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CHOLINESTERASES FROM PLANT TISSUES

VI. PRELIMINARY CHARACTERIZATION OF ENZYMES FROM *SOLANUM MELONGENA* L. AND *ZEA MAYS* L.

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Summary

Enzymes capable of hydrolyzing esters of thiocholine have been assayed in extracts of *Solanum melongena* L. (eggplant) and *Zea Mays* L. (corn). The enzymes from both species are inhibited by the anti-cholinesterases neostigmine, physostigmine, and 284c51 and by AMO-1618, a plant growth retardant; and they both have pH optima near pH 8.0. The enzyme from eggplant is maximally active at a substrate concentration of 0.15 mM acetylthiocholine and is inhibited at higher substrate concentrations. On the basis of this last property, the magnitude of inhibition by the various inhibitors, and the substrate specificity, we conclude that the enzyme from eggplant, but not that from corn, is a cholinesterase.

Introduction

Acetylcholine [1,2] and a cholinesterase (E.C. 3.1.1.8) [3] with high affinity (K_m = 84 μM) for acetylcholine have been identified in extracts of *Phaseolus aureus* Roxb., the mung bean. A survey [4] of approximately sixty plant species from fifteen families identified acetylthiocholine-hydrolyzing activity in species from two families in addition to Leguminosae, which includes *P. aureus*: Gramineae (*Zea mays* L., corn) and Solanaceae (*Solanum melongena* L., eggplant; *S. tuberosum* L., potato; and *Lycopersicon esculentum* (L.) Mill., tomato). We report here data which characterize enzymes from two of the species, *S. melongena* and *Z. mays*.

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Abbreviations and symbols: AMO-1618, 2 isopropyl-4-dimethyl-amino-5-methylphenyl-1-piperidine-carboxylate methyl chloride; DTNB, 5,5'-dithio bis-(2-nitrobenzoic acid); 284c51, 1,5-bis-(4-allyldimethylammonium phenyl) pentan-3-one dibromide.

Materials and Methods

S. melongena variety Black Beauty was sown in soil, grown in a greenhouse, and harvested after approx. 60 days. An extract of the roots was prepared using the method described by Riov and Jaffe [3] for purifying cholinesterase from mung beans, but omitting the use of Sephadex G-200 [4]. *Z. mays* variety Hulting X441 was soaked overnight in running tap water, placed on wetted filter paper in petri dishes, grown in a darkened room, and harvested after 4 days. The coleoptiles together with the embryonic shoot axis were excised at the cotyledonary node and homogenized in 10 mM potassium phosphate (pH 7.0), using the equivalent of 2-ml buffer/g fresh weight, with a VirTis homogenizer and then a Polytron homogenizer. The homogenate was filtered through Miracloth and the filtrate was centrifuged for 2 h at 20 000 × *g*. The supernatant fluid was dialyzed overnight against 10 mM potassium phosphate (pH 7.0). The methods for preparing subcellular fractions have been previously described [5]. A modification [5] of Ellman's method [6] was used to assay the hydrolysis of thiocholine esters. Acetylthiocholine chloride, propionylthiocholine chloride, butyrylthiocholine iodide, 5,5′-dithio-bis-(2-nitrobenzoic acid), physostigmine sulfate, neostigmine bromide, and choline chloride were purchased from Sigma Chemical Co.; 284c51 was purchased from Burroughs, Wellcome, and Co.; and AMO-1618 was purchased from Enomoto and Co.

Results

The rates of hydrolysis of three esters of thiocholine by the enzymes from eggplant and corn are given in Table I and can be summarized: for eggplant, P > A > B; and for corn, A $\cong$ P $\gg$ B where A, P and B are acetylthiocholine, propionylthiocholine and butyrylthiocholine, respectively. The relative rates of hydrolysis by the cholinesterase from mung bean are A > P $\cong$ B. [3] The enzyme from corn differs from the enzymes from mung bean and eggplant by hydrolyzing butyrylthiocholine at a very low rate relative to acetylthiocholine.

The enzymes from both eggplant and corn are inhibited (Table II) by several compounds which inhibit acetylcholinesterase (EC 3.1.1.7) [7–11],

TABLE I

HYDROLYSIS OF THIOLESTERS BY EXTRACTS OF *Z. MAYS* AND *S. MELONGENA*

The activity of the corn extract corresponds to the total enzyme solubilized from the original material (see Table III). For the eggplant, the data refer to an extract that underwent further purification during which a considerable proportion of the activity was not recovered (compare with Table III).

