The fungicide chloraniformethan and the herbicide dichlofopmethyl affect calmodulin-dependent cyclic nucleotide phosphodiesterase from bovine brain

Cornelia Hertel* and Dieter Marmé

Institut für Biologie III, Schänzlestr. 1, D-78 Freiburg, FRG

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Calmodulin-stimulated phosphodiesterase from bovine brain is inhibited by the fungicide chloraniformethan; the activity in the absence of calmodulin is not affected. The herbicide dichlofopmethyl enhances the basal activity and inhibits the calmodulin stimulation.

Bovine brain *Calmodulin* *Fungicide* *Herbicide* *Phosphodiesterase* *Calcium*

1. INTRODUCTION

Calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE) (EC 3.1.4.17) has been identified in various tissues, including bovine brain [1]. It is postulated that the PDE together with the adenylate cyclase regulates the cytoplasmic concentration of the second messenger cyclic AMP [2]. Since the regulatory properties of calmodulin depend on the Ca^{2+} activity in the cell, the calmodulin-dependent PDE plays an important role in the cooperation between the two second messengers, Ca^{2+} and cyclic AMP. Thus, the regulation of a great variety of cellular processes depends on the activity of the calmodulin-dependent PDE.

The stimulation of PDE by calmodulin can be inhibited by several substances, such as phenothiazines [3], local anesthetics [4], R 24571 [5], and W 7 [6].

Here, we describe the effect of two substances, the fungicide chloraniformethan and the herbicide dichlofopmethyl on Ca-calmodulin-dependent PDE. Chloraniformethan inhibits the Ca-calmodulin-dependent stimulation of the PDE comparable to the phenothiazine fluphenazine and to R 24571. Dichlofopmethyl stimulates the basal activity of the phosphodiesterase and prevents an additional stimulation by calmodulin.

Chloraniformethan is a fungicide [7] which inhibits the regeneration of flagellae in *Chlamydomonas reinhardii* (C. Fedtke, personal communication). Dichlofopmethyl inhibits several metabolic processes in plant cells, such as chlorophyll synthesis, photosynthesis and photosynthate translocation [8]. Both substances inhibit Ca^{2+} uptake into plant mitochondria and thus interfere with the regulation of the cytoplasmic Ca^{2+} activity [9].

2. MATERIALS AND METHODS

All chemicals used were of reagent grade. Chloraniformethan and dichlofopmethyl were obtained as gifts from Bayer Leverkusen. Fluphenazine was obtained from Heyden, R 24571 from Janszen Pharmaceutical. Phosphodiesterase was prepared from bovine brain as in [10]. Calmodulin from bovine brain was prepared as in [11].

The activity of phosphodiesterase was determined following the two-step method in [12]. Control experiments were carried out to make sure that the

* Present address: Friedrich Miescher-Institut, PO Box 2543, CH-4002 Basel, Switzerland

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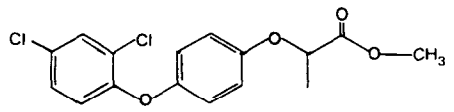
pesticides had no influence on the alkaline phosphatase used in the second step of the assay (not shown). The pesticides were dissolved in dimethylsulfoxide (DMSO), giving a final concentration of 1% DMSO in the assay. The control contained always the same amount of DMSO.

3. RESULTS AND DISCUSSION

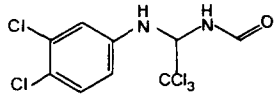
Chloraniformethan (fig.1) is a pesticide which has been shown to exhibit high antifungal activities [7]. Evidence has been given that it interferes with microtubule-dependent processes, such as inhibition of the regeneration of flagellae in *C. reinhardii* (C. Fedtke, personal communication). This effect is probably caused by an increase of cytoplasmic Ca^{2+} activity and not by direct interaction with microtubules [9].

In fig.2A evidence is presented that chloraniformethan inhibits the stimulation of PDE by calmodulin, while the activity in the absence of calmodulin is not affected. Increasing the calmodulin concentration overcomes the inhibitory effect of chloraniformethan. At a concentration of 1.2×10^{-9} M calmodulin, calmodulin-stimulated PDE activity is inhibited by 50% at about 2×10^{-5} M chloraniformethan. In the presence of 3.6×10^{-6} M calmodulin as much as 3×10^{-4} M chloraniformethan is necessary to inhibit calmodulin-stimulated PDE-activity by 50%.

