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cDNA cloning and expression analysis of red color-related hemocyanin gene in shrimp (*Litopenaeus vannamei*)

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Statement of the Problem: A red color-related protein derived from *Litopenaeus vannamei* shell, termed LvPBP75, was identified as hemocyanin by MALDI-TOF-MS. However, hemocyanin has been reported to be correlated with oxygen transportation and inner defense functions. To make a further understanding of LvPBP75, the primary structure and mRNA expression of this red color-related hemocyanin in *Litopenaeus vannamei* were studied.

Methods: Total RNA was isolated from muscle, epithelium, intestine, hepatopancreas, eyestalk, stomach, heart, and nerve using Trizol reagent. Poly A⁺ RNA was isolated from the epithelium, and the full-length cDNA sequence of LvPBP75 was amplified using Marathon Kit and Ex Taq polymerase. The expression of LvPBP75 was measured by quantitative RT-PCR.

Results: A full-length cDNA of 2,183 bp including an open reading frame of 1,986 bp that encodes 662 amino acid residues was cloned. Predicted signal peptide was found between segment of Met1 and Ala20. Arthropod hemocyanin landmark peptides were corresponded to segments of Tyr197-Thr216 and Thr387-Phe395. A putative tyrosinase cooper-binding domain was located between segment of Asp391-Asp402. The amino acid fragments determined by MALDI-TOF-MS were all recognized in the deduced amino acid sequence. Multiple sequence alignment showed ~80% identity between LvPBP75 and hemocyanin or its subunits derived from the hepatopancreas of *L. vannamei*. According to the quantitative RT-PCR results, mRNA expression level of LvPBP75 showed a decreasing order in hepatopancreas, heart, stomach, epithelium, intestine, eyestalk, nerve, and muscle. These results suggest that the LvPBP75 found in *Litopenaeus vannamei* shell is composed of hemocyanin, and the functions of hemocyanins are correlated with the tissues where they are existing. Investigation on the combination mechanism between hemocyanin and pigment is now in progress.

Biography

Chuang Pan is a Doctoral course 2nd year student from Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology. His Major research field of interest is Applied Marine Biosciences and Advanced Food Science and Technology.

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