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## Organophosphorus acid anhydrolase in slime mold duckweed and mung bean: a continuing search for a physiological role and a natural substrate

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### Abstract

Recently, and for the first time, a diisopropylphosphorofluoridate (DFP)-hydrolyzing enzyme, i.e. an organophosphorus acid anhydrolase (OPAA), has been reported in a plant-source. Based on this and other suggestive evidence, the ability of three plant sources and a protist to hydrolyze DFP and 1,2,2-trimethylpropyl methylphosphonofluoridate (Soman) were tested, and the effects of  $Mn^{2+}$  and ethylenediamine tetraacetate (EDTA) on this activity. The plants are duckweed (*Lemna minor*), giant duckweed (*Spirodela oligorhiza*), and germinated mung bean (*Vigna radiata*); the protist is a slime mold (*Dictyostelium discoideum*). The tests are based on a crude classification of OPAAAs as 'squid type' (DFP hydrolyzed more rapidly than Soman) and all of the others termed by us, with questionable justification, as 'Mazur type' (Soman hydrolyzed more rapidly than DFP). Of the two duckweeds, *Spirodela oligorhiza* hydrolyzes Soman but not DFP, and *Lemna minor* does not hydrolyze either substrate. In contrast to the report of Yu and Sakurai, mung bean does not hydrolyze DFP and hydrolyzes Soman with a 5-fold stimulation by  $Mn^{2+}$  and a marked inhibition by EDTA. The slime mold hydrolyzes Soman more rapidly than DFP (but does hydrolyze DFP) and the hydrolysis is  $Mn^{2+}$  stimulated. The failure of these plant sources to hydrolyze DFP is similar to the behavior of OPAA from *Bacillus stearothermophilus*. Published by Elsevier Science Ireland Ltd.

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## 1. Introduction

From the very first report of an enzyme (or enzymes) capable of splitting the P-F bond, as for example in the compound diisopropylphosphorofluoridate (DFP) [1], until the Second International Meeting on this subject [2], and now at the Third International Meeting on Esterases Reacting with Organophosphorus Compounds in 1988, the question of a natural substrate and thus a physiological role for these enzymes remains unanswered. The finding of one such enzyme in the nerve tissue of a specific animal class, the cephalopods, has resulted in the terminology, 'squid type OPA', the initials standing for 'organophosphorus acid anhydrolase'. With good evidence this terminology identifies an enzyme characterized by narrow distribution, and a consistent DFP/Soman hydrolysis ratio of about 5. Other properties have also been noted [3]. What is far less justified (and for which the senior author accepts responsibility) is the lumping together of all the other organophosphorus acid anhydrolases (OPAAAs) and even organophosphorus hydrolase (OPH) [4] under the title 'Mazur type' 133.

Taxonomy and the accompanying nomenclature are less important than what they may suggest about the function of such enzymes. For example, it has been noted that cephalopod nerve, and only this tissue, has an unusual anion, 2-hydroxyethanesulfonate (isethionate), at an intracellular concentration of approximately 150 mM. Despite speculation [3,5,6], it was not possible to relate the presence of squid type OPAA and isethionate. Similar questions and speculations, applied to all the other OPAAAs, either never have arisen, or are dismissed as in Mounter's review [7] as 'several hydrolytic enzymes of overlapping specificities'.

Until recently there has been no report of any of the OPAAAs in plants, despite occasional examinations of plant homogenates by us, and possibly by others. Now that has changed. Yu and Sakurai [8] have reported the hydrolysis of DFP by *Vigna radiata* (mung bean), and recently it was said that duckweed (species not specified at that stage) appeared to be causing a degradation of one of the organophosphorus insecticides [9].

This paper confirms in general the work of Yu and Sakurai [8], but differs in some possibly important details. Findings with duckweed that substantiate the communication from Wolfe [9] were also reported, and describe a striking taxonomic difference. Findings with *Dictyostelium discoideum* (a slime mold) was also included, which is, of course, not a mold and is probably more closely related to *Tetrahymena thermophila*, used by Landis et al. [10,11].

## 2. Materials and methods

*Lemma minor* was grown at the Museum of Science, Boston, MA, and had originally been obtained from Carolina Biological, Burlington, NC. *Spirodela oligorhiza* was obtained from the US Environmental Protection Agency, Athens, GA. *Vigna radiata* was grown to a height of about 5 cm from seeds obtained from the Vermont Bean Seed, Fair Haven, VT. The plant materials were homogenized in

buffer to give a 20% w/v suspension. *Dictyostelium discoidium* was obtained as frozen 10% suspensions from Dr Richard H. Kessin, Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, NY. The cells were disrupted by repeated freezing and thawing at dry ice temperature.

