魚類血清に含まれる抗菌性L-アミノ酸オキシダーゼの性状と機能

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Background

L-amino acid oxidase (LAO) is one of the potent oxygen radical species generator. LAO oxidizes L-amino acid to α-keto acid and generates hydrogen peroxide as a by-product. This enzyme shows a variety of bioactivities such as apoptosis, antiprotozoal and antibacterial activity. Those activities are because of the resulted-hydrogen peroxide. In fish species, antibacterial LAO was firstly found from fish body surface mucus and it may protect bacterial invasion at fish skin (Kitani et al., 2007). After this, fish LAO was isolated from the serum and it was the first report that the LAO found from animal blood (Kitani et al., 2010).

The LAO related host defense mechanisms of animals —including fishes are still unclear, even blood LAO. Previously, I surveyed that the species-specificity of LAO in fish blood and found the red-spotted grouper *Epinephelus akaara* (Fig. 1) serum containing the enzyme (EaLAO) and hydrogen peroxide mediated antibacterial activity against *Aeromonas salmonicida* and *Listonella anguillarum*. Isolated enzyme was 440 kDa acidic protein that consisted of 70 kDa subunits with L-tryptophan, L-methionine and L-phenylalanine oxidation activity. Also, the N-terminal and internal peptide amino acid sequences of the isolated enzyme were similar to known LAOs. This result was the first finding of LAO from *E. akaara*.

Purpose

However, the host defense mechanism of fish blood LAO is not fully examined. The new knowledge of blood LAO may become an important key to understand the first-line host defense system of animals. In this study, to gain more insight into the biological meaning of fish blood LAO, I try to reveal primary structure, tissue distribution and physiological responses of EaLAO.

Methods

First, cDNA cloning and sequence analysis of the EaLAO gene (*ealao*) was carried out. The partial nucleotide sequence was amplified by degenerate PCR. The degenerate primers were designed based on the N-terminal and internal peptide amino acid sequences of EaLAO. The full-length nucleotide sequence of *ealao* was attempted by both 3' RACE and 5' RACE method. Second, the expression level of *ealao* in *E. akaara* tissues -- dorsal skin, abdominal skin, gill, stomach, intestine, liver, spleen head kidney, trunk kidney, brain, pyloric caeca, and whole blood were measured. LAO activity of tissue extracts from those tissues was also measured. Finally, *ealao* alteration by the lipopolysaccharide (LPS) injection, pathogen exposure and traumatic damages were examined. In the LPS injection work, 1 mg of LPS was administrated to each fish by intraperitoneal (IP) injection. The pathogen exposure experiment, formalin killed vaccine of *A. salmonicida*, *L. anguillarum* and *Vibrio harveyi* were individually prepared and 100 μL of vaccines were IP injected to each fish. The 10^6 CFU of live *V. harveyi* injection was also examined. The LPS and bacterial exposure experiment, *ealao* alterations in spleen were monitored. In traumatic damages experiments, two different types of experiments were performed; a) the test fish skin was trepanned 30 times/fish using lancets, b) the blood was collected as much as possible. After both treatments, blood LAO activity and *ealao* alteration in skin, spleen and head

kidney were monitored. The quantitative PCR of all gene expression profiling was amplified with *ealao*, interleukin 1 β (*il1b*) and reference gene (component of oligomeric Golgi complex 5, *cog5*) specific primer set using SYBR green I chemistry. LAO activity of serum or plasma from all experiment was also evaluated by the generation of hydrogen peroxide with L-tryptophan using *o*-phenylenediamine / peroxidase method and measured the absorbance at 492 nm.

Results and Discussions

Partial nucleic acid sequence and amino acid sequence of EaLAO was clarified (555 bp, 185 AA). That sequence showed the high similarity of known fish LAO. The partial predicted amino acid sequence includes signal peptide (Met^{1} - Ala^{20}), flavin adeninedinucleotide binding site (Arg^{115} – Thr^{122}) and N-glycosylation site (at least three sites, Asn^{45} – Ser^{48}, Asn^{62} – Asp^{65}, Asn^{80} – Gln^{83}). During the cloning work, I found two of the other types of LAO genes (*ealao2* and *ealao3*). These results suggested that the *E. akaara* has some of the *ealao* paralogues. The functional differences of the *ealao*s have to be clarified.

The tissue distribution of *ealao* was dominantly detected in dorsal skin, abdominal skin, gill and liver. The LAO activity of each tissue extract was detected in dorsal skin, abdominal skin, gill and serum. Interestingly, the LAO gene was not detected in hematopoietic tissues (spleen and kidney) and whole blood cells.

The LPS injection, *il1b* was increased eight times at six hours rather than the initial group. However, *ealao* was not significantly altered. Similarly, LAO activity in serum was not changed by the LPS injection. Bacterial vaccine and live bacteria injection also showed less alteration of *ealao*. Both injections induced the *il1b*; this result meant that the bacterial response of test fish was formed. Those results suggested that bacteria related substances and bacteria cell bodies are not contributing LAO response.

The *ealao* in the skin was not induced by skin trepanation; in contrast, loss of blood showed interesting responses. The *ealao* expression level was increased than the control group at one day after bled. This alteration was calmed three days after treatment. LAO activity of plasma was increased at three days after treatment. Those results suggested that *ealao* is induced by the the blood loss and synthesizes the LAO protein to recover the normal concentration in three days.

Conclusions

Taken together, the EaLAO may be generated in the liver during the healthy condition to maintain blood LAO concentration and stands by the intruding bacterial pathogens. In case of severe wounds that cause the blood loss, the *ealao* production switches to the head kidney for the recovery. In addition, EaLAO similar gene was detected in the skin and gills of the naïve grouper. Those LAO productions could protect bacterial infection from body surface tissues. In conclusion, LAO may constantly fight against bacterial infection in the entire body of the red-spotted grouper.

References

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