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Demonstration of the mucosal lectins in the epithelial cells of internal and external body surface tissues in pufferfish (*Fugu rubripes*)

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Abstract

We have previously identified two novel mannose-specific lectins, skin- and intestine-type pufflectins, in the pufferfish, *Fugu rubripes* [J. Biol. Chem. 278 (2003) 20882]. In the present study, the localization of the lectins and their producing cells were analyzed with antibody and anti-sense probe that recognize both types of pufflectin. Using immunohistochemistry, pufflectins were detected exclusively in epithelial cells in the skin, gills, oral cavity wall and esophagus, whereas in both mucous and epithelial cells in the intestine. Messenger RNAs for pufflectins were detected only in epithelial cells of these tissues with in situ hybridization, suggesting that epithelial cells are able to produce the lectins. Pufflectins are produced and distributed in cells that cover the external and internal body surfaces, which might mean that *Fugu* have a common immunological system on both surfaces.

Keywords: Lectin; Pufferfish; Body surface; Skin mucus; Epithelial cell; Mucous cell; Immunohistochemistry; In situ hybridization

1. Introduction

The external body surface plays a very important role in the prevention of pathogen invasion, especially in fish, which are continuously exposed to an aquatic environment that serves as a perfect medium for pathogenic microorganisms. In addition to the mechanical barrier of scales, the skin surface of fish is protected by the secretion of mucus. Fish skin mucus contains many molecules that may act as defense factors [1,2]. Lectins, which are saccharide-binding proteins other than antibodies and enzymes [3,4], are also considered to be included in this category [1,2].

Fish skin can be regarded as a continuous single plane with the surface of the digestive tract, since both are composed of similar types of cells such as mucous

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and epithelial cells. Furthermore, both surfaces of fish are covered with mucus. In mammals, mucosal immune system protects the internal epithelial surfaces of organs such as digestive organs and lungs [5,6]. This system is known to be independent of the inner immune system. In addition to the internal surface, fish may have a unique mucosal immune system on the external body surface. Comparison of the skin with the digestive organs would be significant for the development of mucosal immunology in lower vertebrates.

The pufferfish (*Fugu rubripes*), belongs to the Tetraodontiforme, is a biologically important model species for which a draft genome sequence has recently been published [7]. From the skin mucus of this species, we have purified a novel mannose-specific lectin, pufflectin, which is a homodimer of 13 kDa subunits [8]. This lectin shows sequence homology (~30% identity) to the mannose-specific lectins of monocotyledonous plants. Pufflectin can bind to the parasitic trematode, *Heterobothrium okamotoi*, suggesting that this lectin contributes to the self-defense system. This lectin is, furthermore, found to agglutinate some types of bacteria isolated from rearing water of *Fugu* (Mano, personal communication). Pufflectin has at least two cDNA isotypes: the skin-type (pufflectin-s) and the intestine-type (pufflectin-i), which share 91.4% amino acid sequence identity [8]. Pufflectin-s is expressed in the skin, gill, oral cavity wall and esophagus but not in the intestine, while pufflectin-i is expressed in the intestine only [8].

Among teleosts, cells containing skin mucus lectins have been identified in only two species of Anguilliformes. Suzuki and Kaneko [9] demonstrated that a skin mucus lectin of the Japanese eel (*Anguilla japonica*) is contained in club cells. Congerins, which are lactose-binding galectins of the conger eel (*Conger myriaster*), are also distributed in club cells [10]. However, *Fugu* does not have club cells [11,12]. Thus, investigation of the cellular locations of two mucosal lectins in *Fugu* contributes to the understanding of fish lectins. In the present study, we raised antibody against pufflectins, and immunohistochemistry was carried out in several mucosal tissues of *Fugu*. In situ hybridization analysis was also performed to identify cells producing lectin mRNA.

2. Materials and methods

2.1. Fish

Fugu of about 150 g were kindly supplied by the Central Research Institute of the Electric Power Industry, Abiko Research Laboratory (Japan). Fish were kept in a 500 l seawater tank at 17–20 °C and anesthetized with 2-phenoxyethanol (Wako) prior to sacrifice.

2.2. Purification of pufflectin-s

To obtain antigen for immunization, pufflectin-s was purified with mannose-affinity chromatography as previously described [8]. In brief, collected skin mucus was homogenized with an equal volume of 10 mM phosphate buffered-saline, pH 7.4 containing 0.9 mM $CaCl_2$ and 0.33 mM $MgCl_2$ (PBS (+) buffer) and centrifuged at 15,000×*g* for 30 min at 4 °C. The supernatant (skin mucus extract) was applied to mannose-epoxy-activated sepharose 6B pre-equilibrated with PBS (+) buffer. After washing with the same buffer, the bound pufflectin-s was eluted with PBS (+) buffer containing 50 mM mannose. The flow-through fraction of the chromatography was also stored at 4 °C for immunological analyses.

2.3. Preparation of the polyclonal antibody for pufflectin-s

Two rabbits were subcutaneously injected with 0.3 mg purified pufflectin-s together with Freund's complete adjuvant (Rockland). Booster injections with the same amount of antigen were given with Freund's incomplete adjuvant (Rockland) at 7-day intervals. One week after the last injection, exsanguinations were conducted from blood vessel of the ear, and sera were obtained by centrifuging. At first, we used the antiserum in the bellow described Western blotting to verify the specificity, but a little cross-reaction to other proteins was observed. The antiserum was, therefore, pre-absorbed with the flow-through fraction from the affinity chromatography to block non-specific or cross-reactions to other proteins present in the skin mucus.