DFP was obtained from Sigma, St. Louis, MO. Soman was used as described [3], in facilities at the Marine Biological Laboratory. In brief, DFP or Soman hydrolysis was followed with a fluoride-sensitive electrode; 0.5 ml of plant homogenate (equivalent to 100 mg tissue), or 0.025 ml of the slime mold suspension (equivalent to 2.5 mg cells), was added to the reaction vessel so as to give a final volume of 5.0 ml, 3 mM in DFP or Soman, with or without other additives.

### 3. Results

Table 1 shows the hydrolysis of DFP and Soman by the three plant species. The additives,  $Mn^{2+}$  and ethylenediamine tetraacetate (EDTA), were chosen to permit comparisons with other OPAAAs [3], and with the report of Yu and Sakurai [8]. In brief, there appears to be little or no measurable hydrolysis of DFP by the whole homogenates of all three of the plants tested. *Vigna radiata* does hydrolyze Soman, and the hydrolysis is stimulated about 5-fold by  $Mn^{2+}$  and inhibited about 80% by EDTA. The sharp difference between two such superficially similar members of the family Lemnaceae (see Fig. 1), namely *Lemna minor* and *Spirodela oligorhiza*, is self-evident and will be discussed elsewhere.

Table 2 shows the hydrolysis of DFP and Soman by *Dictyostelium discoidium* (Soman/DFP  $\approx$  5) and about a 3-fold stimulation of these hydrolyses by  $Mn^{2+}$ . There is only a little inhibition of the *Dictyostelium* OPAA by EDTA, but since there was a marked stimulation of this enzyme activity by added  $Ca^{2+}$  ( $\approx$  75%; data not shown), there may have been little or no  $Ca^{2+}$  in the *Dictyostelium* preparation to begin with, perhaps due to the method of harvesting the cells. For comparison, Table 2 also presents published data on *Tetrahymena thermophila* [10], and a kind of 'average' for squid (*Loligo pealei*) nerve tissue [3,10], in that one of these sources [3] is already an 'average' of many reports from the laboratory of the senior author.

Table 1  
Organophosphorus acid anhydrolase (OPAA) activity of homogenates of three plant species

P-F Compound/additive	$\mu M$ P-F compound hydrolyzed $h^{-1} g^{-1}$ fresh tissue <sup>a</sup>		
	<i>Spirodela oligorhiza</i>	<i>Lemna minor</i>	<i>Vigna radiata</i>
DFP/none	0	0	0
DFP/ $Mn^{2+}$	0, 0	0	0.6, 1.2
Soman/none	11.1, 12.9	0, 0	5.1, 9.6
Soman/ $Mn^{2+}$	9.0, 11.7	0	25.2, 44.7
Soman/EDTA	1.5, 5.7		1.5

<sup>a</sup> All values are individual results; where a value is missing, no determination was made.

