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Review

The buccal gland of *Lampetra japonica* is a source of diverse bioactive proteins

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ABSTRACT

The parasitic phase lampreys (*Lampetra japonica*) are bloodsuckers in the marine, and their buccal gland secretion (lamphredin) contains various regulators such as anticoagulants, ion channel blockers, and immune suppressors like those from leeches, insects, ticks, vampire bats, and snakes. This review focuses on the functions and characteristics of the active proteins from the buccal gland of *L. japonica* for the first time, and provides new insights into the parasitic mechanisms of lampreys and the possibilities of developing drugs such as novel anticoagulants, thrombolytic agents, local anesthetics, and immunosuppressants.

1. Introduction

Lampreys are extant representatives of the superclass Agnathans in the class of Cephalaspidomorphi, an ancient group of jawless vertebrates whose habitat and body structure have been highly conserved for at least 350 million years [1]. In 2009, Nikitina et al. put forward that lampreys can be used as an ideal animal model for investigating early vertebrate evolution and embryo development due to their unique phylogenetic position near the root of the vertebrate tree and the relative ease of obtaining mature adults and embryos [2]. Recently, more and more researchers have focused on lampreys to study the evolution of the adaptive immune system and have provided valuable insights [3–5].

Lampreys are aquatic, eel-shaped animals which are known as bloodsuckers in the marine. Some species live in the freshwater for their entire lives, while others, including the sea lamprey (*Petromyzon marinus*) and the Japanese lamprey (*Lampetra japonica*), usually live a unique semi-parasitic life cycle. Non-parasitic phase ammocoetes (larvae) mainly live in the freshwater and feed on trapped food particles. After metamorphosis, parasitic phase lampreys migrate to the ocean, where they attach themselves to the body surface of the host fishes through their sucker-like oral disc and feed on the blood and body fluids of the hosts for days. At last the non-parasitic phase adult lampreys return to freshwater streams and rivers to spawn and die. Like many parasitic animals, the parasitic phase lampreys also have to evolve varied strategies to subvert the host defense mechanisms effectively in favor of their own well-being. To help parasitic animals live better, their salivary glands usually secrete various active regulators, some of which have been used as drugs to treat patients for years. For example, hirudin from the salivary gland of Mexican leeches has been used to treat clotting diseases [6]. Similarly, lampreys contain a pair of glands, named buccal glands, which may play important roles in parasitic feeding by secreting lamphredin (originally termed by Lennon) [7]. It may help lampreys counteract the hemostasis and nociceptive responses, suppress the immune response, inhibit the reactive oxygen species (ROS) production, and induce vasodilatation of the host fishes. Although there are extensive studies on secretion from the salivary glands of parasitic animals such as leeches (Annelides class), insects (Arthropoda class) and vampire bats (vertebrate mammals) [8,9], the biochemical nature of

Abbreviations: AMCases, acidic mammalian chitinases; AP, action potential; bFGF, basic fibroblast growth factor; BGSP-1, buccal gland secretion protein 1; BGSP-2, buccal gland secretion protein 2; CAM, chicken chorioallantoic membrane; CRBGP, cysteine-rich buccal gland protein; CRD, cysteine-rich domain; CRISP, cysteine-rich secretory protein; DRG, dorsal root ganglion; DTT, dithiothreitol; ECV304, Human umbilical vein endothelial cell line; EDTA, ethylenediaminetetraacetic acid disodium salt; EST, expressed sequence tag; *L. japonica*, *Lampetra japonica*; LPS, lipopolysaccharide; NIF, neutrophil inhibitory factor; PAGE, polyacrylamide gel electrophoresis; PHA, phytohemagglutinin; PR-1 protein, pathogenesis-related protein 1; Prx, peroxiredoxin; RBCs, red blood cells; RBL-2H3, rat basophilic leukemia; RGD, Arg-Gly-Asp; rLj-CRBGP, recombinant *L. japonica* CRBGP; rLj-Prx2, recombinant *L. japonica* Prx2; rLj-RGD3, recombinant *L. japonica* RGD3; rLj-TCTP, recombinant *L. japonica* translationally controlled tumor protein; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TTX-S, tetrodotoxin-sensitive; TTX-R, tetrodotoxin-resistant.

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lamphredin has barely been investigated until 2007 [10]. Recently, some reports have focused on the new proteins with important physiological functions from the buccal gland of *L. japonica* and provided new insights into the development of effective drugs. Here we discuss the current research on the native and recombinant proteins from the buccal gland of *L. japonica*.

2. Lamphredin from *L. japonica*

In 2007, Xiao et al. caught lampreys (*L. japonica*) at the spawning migration stage in Tong River, a branch of Songhua River in Heilongjiang province in China. They observed that the color of the buccal gland secretion (lamphredin) is brown, the same as that of the sea lamprey secretion (*P. marinus*), as previously described, and found the average protein concentration of the secretion is about 120 mg/ml [10]. Both native-PAGE and SDS-PAGE showed that the secretion mainly contained two protein bands, buccal gland secretion protein 1 (BGSP-1, GenBank number: AB294234) and buccal gland secretion protein 2 (BGSP-2, GenBank number: AB300465). In addition, BGSP-1, BGSP-2 and other small peptides can be isolated from the secretion using a Sephadex G-75 column. Measurement of the protein concentration indicates that, on average, the secretion consists of 12% BGSP-1, 36% BGSP-2 and 52% small peptides [10]. To further elucidate the components and the functions of lamphredin at the molecular level, Gao et al. has constructed a cDNA library (2.1×10^6 pfu/mL) from the buccal gland of *L. japonica* and obtained 1323 clones with inserts longer than 100 bp and with good chromatograms. Among the 1323 clones, 653 expressed sequence tags (ESTs) share significant homology with sequences in protein or nucleotide databases of NCBI, including 328 ESTs which are homologous to sequences of Petromazonidae animals. These ESTs were classified into 11 different functional categories, among which the one with the highest proportion was related to protein synthesis [11].

