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SULFOXIDATION OF METHIOCARB IN HIGHER PLANTS

Elizabeth Blée, John E. Casida and Francis Durst

Laboratoire de Physiologie Végétale, CNRS ERA 104, Institut de Botanique, 28 rue Goethe, 67083 Strasbourg, France; and Pesticide Chemistry and Toxicology Laboratory, 114 Wellman Hall, University of California, Berkeley, CA 94720, USA.

Most biotransformations of xenobiotics by plants that have been studied are oxidative. Recently, several studies have described hydroxylation, dealkylation or dechlorination of pesticides (1-3) but little is known about the oxidation of sulfur containing compounds. It was reported that in vivo sulfur containing compounds such as S-ethyl dipropylthiocarbamate (EPTC) or S-propyl dipropylthiocarbamate (vernolate) are oxidized into their corresponding sulfoxides (4,5). For thiocarbamate herbicides this sulfoxidation was suggested to be one of the initial steps in the degradation of these compounds under many environmental conditions (6). But in vitro, few data are available on the enzymes responsible for this type of reaction. Here we report the oxidation of methiocarb (4 methylthio-3,5-xylyl N-methyl carbamate), a sulfur containing insecticide, by microsomes from soybean cotyledons and some data characterizing the nature of the enzyme involved.

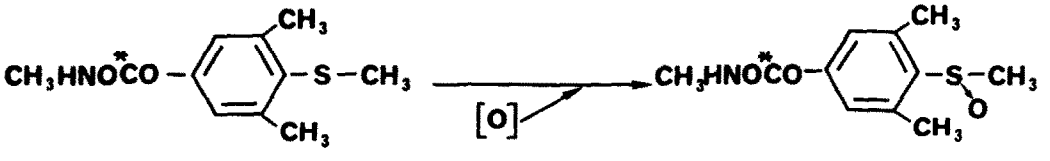
MATERIALS AND METHOD

Preparation of microsomal fraction : Cotyledons of three day old soybean seedlings were homogenized with an ultra-turrax (45 V, 1 min) in 20ml of cold sodium phosphate buffer 50 mM pH 7.2 containing 6% (w/w) of polyclar. The homogenate was squeezed through blutex (50µm). The filtrate was then centrifuged at 10.000 xg for 10 min and the pellet was discarded. The supernatant was centrifuged at 100.000 xg for 45 min to obtain a microsomal fraction. The microsomes were washed by resuspension in 0.1 M sodium pyrophosphate buffer pH 7.2 and by recentrifugation as described above. The microsomes were finally resuspended in 0.1 M sodium citrate buffer pH 5.5 and immediately used.

Oxidation of methiocarb by soybean microsomes : ^{14}C methiocarb (50.000 cpm) was incubated generally for 20 min at 30° with microsomes (0.4 mg protein) in 0.1 M sodium citrate buffer pH 5.5 (final volume : 0.2 ml). The reaction products were extracted with diethyl ether and separated by TLC (SiO_2) using chloroform, ethyl acetate (1:2, v/v) as solvent. Methiocarb and its sulfoxide were scraped off the plates and counted.

RESULTS AND DISCUSSION

Soybean extracts were found to convert ^{14}C labelled methiocarb into its sulfoxide:



methiocarb **sulfoxide**

no further oxidation into the sulfone was observed. The highest activity was found in the cotyledons and in this organ it was shown to be exclusively associated with the microsomal fraction. The oxidative ability of the membranes was destroyed when microsomes were heated to 60° for 2 min. About 40% of sulfoxidase activity could be solubilized by 0.8% (w/v) octyl

glucoside or 0.1% (w/v) Triton X-100. Higher detergent concentrations resulted in the loss of the activity. It was noted that the solubilized sulfoxidase became highly susceptible to inactivation by proteases such as proteinase K. These observations indicate that the oxidation of methiocarb was not a chemical artifact but was catalyzed by proteins. The oxidation rate of methiocarb was proportional to added microsomes up to 1mg protein per assay.The sulfoxidation followed classical Michaelis kinetics and a Km of 2 mM was determined.Under saturating conditions an average rate of 0.4 nmoles/min/mg protein was found.

Sulfoxidation of methiocarb did not require cofactors such as NADPH or NADH,which seems to rule out the participation of classical P450 or FAD dependent monooxygenases. Sulfoxidase did not act however as a peroxidase:H_2O_2 was not a cosubstrate,no effect of inhibitors of peroxidative reactions (KCN,catalase) could be observed and classical peroxidase such as horseradish peroxidase did not metabolize methiocarb.

The oxidation of methiocarb could therefore be catalyzed by a cooxidation reaction such as is known to occur with lipoxygenase or cytochrome P450.In this case the oxygen donors could be hydroperoxides in which soybean cotyledons are particulary rich.In order to test this point,we have studied the sulfoxidation of methiocarb in the presence of hydroperoxides. Oxidation of methiocarb by soybean microsomes was stimulated when organic hydroperoxides were present in the reaction mixture.For example,a 20 fold stimulation was observed in the presence of 1mM cumene hydroperoxide for 1 min.A similar increase in sulfoxidation (3 fold) occured in the presence of fatty acid hydroperoxides liberated from phospholipids after treatment of the microsomes with phospholipase A_2.However lipid peroxidation alone did not explain the sulfoxidation reaction,since no effect of radical scavengers (EDTA,α-tocopherol,mannitol) could be detected even when given during the preparation of the microsomes.

Cumene hydroperoxide initiated paracatalytic inactivation of sulfoxidase previously observed for mixed-function oxygenases.Our results suggest that cytochrome P450,functioning as peroxidase,could be involved in the sulfoxidation of methiocarb.In favor of this hypothesis we have observed that 1-aminobenzotriazole (ABT) a suicide inhibitor of this type of enzyme (7) inhibits sulfoxidation of methiocarb by a mechanism-based reaction.

To substantiate our hypothesis we are presently studying the same reaction in plant materials which are poor in endogenous hydroperoxides.It is expected that in this case the sulfoxidation reaction will be stimulated by the presence of NADPH.Preliminary results obtained with microsomes from Jerusalem artichokes seem to bear out this point.

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