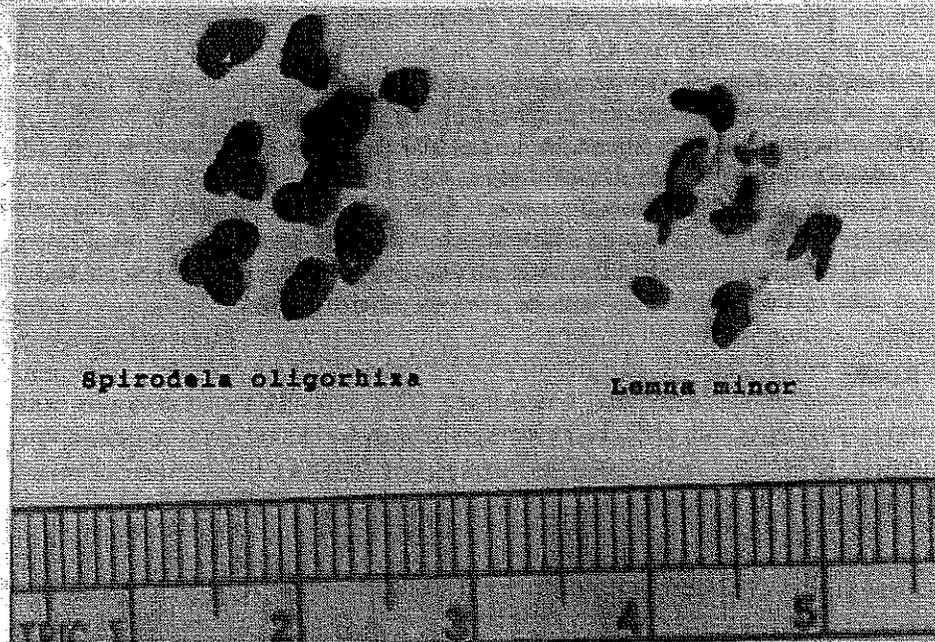


Fig. 1. *Spirodela oligorhiza* and *Lemna minor*. Most of the roots were removed, as well as any detritus and discolored leaves, and the remaining material was pressed between paper towels before making a fresh weight determination.

Because a rate of zero, or 'little or no...' as worded above is always subject to question, the primary data is illustrated in Fig. 2. The  $F^-$ -sensitive electrode was standardized with  $2 \times 10^{-5}$  and  $2 \times 10^{-4}$  M  $F^-$ . While the slope differs from day to day, it is always between 57 and 58 mV. Fig. 2 shows, as expected, a higher

Table 2

Organophosphorus acid anhydrolase (OPAA) activity of *Dictyostelium discoideum* compared to other published values

P-F Compound/additive	$\mu$ M P-F compound hydrolyzed $h^{-1} g^{-1}$ fresh tissue	<i>Dictyostelium discoideum</i>	<i>Tetrahymena thermophila</i> <sup>a</sup>	Squid nerve <sup>a</sup>
DFP/none	24, 34.5, 36	46	230	
DFP/ $Mn^{2+}$	78, 90	41	226	
Soman/none	144, 144	477	66	
Soman/ $Mn^{2+}$	456	1097	70	
Soman/EDTA	120, 144		6	

<sup>a</sup> Averages from Refs. [3] and [10]. The value from [3] is itself an 'average' of many reports from the senior author's laboratory, and includes both axoplasm from the giant axon and homogenized optic ganglia, all from *Loligo pealei*.

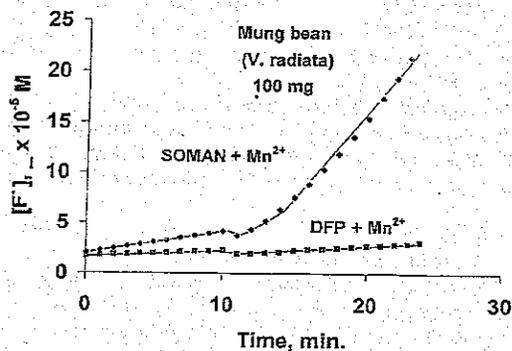


Fig. 2. The enzymatic hydrolysis of Soman and diisopropylphosphorofluoridate (DFP) by *Vigna radiata* (mung bean) homogenate. Although the DFP enzymatic hydrolysis rate appears to be zero, i.e., not greater than the non-enzymatic rate of DFP hydrolysis, the use of a regression equation permits the calculation of the extremely low rate given in Table 1. The dip at approximately 10 min marks the addition of homogenate.

non-enzymatic rate of hydrolysis for Soman than for DFP. The slight downward deflection at about 10 min marks the addition of 0.5 ml of a 20% plant tissue homogenate. Thereafter, the hydrolysis of Soman is evident, whereas either by inspection or by the calculation of a regression equation there appears to be little or no hydrolysis of DFP by *Vigna radiata*.

#### 4. Discussion

Based on either published work [8] or personal communication [9], a characterization of OPAA-like activities of three plants was undertaken. For comparison with work by Landis and associates [10,11], a protist was also examined. While there are minor differences and inconsistencies, it appears that the OPAA's of two of the plants and the protist (the slime mold) would fall into the catch-all category of 'Mazur type' [3]. The enzyme levels in the plants range from very low to zero, or at any rate unmeasurable, in *Lemna minor*, to levels comparable to others reported when Soman is the substrate. The most striking feature of the observations is that within a single family, Lemnaceae, two superficially similar species show a marked difference with respect to Soman hydrolysis. The interest is less in the practical ability of *Spirodela oligorhiza* to detoxify a nerve gas (the level of activity is too low) than in the question: what else does this species have (or lack) that *Lemna minor* lacks (or has)? Behind this is the question: what is the natural substrate and physiological role for the OPAA's?

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