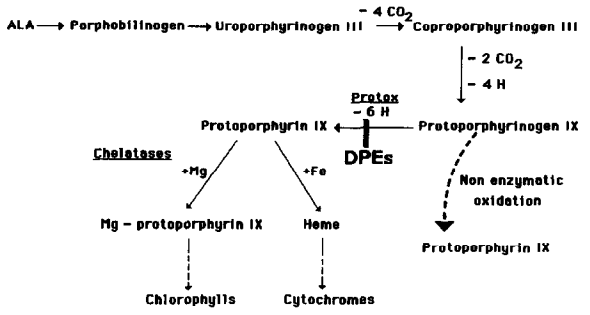

Fig.3. Postulated mechanism of action of diphenyl ether-type herbicides (DPEs).

although chemically unrelated, have the same target enzyme. Work is in progress to determine whether these molecules compete with the substrate and have the same binding site on protoporphyrinogen oxidase.

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REFERENCES

[1] Matringe, M., Camadro, J.M., Labbe, P. and Scalla, R. (1988) Biochem. J., in press.
[2] Matringe, M. and Scalla, R. (1988) Plant Physiol. 86, 619–622.
[3] Lydon, J. and Duke, S.O. (1988) Pestic. Biochem. Physiol. 31, 74–93.
[4] Witkowski, D.A. and Halling, B.P. (1988) Plant Physiol. 87, 632–637.
[5] Duke, S.O., Lydon, J. and Paul, R.N. (1988) Weed Sci., in press.
[6] Sandmann, G. and Böger, P. (1988) Z. Naturforsch. 43c, 699–704.
[7] Matringe, M. and Scalla, R. (1988) Pestic. Biochem. Physiol. 32, 164–172.
[8] Derrick, P.M., Cobb, A.H. and Pallett, K.E. (1988) Pestic. Biochem. Physiol. 32, 153–163.
[9] Falk, J.E. (1964) in: Porphyrins and Metalloporphyrins, BBA Library, vol.2, Elsevier, Amsterdam.
[10] Prado, A.D., Chereskin, B.M., Castelfranco, P.A., Franceshi, V.R. and Wezelman, B.E. (1980) Plant Physiol. 65, 956–960.
[11] Jackson, C. and Moore, A.L. (1979) in: Plant Organelles (Reid, E. ed.) pp.1–12, Horwood, Chichester.
[12] Camadro, J.M., Urban-Grimal, D. and Labbe, P. (1982) Biochem. Biophys. Res. Commun. 106, 724–730.
[13] Johnson, D. and Lardy, H. (1967) Methods Enzymol. 10, 94–96.

[14] Jacobs, N.J. and Jacobs, J.M. (1982) Enzymes 28, 206–219.
[15] Labbe, P., Camadro, J.M. and Chambon, H. (1985) Anal. Biochem. 149, 248–260.
[16] Kapas, A., Sassa, S. and Anderson, K.E. (1983) in: The Metabolic Basis of Inherited Disease (Stanbury, J.B. et al. eds) pp.1301–1384, McGraw-Hill, New York.
[17] Matringe, M. and Scalla, R. (1987) in: Proceedings, 1987 British Crop Protection Conference – Weeds, vol.3, pp.981–988.
[18] Kouji, H., Masuda, T. and Matsunaka, S. (1988) J. Pestic. Sci. 13, 495–499.