Purification and Characterization of Heat Shock Protein Induced in Larval Hemolymph of the Wax Moth, Galleria mellonella in Response to Thermal Stress

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Heat shock proteins (Hsp) induced in the last larval hemolymph of *Galleria mellonella* reared at altered condition of high temperature were detected on non-SDS polyacrylamide gel. Comparing on SDS-PAGE gel with protein bands of hemolymph from wax moths reared in normal (26°C) and high temperature (45°C), the Hsp band could not be distinguished from normal hemolymph proteins. Since the Hsp co-migrates with major hemolymph protein of normal insect. The intensity of Hsp in hemolymph notably increased over a period of 12 h at high temperature. To purify Hsp from hemolymph proteins, we adapted the Sephadex G-100 gel filtration, DE-52 anion exchange chromatography and finally Mono-Q anion exchange FPLC. The M_r and pI value of Hsp was estimated as 73 kDa by SDS-PAGE and below 5.0 by isoelectric focusing gel, respectively, indicating the Hsp is an acidic protein. The amino acid composition and N-terminal sequence of Hsp were determined and compared with Hsps examined in other animals.

E112 Halocynin: Noble Antimicrobial Peptide from the Hemocytes in Korean Ascidian, *Halocynthia roretzi*

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It has been confirmed that the hemocytes in *Halocynthia roretzi* (called as rd in Korean) contain copious amounts of antimicrobial cationic peptides. Recently, we isolated and purified the major peptide from acid extracts of hemocytes, and temporarily named as 'halocynin'. *In vitro* antimicrobial assays, it has been proved that halocynin has potent antimicrobial activities against gram (-), (+) bacteria and fungus *Candida albicans*. Unlike clavanins, antimicrobial peptides that were originally found in protochordata *Styela clava*, halocynin has more cationic amino acids such as Arg and Lys. Therefore the antimicrobial activities of halocynin could remain at neutral pH. The molecular mass, N-terminal amino acids sequences and amino acid composition of halocynin were determined. Moreover, the hemolytic activity of halocynin on human erythrocytes was compared with those of mellitin, clavanin A and clavanin AK. Our finding of halocynin, an antimicrobial peptide from *halocynthia roretzi*, which is known as a favorite sea food in korea, may result in advent of ideal peptide antibiotics for korean people.