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Minireview

Proteases and Protease Inhibitors in Bee Venoms

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Abstract Bee venom is a complex mixture of various toxic components, including enzymes and peptides. While bee venom components can trigger local and systemic allergic reactions, they are also employed in traditional and alternative medicine for treating various diseases and managing venom allergies. Recent studies have shown a growing interest in bee venom components' diverse biological, toxicological, and pharmacological effects. This study focuses on the roles of proteases and protease inhibitors in the venoms of honeybees and bumblebees. We conducted a review of the fibrinogenolytic activity exhibited by the serine proteases in bee venom as well as the multifunctional bee venom peptides that possess antifibrinolytic, anti-elastolytic, and antimicrobial properties.

Keywords Honeybee, Bumblebee, Protease, Protease inhibitors, Venom

INTRODUCTION

In nature, there are some animals that possess venoms. Venomous animals include snakes, frogs, scorpions, bees, ants, and wasps. These animals use venom as a tool to capture prey or as a defense mechanism against predators. Venoms from such animals are known to cause severe harm to humans and have encouraged substantial research into the treatment and prevention of venom-related injuries. On the other hand, the venom of bees, particularly honeybees, has been utilized for pain relief and to treat conditions like arthritis through a traditional practice known as bee sting therapy (Son *et al*., 2007; Chen and Lariviere, 2010). In recent times, advancements in biotechnology have not only shed light on the pharmacological effects of venom components but have also led to the development and commercialization of various cosmetics using venom extracts from snakes and bees.

Considering these aspects, venoms can be seen as a double-edged sword. Just as medicines can sometimes have side effects, venoms can paradoxically possess medicinal properties. This review article focuses on bee venom components, such as proteases, protease inhibitors, and low-molecular-weight peptides, to explore the utilization of bee venom from both a human perspective and the perspective of the bees themselves.

MAIN COMPONENTS OF BEE VENOM

Two of the more commonly encountered species of bee are the honeybee (*Apis mellifera*), which is a beneficial insect for humans, and the bumblebee (*Bombus* spp.), which is commonly used in greenhouses and fields to pollinate crops (Velthuis and van Doorn, 2006). Venoms are commonly found in honeybees and bumblebees, which are readily encountered by humans and other animals. These bees use their venom as a defensive weapon against intruders. The quantity of venom protein released by honeybees in a single sting ranges from 50 to 140 μg, which is approximately five times higher than that released by bumblebees (10–31 μg per sting) (Hoffman and Jacobson, 1984; Schumacher *et al*., 1994). Unlike honeybees, bumblebees can sting multiple times without losing their stingers. Bee venom is known as a

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complex mixture of biologically, toxicologically, and pharmacologically active substances, including proteins, enzymes, and low-molecular-weight compounds (Son *et al*., 2007; Chen and Lariviere, 2010; Danneels *et al*., 2015). While bee venom can occasionally induce life-threatening allergic reactions because of the presence of various allergenic and non-allergenic molecules, it generally triggers acute local inflammatory responses.

The two major components of honeybee venom are melittin and phospholipase A_2 (Fig. 1). Melittin, the most abundant component in bee venom, constitutes approximately 50% of its dry weight. Melittin consists of 26 amino acids and exhibits antibacterial and lytic activities (Habermann, 1972; Cruciani *et al*., 1991). Phospholipase A_2, which accounts for approximately 12% of the dry weight of bee venom, is the most extensively studied toxic enzyme. Phospholipase A_2 is known for its ability to induce pain and inflammatory responses (Hartman *et al*., 1991; Landucci *et al*., 2000; Six and Dennis, 2000). Bumblebee venom contains three major components: bombolitin, phospholipase A_2, and serine proteases (Fig. 1). Bombolitin is the most abundant component in bumblebee venom and exhibits structural and biological similarities to melittin (Choo *et al*., 2010a; Qiu *et al*., 2012). Phospholipase A_2 is also an important toxic component in bumblebee venom (Xin *et al*., 2009). Remarkably, bumblebee venom, unlike honeybee venom, includes serine proteases as major components that display fibrinogen and fibrinolytic/fibrinogenolytic activity (Choo *et al*., 2010b, 2011; Qiu *et al*., 2011). In addition, bee venom contains serine protease inhibitors with antifibrinolytic activity and low-molecular-weight peptides, such as secapin and inhibitor cysteine knot (ICK) peptides (Park *et al*., 2014; Lee *et al*., 2016). These serine protease inhibitors and low-molecular-weight peptides have been reported to exhibit antifibrinolytic and antimicrobial activities (Lee *et al*., 2016; Yang *et al*., 2017; Kim *et al*., 2022).

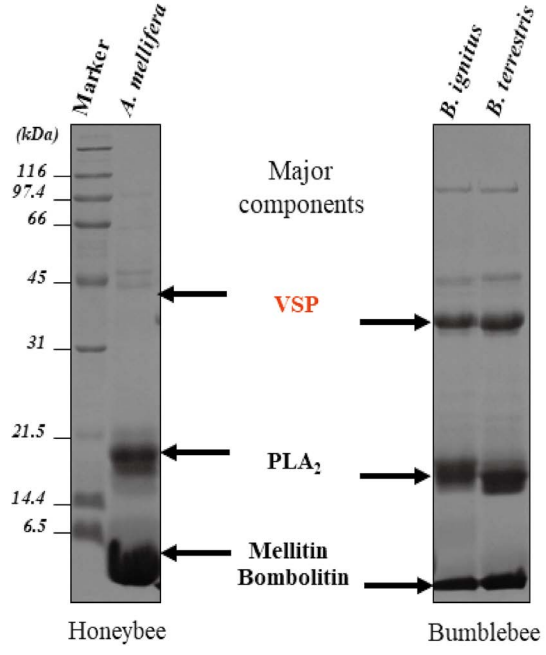

Fig. 1. Protein electrophoresis analysis of honeybee and bumblebee venoms. VSP, venom serine protease; PLA_2, phospholipase A_2.

