

Purification and Characterization of an Inducible Antibacterial Peptide from the Larvae of *Protaetia brevitarsis*

Hye-Suk Yoon, In-Hee Lee¹ and Hak-Ryul Kim

Department of Biology, Korea University,

¹Department of Biochemistry, Hoseo University

Three types of antibacterial peptides (protaetins 1, 2 and 3) have been isolated and purified from the larval hemolymph of *P. brevitarsis* using gel permeation chromatography, preparative acid-urea PAGE and reverse-phase FPLC. Acid extracts of the cell-free hemolymph prepared by mixing with 10% acetic acid were used as starting materials for purification. Ultrasensitive radial diffusion and overlay assays were carried out to monitor the antibacterial fractions through purification steps. Of three antibacterial peptides, protaetin 1 has been successfully purified to homogeneity.

The N-terminal sequence of protaetin 1 was determined by gas-phase Edman degradation and the molecular mass was also measured to be 9283.95 Da by MALDI-TOF mass spectrometry. From antibacterial radial diffusion assay, we could confirm that the antibacterial activities of protaetin 1 were effective against Gram positive and Gram negative bacteria. Although the other two antibacterial peptides (protaetin 2, 3) were not completely purified, the molecular masses of protaetin 2 and 3 were estimated as 7 and 12 kDa on 16.5% Tricine SDS-PAGE, respectively. Further works including purification of protaetin 2 and 3 and cDNA cloning for protaetin 1 are undertaking.