Properties and Purification of Acetylcholinesterase of *Spodoptera exigua*

Yun-Jung Kang and Yonggyun Kim

Laboratory of Insect Physiology, School of Bioresource Sciences,
College of Natural Sciences, Andong National University, Andong, Korea

Acetylcholinesterase (AChE, EC 3.1.1.7) catalyzes a hydrolytic cleavage of acetylcholine which is a neurotransmitter in insect central nervous system. Modification of AChE is a major mechanism for organophosphate and carbamate-resistant insects. Significant differences in inseciticide susceptibilities between early and late larval stages have been reported and were correlated with the variation of AChE activities. With unpurified AChE extracts, we could get a developmental polymorphism of AChE on 8% nondenaturing PAGE: AChE1 and AChE2. AChE1 was a major band at 3rd larval instar, while AChE2 was a major in adult. The 5th instar larvae had both AChEs with very little activitis. Addition of 0.2% Triton-X to the gel resulted in an increased activity of AChE1 in adults. To solve this physiological phenomenon, AChE purification was needed. This study was designed as a preliminary step for optimal AChE purification. First, we analyzed the substrate specificity of AChE using acetylthiocholine iodide (ATC), propionylcholine iodide (PTC), butyrylcholine iodide (BTC), and acetyl (4-methyl) thiocholine (AMTC) iodide. ATC and AMTC were more preferable substrates than PTC, while little BTC was catalyzed by AChE of *S. exigua*. Then we compared the effects of NaCl and Triton-X on the AChE solubilization. Triton-X (0.2%) increased the total AChE activity, but NaCl did not. Adult head extract with 0.2% Triton-X was subjected to an affinity chromatography using a ligand of 3-carboxyphenyl dimethyl ammonium iodide. Most AChE was eluted after the addition of 0.05M tetraethyl ammonium iodide. Though its yield was low (3.4%), the degree of AChE purification was about 115-fold.