Note

Tissue-specific Expression of Rhamnose-binding Lectins in the Steelhead Trout (Oncorhynchus mykiss)

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Received January 17, 2002; Accepted February 22, 2002

Tissue-specific expression of three L-rhamnose-binding lectins, named STL1, STL2, and STL3, in the steelhead trout (Oncorhynchus mykiss) was investigated. STL2 and STL3 mRNAs were restricted in the oocytes. In contrast, STL1 mRNA was detected only in the liver. The transcription of STL2 and STL3 started in previtellogenic oocytes. These results showed distinct expression profiles of rhamnose-binding lectins in the fish.

Key words: animal lectin; lectin; rhamnose-binding lectin; steelhead trout

Three L-rhamnose-binding lectins, named STL1, STL2, and STL3, were isolated from steelhead trout (Oncorhynchus mykiss) eggs, and characterized.1–4) They are composed of two (for STL2 and STL3) or three (for STL1) tandemly repeated carbohydrate-recognition domains (CRDs) that consist of about 95 amino acid residues. L-Rhamnose-binding lectins from catfish (Silurus asotus) eggs (SAL) and sea urchin (Anthocidaris crassispina) eggs (SUEL) are also composed of the CRD motif, indicating that L-rhamnose-binding lectins form a novel animal lectin family and may have important biological functions in the eggs of marine animals.5,6) Immunohistochemical study showed that STL1 protein was localized not only in the ovary but also in the tissues of the immune system such as serum, spleen, leukocytes, and thrombocytes.4) In contrast, STL2 and STL3 proteins were restricted to the ovary. In the oocytes, STL proteins were mainly localized in the cortical vesicles, and released into the perivitelline space just after fertilization. Taking into account these results together with that STLs agglutinate Gram-negative and Gram-positive bacteria by recognizing their lipopolysaccharides and lipoteichoic acids, respectively, STLs may be involved in the innate immunity of fish.3)

Tissue-specific expression of STLs was examined by northern blot analysis in this study. Digoxigenin-labeled STLs sense and antisense RNA probes were prepared by in vitro transcription with the cDNAs of STLs full open reading frames (STL1, 936 bp; STL2, 654 bp; STL3, 651 bp) and a DIG RNA Labeling kit (SP6/T7) (Roche Diagnostics, Tokyo, Japan). Total RNAs were prepared from various tissues of steelhead trout in the previtellogenic stage using Isogen (Nippon Gene, Tokyo, Japan). Twenty micrograms of total RNA were separated on a formaldehyde-agarose gel, transferred onto a Hybond N+ niron membrane (Amersham Pharmacia Biotech), and the membrane was hybridized at 65°C overnight with digoxigenin (DIG)-labeled STLs antisense RNA probes. A positive signal with the STL1 probe was observed only in the liver (Fig. 1A). In contrast, positive signals with STL2 and STL3 probes were observed only in the ovary. Cross-hybridization should be minimal, because the hybridization was done at high temperature (65°C) and the nucleotide sequence similarities of STLs probes were relatively low (34 to 47%). These results indicate that the STL1 gene is transcribed specifically in the liver, and the STL2 and STL3 genes are transcribed specifically in the ovary.

The expression profile of STLs during embryonic development was examined by northern blot analysis. The positive signals with STL2 and STL3 antisense RNA probes were detected in the previtellogenic and vitellogenic oocytes, but not in the fertilized eggs and larvae of fishes (Fig. 1B). The RNA prepared from immature ovary consisting mainly of previtellogenic oocytes had a stronger signal than that prepared from mature ovary consisting mainly
Fig. 1. Northern Blot Analysis.
Twenty micrograms of total RNA from various tissues were loaded onto a 1.5% agarose gel, transferred onto a membrane, and hybridized with DIG-labeled STL1 (a), STL2 (b), and STL3 (c) antisense RNA probes, respectively. (d) Ethidium bromide-stained pattern. Arrowheads indicate the positions of ribosomal RNA. (A) Tissue-specific expression of STLs in the steelhead trout. (B) Changes in the STLs mRNA levels of steelhead trout eggs during the embryonic development. Lane 1, Previtellogenic oocytes; lane 2, vitellogenic oocytes; lane 3, day 21 after fertilization (organogenesis); lane 4, day 28 after fertilization (hatching); lane 5, day 35 after fertilization (yolk absorption).

Fig. 2. In situ Hybridization.
Steelhead trout ovary was hybridized with DIG-labeled STLs sense and antisense RNAs probes, respectively. Scale bar is 100 nm.
of vitellogenic oocytes. In contrast, the positive signals with the STL1 antisense RNA probe were detected in the hatched larvae, but not in the previtellogenic and vitellogenic oocytes, or fertilized eggs.

In situ hybridization was done to identify the expression site of STLs in the ovarian tissues. The steelhead trout ovarian fragment was fixed in 4% paraformaldehyde (PFA) in 10 mM phosphate-buffered saline (pH 7.4) (PBS), and deparaffinized with xylene and ethanol. The tissues were embedded in paraffin wax, sectioned 5 μm in thickness, and fixed onto microscope slides. Hybridization was done with DIG-labeled STLs sense and antisense RNA probes, respectively, as described by Oba.7) The positive signals were observed with STL2 and STL3 probes only in the cytoplasm of previtellogenic oocytes, but not in the vitellogenic oocytes and other ovarian cells (Fig. 2). However, STL2 and STL3 mRNAs were indeed present in the vitellogenic oocytes when an equal amount of total RNA was analyzed by Northern blot. This discrepancy might be caused by the dispersion of STL2 and STL3 mRNAs in growing vitellogenic oocytes during yolk absorption. No signal was detected in the ovarian tissues with the STL1 antisense probe or STLs sense probes (Fig. 2). These results suggest that STL1 was expressed in the liver, and then transported to the immune system and ovarian cells via the blood stream. On the other hand, STL2 and STL3 were expressed specifically in the oocytes via the blood stream. Genomic Southern blot analysis was done using STLs RNA probes. DNA was prepared from the liver of female steelhead trout. Ten micrograms of DNA were digested with Eco RI, Hind III, and Bam HI, respectively, separated on a 1.0% agarose gel, transferred onto a membrane, and hybridized with DIG-labeled STL1 (B), STL2 (C), or STL3 (D) antisense RNA probes. (A) Ethidium bromide-stained pattern. The lines on the left show the positions of size markers (kilobases).

Fig. 3. Genomic Southern Blot Analysis.

Ten micrograms of DNA digested with Eco RI (lane 1), Hind III (lane 2), and Bam HI (lane 3), respectively, were loaded onto a 1.0% agarose gel, transferred onto a membrane, and hybridized with DIG-labeled STL1 (B), STL2 (C), or STL3 (D) antisense RNA probes. (A) Ethidium bromide-stained pattern. The lines on the left show the positions of size markers (kilobases).

References