We compared the effect of chloraniformethan with two well-known inhibitors of calmodulin-stimulated PDE, fluphenazine and R 24571 (fig.2B,C).



dichlofopmethyl



chloraniformethan

Fig.1. Structures of chloraniformethan and dichlofopmethyl.

Fluphenazine inhibits calmodulin-stimulated PDE by 50% in the same concentration range as chloraniformethan (5×10^{-6} M fluphenazine at 1.2×10^{-9} M calmodulin), while less R 24571 is necessary (4×10^{-8} M R 24571 at 1.2×10^{-9} M calmodulin). Increasing the calmodulin concentration overcomes the inhibitory effect of these drugs similarly to the results demonstrated with chloraniformethan.

Dichlofopmethyl (fig.1) is a herbicide which has been shown to interfere with auxin-dependent processes [13] and several metabolic pathways [8]. It also inhibits plant mitochondrial Ca^{2+} uptake [9]. Fig.3 shows that dichlofopmethyl in contrast to

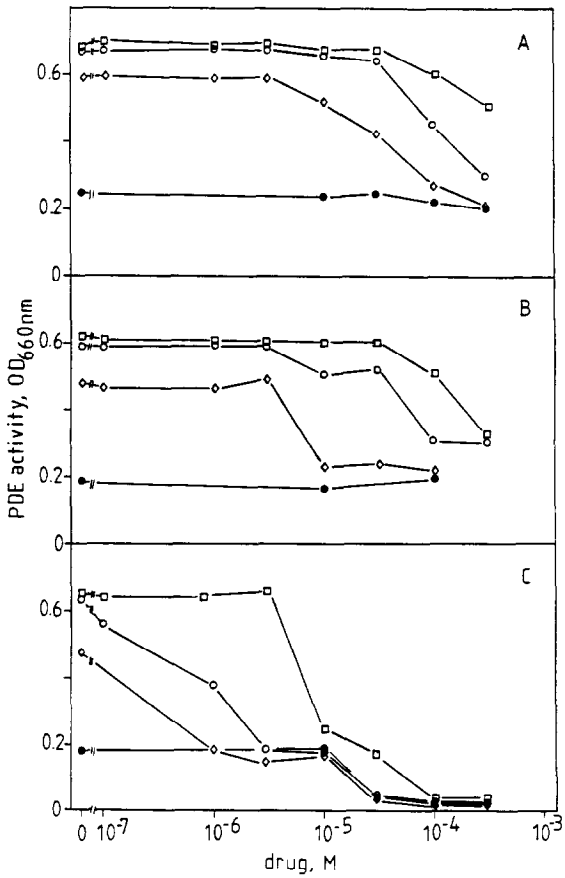


Fig.2. Effects of (A) chloraniformethan; (B) fluphenazine; (C) R 24571, on basal (●) and calmodulin-stimulated activity of cyclic nucleotide phosphodiesterase from bovine brain at different calmodulin concentrations: (□) 3.6 μM; (○) 36 nM; (◇) 1.2 nM calmodulin. 3 munits PDE were used/assay.

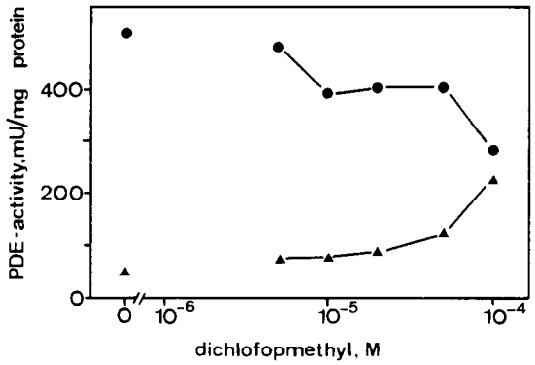


Fig.3. Effect of dichlofopmethyl on basal (▲) and calmodulin-stimulated (●) activity of cyclic nucleotide phosphodiesterase from bovine brain.

chloraniformethan stimulates the basal PDE-activity. At a concentration of about 2×10^{-5} M the basal activity is stimulated 2-fold. At 10^{-4} M the stimulation is about 5-fold. At this concentration the calmodulin-stimulated activity is decreased to about 50% of the control.

One may conclude that chloraniformethan and dichlofopmethyl modulate calmodulin-dependent enzyme activities. Calmodulin has been shown to exist in plants [14] as well as in fungi [15]. At present we cannot decide whether the fungicidal and herbicidal properties of these substances are due to the interference with calmodulin-dependent processes. Both substances may serve as tools suited to investigate calmodulin-mediated reactions in the intact cell.

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