

Thesis Title Study on the Purification of *N*-acetyltransferase
in the Optic Lobe of Giant Freshwater Prawns,
Macrobrachium rosenbergii de Man

Name Vimolsri Manasan

Degree Master of Science (Anatomy)

Thesis Supervisory Committee

Boonsirm Withyachumnarnkul, M.D., Ph.D.
Jisnuson Svasti, Ph.D.
Prasert Sobhon, Ph.D.

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Abstract

N-acetyltransferase has been extracted and partially purified from the optic lobe of *M. rosenbergii* de Man. The methods employed for the isolation involved a number of column chromatography: gel filtration on Sephadex G-100, disulfide-exchange chromatography on Sepharose-cystamine and affinity chromatography on Sepharose-CoA. Each procedure was compared by using the homogenate of the optic lobe as a starting material. The results from gel filtration show the existence of polymeric form of the enzyme. By elution on Sephadex G-100 in the presence of sodium phosphate buffer (0.01 M or 0.1 M, pH 6.5), MW of optic lobe NAT were estimated to be 51.5 kD and 25.5 kD, for the high-MW form and the low-MW form, respectively. All methods employed gave good purification of NAT with reasonable recovery of the activity. However the activity declined rapidly after purification resulting in a low recovery yield. Analysis by SDS-PAGE of each purified preparation demonstrated that they were still heterogenous, and there was no evidence to conclude which band is NAT.

Attempt to stabilize activity of the purified enzyme at low temperature of difference degree, i.e., 4°C, -20°C and -70°C recorded that the enzyme was best stabilized at 4°C. At that temperature, the activity declined by 70 % after 24 hours. The effect of acetyl CoA, tryptamine and BSA to protect the enzyme activity were also examined. BSA was effective in stabilizing the enzyme activity during storage for 24 hr while acetyl CoA and tryptamine were much less effective. However, BSA could protect the activity only for a short term storage. When further purification was performed by combination of gel filtration and affinity chromatography, the recovery yield was not increase satisfactorily. These data suggested the some unknown factor in the optic lobe homogenate acts as stabilizer of NAT activity.