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Cholinesterases in animals and Arabidopsis Thaliana

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Abstract:
Cholinesterases are classified into two major categories of vertebrates: Acetylcholinesterase (AChE) and butrylcholinesterase (BChE). AChE belongs to the esterase family within higher eukaryotes which acts on different types of esters. The most well-known biological role of AChE is the transmission at cholinergic synapse in animals by rapid hydrolysis of acetylcholine. AChE has also been found in the organisms that lack a nervous system like bacteria and protozoa, slime moulds and plants. This indicates that the need for AChE arose very early in evolution. This review focuses on historical background of discovery of acetylcholinesterase in plants.

1. Introduction

Acetylcholine is a well-known neurotransmitter in animals. There are two enzymes that needed for acetylcholine metabolism: Choline acetyl-transferase (ChAT), which catalyzes the synthesis of acetylcholine and Cholinesterase (ChE) which catalyses hydrolysis of acetylcholine into choline and acetate. ChE are the enzymes that hydrolyses choline esters at a higher rate than any nonholinester and are inhibited by low concentration (10^{-5} - 10^{-6} M) of organophosphate (OP) and carbamate compounds. Cholinesterases are classified into two major categories or vertebrates. One is acetylcholinesterase or true cholinesterase (AChE; EC.3.1.1.7) and the other one is butrylcholinesterase or pseudocholinesterase (BChE; EC.3.1.1.8). The basis for this classification is that AChE hydrolyses acetyl esters of choline at a higher rate than butryl or propionyl esters and is inhibited by supraoptimal concentration of ACh. The contrast holds true for butrylcholinesterase. But cholinesterases from invertebrate's exhibit intermediate properties of both AChE and BChE thus cannot be classified into either of these categories (19). Neurons which synthesizes, store and secrete neurotransmitters are called cholinergic. The four elements necessary for a cholinergic system are: Acetylcholine (ACh), AChE, AChR and ChAT. AChE is a serine hydrolase, which belongs to the esterase family within higher eukaryotes. This family acts on different types of esters. The most well-known biological role of AChE is the transmission at cholinergic synapse in animals by rapid hydrolysis of acetylcholine. Apart from neuronal locations, acetylcholine and its metabolizing enzyme have also been found in non-neuronal locations like RBC, placenta, amniotic fluid and developing fetus where there is no nervous system, thus becomes very puzzling for scientists (18). But evidences are there to support the involvement of AChE in cell division and differentiation during embryo development (21). BChE has been found in serum, liver, intestine, and megakaryotes. The role of BChE is also ambiguous. AChE has also been found in the organisms that lack a nervous system like bacteria and protozoa (19, 18), slime moulds and plants (5). This indicates that the need for AChE arose very early in evolution.

2. Physiological Role of ACh in Plants

The physiological role of acetylcholinesterase in plants and non-neuronal locations is no yet fully understood. Thus, it attracts the interest of a great number of scientists. In plants, a number of physiological roles are assigned to ACh. These include plant growth, germination, flowering, generation of bioelectric potential, photochemical reaction (4) stomatal opening (11*)* and pollen-pistil interaction (10).

3. Isolation and Characterization of Acetylcholinesterase from Plants

Dettbarn (1962) was the first to study AChE in plants to understand the bioelectric potential. He discovered AChE activity in *Nitella jlexis* and suggested a possible similarity in the bioelectrogenesis in plants and animals. Later, AChE was also found in yeast *Saccharomyces cerevisiae.* In lichen *Parmelia caperata* (Raineri and Modensi, 1986), fungus *Aspergillus niger* (17). AChE was first isolated, purified and extracted from bacteria *Pseudomonas jluorescens* (2, 3). In plants, AChE was first isolated, partially purified and characterized from mung bean (14). Till date, AChE has been isolated, purified and extracted from plants like *Phaseolus aureus* (14), *Phaseolus vulgaris* (12, 1), *Pisum sativum* (9) and *Cicer arientum* (8), *Robinia pseudoacacia* (15), *Zea*

mays (15), *Urtica dioica* (15).

4. Localization of AChE in Plants

By electorphoretic homogenization of AChE, it has been found that AChE is localized in the roots of *Vigna radiata (Phaseolus aureus),* the roots of *Solanum melongena* and coleoptile of *Zea mays* (5), roots of *Pisum sativum* (9), roots of *Phaseolus vulgaris* (13), hypocotyl hook of *Phaseolus vulgaris* (1), roots of *Cicer arietinum* (8), shoots of *Pisum sativum* (22), latex of *Synadenium grantii* (6) chloroplast of *Pisum sativum* (15), roots of *Pisum sativum* (20), chloroplasts of *Convolvulus arvensis, Pisum sativum, Robenia pseudoacacia, Urtica dioca, Vigna radiata, Zea mays* (16), leaves of *Calystegia sepium*, mycelium of *Aspergillus nigrum* (17), plasmalemma (root, cotyledon, stomata, pollen tube, pistil-stigma, thallus), cell wall (hypocotyl, root, callus, cotyledons, thallus, etiolated seedling, stomata, pistil stigma) and partly in the cytoplasm of root cells, cotyledon, exine of pollen, and nucleus of root cells in *Pisum sativum* (12). It has been found that AChE has a K_m of 150 µM. Species that belong to family Solanaceae shows highest activity in crude extract. This is similar to acetylcholinesterase activity in giant axon of squid (7).

Although the genes for ChE from several animals have been cloned and characterized (data available on ESTHER database). In 1995, presence of one copy of AChE gene in *Arabidopsis thaliana* was indicated (11). This group reported that a probe prepared from *Torpedo* AChE c-DNA hybridizes to *Arabidopsis thaliana* DNA sequence under permissive hybridization conditions. At present, several genes in Arabidoopsis, which show high homology with Cholinesterases in animals, have been annotated as carboxylesterases. In 2003, Marshall reported carboxylesterase gene family from *Arabidopsis thaliana* but still little is known about role of carboxylesterases in plants.

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