3. Buccal gland secretion protein 1 (BGSP-1) as a fibrinogenase

BGSP-1 is one of the most abundant proteins in the buccal gland of *L. japonica* at the spawning migration stage. Sequence analysis indicates that BGSP-1 shares high homology with the plasma albumin pre-peptide from *P. marinus*, and its molecular weight is 159,909 Da. BGSP-1 from lamphredin acts as an α-fibrinogenase, which is also ubiquitously distributed in many snake venoms [12–15], because BGSP-1 can rapidly degrade the alpha chain of human fibrinogen by cleavage at Ala_{10}-Glu_{11} and His_{368}-Ser_{369}, but slowly degrade the beta chain and hardly degrade the gamma chain. In addition, lamphredin and BGSP-1 prefer to cleave the C-terminal region of the alpha chain of fibrinogen over the N-terminal region; this manner of cleavage may contribute to prolonging the clotting time [16–18]. Thus, the function of BGSP-1 on anticoagulation is mainly due to its ability to degrade fibrinogen, and subsequently prevent the formation of fibrin clots. This property of BGSP-1 in lamphredin could keep the lampreys feeding on the host fishes for a long time and sucking the blood conveniently.

As reported [10], the fibrinogenolytic activity of BGSP-1 is ~70.6 U (*Km*, 2.3×10^{-6} mol L^{-1}; *Kcat*, 2.77×10^9 mol s^{-1}), and the optimum temperature for the fibrinogenolytic activity is around 45 °C. Further study shows that lamphredin and BGSP-1 could be inactivated in the presence of a metal chelating agent EDTA. However, addition of Ca^{2+} or Mg^{2+}, but not Zn^{2+}, could restore the fibrinogenolytic activity. This suggests that BGSP-1 may act as a metalloproteinase in the buccal gland. Besides, BGSP-1 in lamphredin cannot degrade human serum albumin and hemoglobin, which are the most abundant proteins in the plasma. This suggests that the highly specific fibrinogenolytic activity of lamphredin and BGSP-1 is not only critical for the survival of *L. japonica*, but can also serve as a very promising candidate for a highly specific anticoagulant.

4. Cysteine-rich buccal gland protein (CRBGP) mediates important physiological functions

Buccal gland secretion protein 2 (BGSP-2) is the other abundant protein in the buccal gland of *L. japonica* at the spawning migration stage. BGSP-2 shares high homology with the cysteine-rich secretory protein (CRISP) family, characterized with 16 highly conserved cysteine residues. Thus, BGSP-2 was further named as cysteine-rich buccal gland protein (CRBGP). Xiao et al. has obtained the native CRBGP with the molecular weight of 25,660 Da and its polyclone antibody with a titer of 1×10^4 [10]. Furthermore, Yu et al. cloned the CRBGP gene from a cDNA library of buccal gland from *L. japonica* and obtained the recombinant protein with an apparent molecular weight of 31,600 Da [19].

4.1. CRBGP as a Na^+ channel blocker

Chi et al. firstly reported that the native CRBGP from the buccal gland of *L. japonica*, a member of the pathogenesis-related protein 1 (PR-1 protein) subfamily, is able to reduce the amplitude and firing frequency of action potentials (APs) generated from the hippocampal neurons and dorsal root ganglion (DRG) neurons through blocking the voltage-dependent Na^+ channels [20]. It is likely that this CRBGP (1.7 mM), which could suppress the neuronal excitability, accounts for how the lampreys make the host fishes lose nociceptive responses and keep sucking the blood without being awared during their long parasitic life cycle. Therefore, CRBGP is another advantageous factor which might facilitate the blood-sucking habit of lampreys. Like many ion channel blockers from the CRISP family [21], CRBGP could also block the K^+ channel of the hippocampal neurons, which may be partly responsible for the increase in the AP duration and reduction of the firing frequency of the AP in the neurons. Chi et al. suggests that lampreys are under less evolutionary pressure to improve their preying abilities because the voltage-gated Na^+ channels are encoded by a group of highly conserved genes. In addition, Chi et al. also found that intracellular CRBGP blocks the peak Na^+ current strongly. This is consistent with the extensive studies showing that Na^+ channel blockers typically access their binding site within the channel pore from inside [22], and the blockage induced by CRBGP is slightly reversible after washout. Further studies show that CRBGP can suppress not only the tetrodotoxin-sensitive (TTX-S) Na^+ channel currents, but also the tetrodotoxin-resistant (TTX-R) Na^+ channel (NaV1.8) currents of the DRG neurons which play important roles in nociception, suggesting that CRBGP has the ability to inhibit a broad spectrum of voltage-dependent Na^+ channels. Thus, as a Na^+ channel blocker, CRBGP from the lampreys has vast potential for future development as a local anesthetic.

4.2. CRBGP as a muscle contraction inhibitory factor

At the same time, Ito et al. [23] isolated and characterized the lamprey CRISP (CRBGP) independently. They found that the native CRBGP could suppress depolarization-induced contraction of rat tail arterial smooth muscle, another advantageous factor for long time feeding on the vertebrate blood due to its vasodilation effect. They inferred that the suppression of muscle contraction was probably through blockade of an L-type Ca^{2+} channel. However, Chi et al. pointed out that Ito et al. did not show any direct evidence, such as electrophysiological study, and that many factors could