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Molecular cloning and characterization of two ferritin subunits from the disk abalone (*Haliotis discus discus*)

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Introduction

Iron is an essential element for all living organisms for many biological processes and constituent of metalloproteins such as enzymes, electron transfer complexes and oxygen carriers. But in the other hand excessive accumulation of free iron in the cells are potentially toxic since it promotes serious hazards like oxidative injury [Andrews et al., 1992].Therefore, cells have evolved homeostatic mechanisms that regulate transport, storage, and mobilization of cellular iron to prevent the deleterious effect of excessive iron. Many proteins are involved in iron metabolism and among them ferritin plays the most important roll in iron storage and detoxification.The abalone (*Haliotis discus discus*) belongs to class gastropoda and one of the most popular mollusksin worldwide aquaculture. Therefore, our objective was initiating this study to illustrate different ferritin sub units in abalone by molecular cloning, sequence analyses, expression and protein purification.

Materials and methods

Two sub units of abalone (*Haliotis discus discus*) ferritin AbF1 and AbF2 were obtained from the abalone cDNA library. AbF1 and AbF2 sequences were analyzed comparing with other known ferritin sequences available at NCBI database. The primers were designed to amplify the coding sequence of 621 bp and 549 bp fragments of the two ferritin sub units respectively. Polymerase chain reaction (PCR) was performed to amplify the relevant coding sequences. Then purified PCR products of both AbF1 and AbF2 were ligated same way in to pMAL-c2X expression vector. Finally, this ligated plasmid DNA was transformed into competent *E Coli* 10G cells for multiplication. The plasmid was transformed into *E coli* K12 (TB1) protein expression cells and the culture was induced with 3 μl (1mM) of iso-propyl β -thio-galactopyranoside (IPTG) at 30 ℃ for 3 hrs. The two-ferritin protein sub units

were purified using pMAL protein fusion and purification system. The purified ferritin were visualized by 10 % SDS-PAGE.

Results and summery

The sequenced AbF1, AbF2 two ferritin sub units were 800 bp and 902 bp in length with 207 aa and 183 aa protein sequence respectively. The predicted protein sizes were 24 kDa and 21 kDa which both match to ferritin heavy chain polypeptide molecular size ranging from 20-28 kDa in different species. Pairewise alignment of AbF1 and AbF2 amino acids showed only 35% identity therefore, considered as two different ferritin sub units functioning in abalone. BLAST p alignment at amino acid level showed AbF1 and AbF2 have highest homologies (76%) with Pearl oyster (*Picnctada fucata*) and (38%) Pacific white shrimp (*Litopenaeus vannamei*) ferritins respectively. The N-terminus of the AbF1 sub unit showed signal peptide sequence by SignalP program with the cleavage site at 20-21 aa position. There was no signal peptide in AbF2 subunit The highly conserved iron responsive element (IRE) domain was present in the 5'untranslated region (UTR) of abalone AbF2 subunit but not present in the AbF1 sub unit. This IRE showed typical stem loop structure, which needed to iron responsive protein fixation. The IRE domain showed highest homology (96.2%) equally to pearl oyster and Pacific oyster ferritins IRE domain.

As expected, AbF1 and AbF2 protein bands were approximately 66.5 kDa and 63.5 kDa respectively since our maltose binding fusion protein size is 42.5 kDa. Therefore, AbF1 and AbF2 protein sizes of 24 kDa, 21 kDa are accordance to the predicted sizes of abalone two ferritin sub units.

References

Andrews, S.C., Arosio, P., Bottke, W., Briat, J.F., Von Darl, M., Harrison, P.M., et al., 1992. Structure, function and evolution of ferritins. *J. Inorg.Biochem.* 47, 161-174