

### 3-3-8. Effect of Molecular Forms and Alteration of Amino Acid Sequences on Enzyme-sensitivity with Insecticides in Acetylcholinesterase from Housefly (*Musca domestica*)

Won Tae Kim, Chung Sei Kim, Dae Joong Im, Kyung Saeng Boo

School of Agricultural Biotechnology, Seoul National University, Suwon, Korea

Acetylcholinesterase (AChE) is responsible for the hydrolysis of acetylcholine, thus terminating the transmission of the nerve impulse, and also is the target of inhibition by organophosphorus (Op) and carbamate insecticides, which have been used for insect pest control for many years. Up to now, insecticide resistance due to the abused use of these two groups of insecticides has been found in many insects. Many researchers reported that AChE sensitivity on insecticides effected op insecticide resistance. Recent AChE molecular studies showed that enzyme-insensitivity on insecticides is related to AChE molecular structure. To know the factors affecting the enzyme-sensitivity with op, purification and molecular cloning of AChE from the housefly were conducted and effect of molecular forms on enzyme-sensitivity with op was investigated.

Affinity chromatography was carried out to purify AChE from housefly-head extracted by LST<sup>1</sup>. Purity was clear by very clear peak shown by CE (Capillary electrophoresis). Substrate specificity was ATC > PTC > BTC. Inhibitory specificity was BW284C51 > eserine > ethopropazine.  $K_m$  and  $V_{max}$  were 0.06mM and 166.9unit/mg for ATC, 0.04mM and 97.8unit/mg for PTC, 0.12mM and 50.5unit/mg for BTC, at 25°C and pH 8.0, respectively. To separate molecular forms, heads of the housefly were treated by LS<sup>2</sup> for the soluble form AChE and HST<sup>3</sup> for the amphiphilic form AChE linked to membrane by glycolipid anchor. It was separated clearly on native-PAGE. For *in vitro* anticholinesterase assay, interaction between op and AChE was investigated by UV-spectro-photometer. It could not be clearly found that purification and molecular form are involved in sensitivity of AChE to insecticides in *in vitro* test. AChE molecular cloning was completely accomplished by RT-PCR. Especially, we could

find in AChE molecular amino acid sequence altered amino acids which is known to be involved in insensitivity of AChE on insecticides.

1. LST : 0.1M sodium phosphate with 0.01M sodium chloride, 1%(v/v) Triton X-100 and protease inhibitor cocktail, pH 8.0.
2. LS : 0.1M sodium phosphate with 0.01M sodium chloride and protease inhibitor cocktail, pH 8.0.
3. HST: 0.1M sodium phosphate with 1M sodium chloride, 1%(v/v) Triton X-100 and protease inhibitor cocktail, pH 8.0.