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cDNA cloning of paramyosin from several kinds of squid mantle muscle

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Introduction

Paramyosin is a fibrous protein and specific in invertebrate muscle [1, 2]. Two α-helical chains of about 100 kDa interact with each other to form a coiled-coil structure [3,4]. Futhermore paramyosin polymerizes with each other to form a core, and myosin binds around it to form thick filaments [5]. It is thought that these aggregation play the same function as thick filaments in vertebrate muscles. The content of paramyosin to musclular protains is different among species and body regions. Actually the content ratios of paramyosin are in the wide range of 5 to 50% in many invertebrates [6].

Squid mantle muscle, which is the main edible part is covered with four layers of outer skin, consequently the texture is hard. Interestingly, the processing properties whether mantle muscles are suitable for raw food as sashimi or processed food, depend on species. In addition, even the muscles of the same squid exhibit different processing characteristics. We have been investigated whether squid meat is available for a surimi-based product because it has soft texture and will be easy to eat for everyone. In processing of squid meat, there are two different points from fish meat. Firstly, squid meat paste could not form setting gel at comparative low temperature. It is thought to involve highly active proteases existed in squid meat [7,8]. Several active ones decompose muscle proteins during processing, resulting in weakening of the thermal gel. Secondary, the gel made from squid meat shows unique texture, which is different from that of kamaboko. It is suggested that the difference between these two products is caused of paramyosin, especially existing in invertebrate muscle. We are also interested in the role of paramyosin on raw and processed food because this protein would attributed to the species-specific differences in texture.

Marine organisms in which the primary structure of paramyosin has been reported are molluscs such as *Haliotis discus* (BAJ61596), *Mytilus galloprovincialis* (O96064), *Octopus bimaculoides* (XP_014783284), *Crassostrea gigas* (XP_011429255), and *Pinctada fucata* (JAS03399). On the other hand, it has not been reported the primary structure of squid paramyosin. Therefore, in this study, the primary structures of paramyosin from several kinds of squids were determined by cDNA cloning and then the structural characteristics were investigated.

Materials and methods

Materials

Frozen mantle muscle from four kinds of squid *Ommastrephes bartramii*, *Dosidicus gigas*, *Sepia esculenta*, and *Gonatus onyx* were purchased from IDO-SYOTEN company (Iwate).

Methods

cDNA cloning of four squids

Firstly, total RNA was prepared from each mantle muscles using ISOGEN II solution (Nippon Gene, Tokyo), and cDNA was synthesized using total RNA and oligo dT primers with a reverse transcriptase. Primers were designed from the conserved sequences of paramyosin of oyster, mussels, abalone, and octopus previously reported. PCR, 3' RACE and 5' RACE method was performed to obtain a DNA fragment, and the fragment was sequenced. First strand cDNA was performed for PCR using a Go Taq Master Mix (Promega, Tokyo). cDNA cloning of full length was performed for PCR using a LA Taq DNA polymerase (Takara, Shiga).

Phylogenetic tree analysis

The deduced amino acid sequences were aligned using ClustalW program (EMBL-EBI: The European Bioinformatics Institute, European Molecular Biology Laboratory). Subsequently, the phylogenetic tree was constructed using the neighbor-joining method on the software Mega6 [9].

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Results

Firstly, four cDNAs encoding paramyosin from the *O. bartramii*, *D. gigas*, *G. onyx*, and *S. esculenta* were cloned. Next the full-length nucleotide and deduced amino acid sequences of four paramyosin cDNAs have been determined. The sequences were deposited to the DDBJ/EMBL/GenBank databases with accession numbers LC272578, LC272579, LC272580, and LC272583 respectively. The *O. bartramii* paramyosin cDNA was cloned up to 2,605 bp which contains an open reading frame (ORF) of 2,574 bp encoding 858 amino acids. The *D. gigas* one was cloned up to 2,691 bp and contains an ORF of 2,640 bp encoding 880 amino acids. The *G. onyx* one was cloned up to 2,609 bp and contains an ORF of 2,574 bp encoding 858 amino acids. The *S. esculenta* one was cloned up to 2,631 bp and contains an ORF of 2,574 bp encoding 858 amino acids. Molecular weights of these four paramyosin were 99.7, 102.4, 99.6, and 99.6 kDa, respectively. *O. bartramii*, *D. gigas*, *G. onyx*, and *S. esculenta* showed relatively low identity of 69%, 67%, 68%, and 70% to *Haliotis discus* paramyosin. Nucleotide identities among four squid paramyosins showed 96% between *O. bartramii* and *D. gigas*, 88% between *O. bartramii* and *G. onyx*, 90% *O. bartramii* and *S. esculenta*, 86% *D. gigas* and *G. onyx*, 87% between *D. gigas* and *S. esculenta*, 89% *G. onyx* and *S. esculenta*. The phylogenetic analysis was performed by using all paramyosin sequence deposited in DDBJ including four squid paramyosins determined in this study. The result showed that four paramyosins formed a group, which was near to that of octopus.

Discussion

In amino acid sequences of four paramyosins, a series of 28-amino acid repeats were found. And the repeats were composed of a unit of seven amino acid residues, (*a*-*g*) [10]. Hydrophobic amino acids are placed at positions *a* and *d*, and charged amino acids are placed at positions *e* and *g*. Therefore, it was suggested that four paramyosin also form the α-helical coiled-coil structure. In addition, an ACD (Assembly competence domain) consisting of 29 residues was present on the C-terminal side [11]. ACD is thought to be essential for filament formation. Squid paramyosins in this study could polymerize through this region in the physiclogical condition.

From result of phylogenetic tree analysis, it was shown that *O. bartramii* and *D. gigas* are close, *G. onyx* and *S. esculenta* are close in squid group. More squid paramyosin sequences have been determined, it may be possible to understand in detail. Now, we are trying to construct the expression system of squid paramyosin, in order to investigate their various character.

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