Substrate	Rate of hydrolysis (nmol/min/g fresh wt equivalent)	
	S. melongena	*Z. mays*
Acethiocholine	0.78	2.80
Propionylthiocholine	0.96	2.58
Butylthiocholine	0.37	0.03

TABLE II

EFFECT OF VARIOUS INHIBITORS ON ACETYLTHIOCHOLINE HYDROLYSIS BY PLANT EXTRACTS

The rate of enzyme-catalyzed substrate hydrolysis was measured at a series of concentrations of the test compounds. The I_{50} values were determined from graphs of these data.

Compound	Concentration	Percent inhibition		
		S. melongena	*Z. mays*	*P. aureus**
Neostigmine bromide	16 nM	I_{50}	0	0
	8 μM	100	I_{50}	100
Physostigmine sulfate	0.1 mM	I_{50}	10.6	26
	0.7 mM	90	I_{50}	45
AMO-1618	0.1 mM	I_{50}	0	32
	10 mM	74	39.2	94
284c51	0.1 mM	0	9	0
	1.0 mM	12	20	20.3
Choline chloride	1 mM	17	0	80**
	10 mM	—	20	165**

* Data from refs 3 and 12.

** Percent activation.

butyrylcholine esterase (EC 3.1.1.8) [8,9,11], and the cholinesterase from mung bean [3]. The enzymes from eggplant and corn are similar to cholinesterase from mung bean with respect to inhibition by neostigmine, physostigmine, and 284c51, although the absolute values for inhibition vary with the plant source; likewise they are less sensitive to the plant growth retardant, AMO-1618. Choline chloride inhibits the enzymes under study here. In this respect these enzymes differ from the mung bean cholinesterase, which is activated by choline [3].

The enzymes from both plants have pH optima near pH 8.0, although the

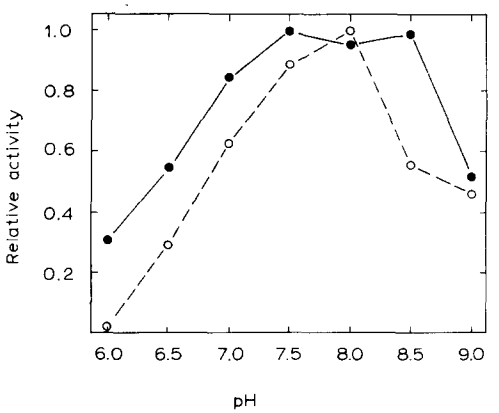

Figure 1. Effect of pH on the hydrolysis of acetylthiocholine by extracts of *Z. mays* and *S. melongena*. Potassium phosphate was used from pH 6.0 to pH 8.0 and potassium borate was used from pH 8.0 to 9.0. *S. melongena*, ———; *Z. mays*, ○- - - - -○.

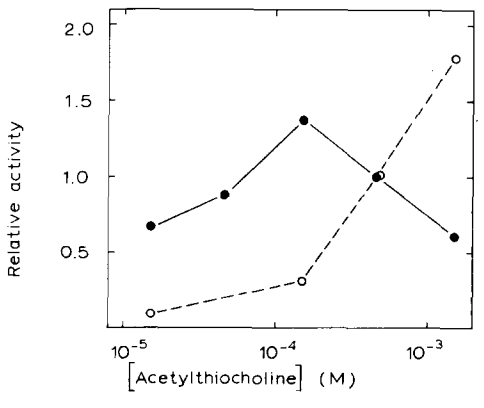

Figure 2. Effect of acetylthiocholine concentration on enzyme activity. *S. melongena*, ———; *Z. mays*, ○- - - - -○.

TABLE III

DISTRIBUTION OF ENZYME ACTIVITY AMONG SUBCELLULAR FRACTIONS

Fraction	Percent of total activity	
	S. melongena	*Z. mays*
Residual material	44.6	33.4
1000 × *g* pellet	2.0	2.9
10000 × *g* pellet	2.4	5.9
100000 × *g* pellet	7.3	0
100000 × *g* supernatant fluid	43.7	57.8
Total activity (nmol/min/g fresh wt. equivalent)	13.72	4.75

optimal range is somewhat broader in the case of the enzyme from eggplant (Fig. 1).

A characteristic of acetylcholinesterase [14,15] and of the cholinesterase from mung bean [3] is inhibition by superoptimal substrate concentrations. The enzyme from eggplant, but not that from corn, also shows inhibition by excess acetylthiocholine (Fig. 2).

Approximately 90% of the neostigmine-inhibited activity from both species is equally distributed between two fractions, the crude cell wall fraction and the 100 000 × *g* supernatant fraction (Table III). This is in contrast to the cholinesterase from mung bean, of which approx. 95% is associated with cell wall material [5].

We conclude that the enzyme from eggplant, but not that from corn, is a cholinesterase. There are important apparent differences, however, between the cholinesterases from eggplant and mung bean (e.g., subcellular localization) and further work is necessary to clarify these differences.

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