Cholinesterases from Plant Tissues

V. CHOLINESTERASE IS NOT PECTIN ESTERASE

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ABSTRACT

Several properties of the cholinesterase from Phaseolus aureus Roxb. and of pectin (methyl) esterases from both Phaseolus aureus and Lycopersicon esculentum (L.) Mill. are contrasted. Cholinesterase activity is inhibited by all of the concentrations of NaCl tested, from 0.05 M to 0.9 M, a property which differs sharply from published data pertaining to pectin esterase. Although crude preparations of cholinesterase contain pectin esterase activity, further purification by gel filtration of the cholinesterase results in a nearly complete elimination of the pectin esterase activity. The activity of neither the pectin esterase from Lycopersicon esculentum nor that from Phaseolus aureus is affected by 25 µM neostigmine, a potent inhibitor of the cholinesterase activity extracted from Phaseolus aureus.

An enzyme with a high affinity for acetylcholine has been identified (12) in extracts prepared from Phaseolus aureus, the mung bean. The enzyme also has other properties in common with acetylcholinesterase (EC 3.1.1.7), including inhibition by relatively low concentrations, roughly 1 µM, of neostigmine and organophosphates such as paraaxon and inhibition by excess substrate (>1 mM) (12). Furthermore, acetylcholine itself has been identified in extracts of Phaseolus aureus (7). Riiov and Jaffe (12) concluded that the enzyme from Phaseolus aureus is a cholinesterase, and subsequently a number of reports (4, 11, 13) have further characterized the ChE3 from Phaseolus aureus.

Because both are cell wall-bound esterases, it seemed possible that ChE is actually PE (EC 1.1.11). On the one hand, both enzymes are esterases, are associated with cell wall material (2, 4, 6), and are present in species of the Leguminosae and Solanaceae (3, 5, 8, 15). Crude preparations of PE from orange hydrolyze acetylcholine, although this ability is nearly lost during further purification of the PE (9). On the other hand, PE activity has been reported in a number of plants, cucumber (1), tobacco (2), and Coleus blumei (10), which contain no apparent ChE activity (5). Also, the pH dependence of the PE activities from Vigna sinensis (3) and Phaseolus vulgaris (15) differs sharply from the pH dependence of ChE from Phaseolus aureus (12). Whereas PE has a broad pH optimum from pH 7 to pH 9 (3, 15), ChE activity has a well defined optimum at pH 8.5 (12).

We report here further experiments which contrast the properties of PE and ChE, and indicate that the two enzymes are different proteins.

MATERIALS AND METHODS

ChE activity was extracted from Phaseolus aureus Roxb. and purified as described previously (4, 12). Briefly, the ChE in a 4% (w/v) (NH₄)₂SO₄ tissue extract was concentrated by adding solid (NH₄)₂SO₄ to 80% saturation, precipitating the ChE and resulting in a 12-fold purification of the enzyme activity. Further purification, resulting in a final 36-fold purification of the enzyme, was achieved by gel filtration of the 80% ammonium sulfate fraction through Sephadex G-200 (12). The assays for ChE activity were performed using acetylthiocholine chloride as substrate and were performed as described previously (4, 12). Control assays contained 25 µM neostigmine bromide, a potent inhibitor of ChE activity (12).

In order to test the effect of NaCl on ChE activity, 0.02 M potassium phosphate, pH 8.0, replaced the 0.5 M potassium phosphate, pH 8.0, which is routinely used in the assays, and different concentrations of NaCl were added.

Commercial PE [prepared from Lycopersicon esculentum (L.) Mill.] was purchased from Sigma Chemical Company and citrus pectin was purchased from Nutritional Biochemicals Corporation. PE activity was measured using a modification of the method of Somogyi and Ramani (14). The enzyme or test sample (0.5--2.0 ml) was added to 25 ml of 0.5% (w/v) pectin in 0.1 M NaCl, pH 8.0, at 25°C and the rate of change of the pH was recorded. The effect of neostigmine bromide on PE activity was tested by preincubating the PE sample with 25 µM neostigmine for 30 min and also including 25 µM neostigmine in the assay medium.

RESULTS AND DISCUSSION

Maximal ChE activity was observed in the absence of NaCl (Fig. 1). There was approximately 25% less activity in the presence of 0.2 M to 0.3 M NaCl, concentrations of NaCl which have been reported to enhance the PE activities from Vigna sinensis (3) and Phaseolus vulgaris (15) by 4- and 18-fold, respectively.

The crude preparations of ChE from Phaseolus aureus contained detectable PE activity but this activity was not affected by 25 µM neostigmine, a concentration of neostigmine

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2 Abbreviations: ChE: cholinesterase; and PE: pectin esterase.

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which completely inhibited the acetylcholine-hydrolyzing activity in these same preparations. After gel filtration through Sephadex G-200, approximately 65% of the ChE applied to the column was collected in the void volume, i.e. the enzyme was excluded from the gel phase (12). The pooled fractions containing ChE activity contained less than 2% of the PE activity applied to the column.

Commercial PE contained no detectable ChE activity; and the PE activity of this sample was not affected by 25 μM neostigmine.

We conclude, on the basis of these experiments and the data summarized in the introduction, that bean root cholinesterase is not pectin esterase.

LITERATURE CITED