Studies on red color-related pigment-binding protein derived from the shell of Pacific white shrimp Litopenaeus vannamei

Doctoral Dissertation Summary

専　攻 Major	応用生命科学	氏　名 Name	Pan Chuang
論文題目 Title	Studies on red color–related pigment–binding protein derived from the shell of Pacific white shrimp *Litopenaeus vannamei* (バナメイエビ殻由来の色素結合タンパク質に関する研究)		

Introduction & Purpose

Crustaceans are cultured widely throughout the world, with 90% of shrimp cultured in Asia. Pacific white shrimp (*Litopenaeus vannamei*) is one of the economically important species of shrimp culture (~70%). It is mainly cultured in Asian countries, both as a food item for domestic consumption and as a valuable export commodity. Although it is well known that *L. vannamei* shell color changes from pale gray to bright red–orange after cooking, the underlying mechanism and red color–related pigment–binding proteins derived from *L. vannamei* shell are poorly understood. The main objective of this research is to illuminate the red color change on the surface of *L. vannamei* by studying the red color–related protein derived from its shell, as well as to help to promote the usage of shrimp by–products.

Materials & Methods

In order to clarify the red color change on *L. vannamei* shell surface, the red color–related protein was first purified from shell by ammonium sulfate precipitation, gel filtration and anion exchange HPLC. The red color–related protein was termed as LvPBP75 (*Litopenaeus vannamei* pigment–binding protein with molecular mass ~75 kDa). Then in order to elucidate the structural properties of LvPBP75, cDNA cloning and circular dichroism spectroscopy analysis were carried out. Meanwhile, to investigate the thermal properties of LvPBP75, studies on the effects of temperature, ion strength, pH, and alcohol

on the red color change of LvPBP75 were carried out. In the next step, in order to demonstrate the pigment–binding property of LvPBP75, tissue distribution of LvPBP75 and reconstruction of artificial LvPBP75 were carried out. Finally, to investigate whether the LvPBP75 is specific in *L. vannamei* or not, the red color–related proteins derived from the shell of *Homarus americanus*, *Marsupenaeus japonicus*, and *Panulirus japonicus* were purified using gel filtration and ion exchange HPLC, respectively.

Results & Discussion

The red color–related protein was purified from the shell of *L. vannamei* using ammonium sulfate precipitation, gel filtration, and anion exchange HPLC. Gel filtration HPLC and SDS–PAGE analysis demonstrated that the pure protein has a molecular mass of ~75 kDa. It was a homogeneous monomer with purity more than 90%. Peptide mass fingerprinting analysis revealed a protein named hemocyanin matched this 75 kDa protein. The pigment was confirmed as astaxanthin. The results suggested that the red color change on *L. vannamei* shell surface is correlated with a novel red color–related pigment–binding protein, LvPBP75, which is consist of hemocyanin and astaxanthin, but not the previously described crustacyanin in lobster shell.

On the basis of the partial amino acid sequences determined by peptide mass fingerprinting, a full–length cDNA of 2,183 bp including an ORF of 1,986 by that encodes 662 amino acid residues was cloned. Blast analysis revealed that it belongs to the hemocyanin family. Circular dichroism analysis illuminated that it was a protein rich in α–helix conformation. This red color–related protein was determined as a thermal sensitive protein. Color changes of this protein can be promoted after being subjected to conditions of high concentrations of NaCl, acidic or alkaline pH, and high concentrations of alcohols. The results suggested a novel function of hemocyanin as binding with pigment and its involvement in *L. vannamei* shell color change.

Tissue distribution revealed that LvPBP75 has the highest expression level in hepatopancreas, mediate level in heart, hemolymph, epithelium, and intestine, and the lowest in eyestalk, nerve, and muscle. Recombinant and structural analysis revealed that astaxanthin could bound to the shell derived hemocyanin and this pigment–binding complex had closely corresponded to *Litopenaeus vannamei* shell color change. But unlike crustacyanin, astaxanthin does not bring any structural changes to the binding complex. Three–dimensional structural analysis revealed a unique spatial structure of this red color–related protein. The results suggested that the pigment–binding ability of hemocyanins has species– or tissue–specificity and their unique structural features play an important role in binding ability.

The 75 kDa protein was correlated with the red color change on the surface of *L. vannamei*, *Marsupenaeus japonicus*, and *Panulirus japonicus*. Meanwhile, 22 kDa protein was correlated with the red color change in *Homarus americanus*. Accordingly, the red color–related pigment–binding protein LvPBP75 is not species–specific among shrimps. The results suggested that the red color change on L. vannamei surface is mainly correlated with LvPBP75 and it is not specific in L. vannamei, further investigation is necessary to understand the red color–related proteins among a variety of crustacean species.

Conclusively, a novel red color–related pigment–binding protein, LvPBP75 was purified from the shell of *L. vannamei* by using ammonium sulfate precipitation, gel filtration, and anion exchange HPLC. It had closely corresponded to *L. vannamei* shell color change under heat treatment and was identified as hemocyanin which could bind with ATX. The pigment–binding ability of hemocyanins has tissue– or species–specificity. Meantime, this red color–related hemocyanin–ATX binding complex was not specific among shrimp species.