SERINE PROTEASES IN BEE VENOMS

Bee venom contains serine proteases, including a conserved catalytic triad (Ser, His, and Asp) that represents the main criterion for the classification of a protein as a serine protease (Choo *et al*., 2010b; Qiu *et al*., 2011). While serine proteases play a major role in bumblebee venom, they are present in trace amounts in honeybee venom (Fig. 2). Serine proteases in bee venom are expressed in the venom gland and are stored in the venom sac, where they are secreted through the stinger. Serine proteases found in bee venom possess various functions (Choo *et al*., 2010b; Qiu *et al*., 2011). In mammals, serine proteases from bee venom have been shown to

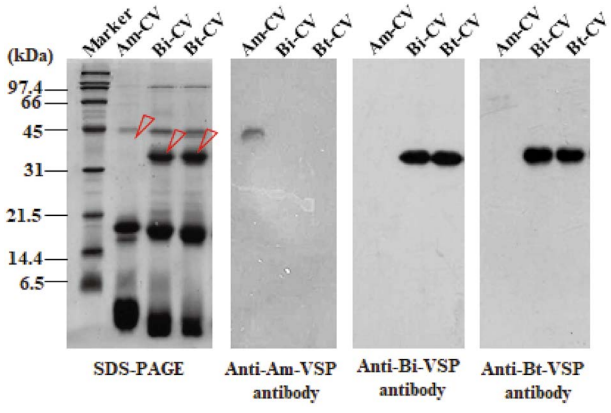

Fig. 2. Serine proteases in honeybee and bumblebee venom (Qiu *et al*., 2011). Am-CV, *Apis mellifera* crude venom; Bi-CV, *Bombus ignitus* crude venom; Bt-CV, *Bombus terrestris* crude venom; Am-VSP, *A. mellifera* venom serine protease; Bi-VSP, *B. ignitus* venom serine protease; Bt-VSP, *B. terrestris* venom serine protease. The arrowheads indicate serine proteases in the venom.

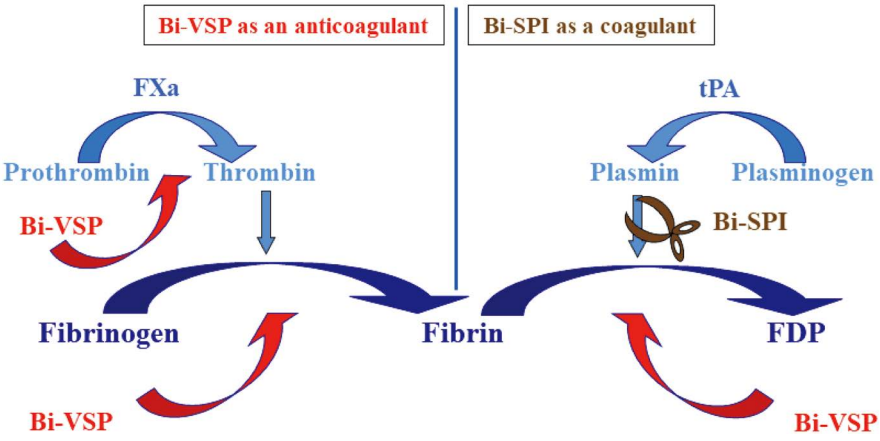

Fig. 3. Mechanisms of fibrinogen and fibrinolytic activity of bee venom serine protease and antifibrinolytic activity of bee venom serine protease inhibitor. Bi-VSP, *Bombus ignitus* venom serine protease; Bi-SPI, *B. ignitus* venom serine protease inhibitor.

activate the conversion of prothrombin to thrombin, act as thrombin-like serine proteases, and play a role as plasmin-like serine proteases (Fig. 3). These functions convert fibrinogen to fibrin and generate fibrin degradation products, demonstrating fibrinogenolytic activity. From a human perspective, the fibrinogen and fibrinolytic activity of bee venom serine proteases suggests their potential application as thrombolytic agents. On the other hand, considering that bee venom is a primary defense mechanism against intruders, the fibrinogen and fibrinolytic functions of serine proteases may contribute to the effective dispersion of bee venom components by lowering fibrinogen levels in mammalian blood, similar to what has been observed with snake venom serine proteases (Koh *et al*., 2001; Swenson and Markland, 2005; He *et al*., 2007), thus contributing to the efficient spread of the bee venom components through the bloodstream (Choo *et al*., 2010b; Qiu *et al*., 2011).

In addition, bee venom serine proteases are involved in the formation of melanin, which is part of the humoral immune response in insects (Choo *et al*., 2010b). In insects, melanin is formed locally at the wound or microbial infection sites to aid in wound healing or killing infectious microbes. Bee venom serine proteases act as prophenoloxidase-activating enzymes that activate the conversion of prophenoloxidase into phenoloxidase. This function leads to a rapid and excessive melanin formation in the hemolymph of insects injected with bee venom, ultimately killing them. This strategy, which manipulates the innate immune system of insects, demonstrates a potential for pest control by disrupting insect physiology. As an example, introducing the gene of serine proteases from bumblebee (*B. ignitus*) venom into the entomopathogenic fungus *Beauveria bassiana* increased the insecticidal and virulence effects of the fungus (Kim *et al*., 2013b).

PROTEASE INHIBITORS IN BEE VENOMS

Protease inhibitors from the venoms of honeybees and bumblebees have been well-documented (Choo *et al*., 2012; Kim *et al*., 2013a, 2021, 2022; Qiu *et al*., 2013; Wan *et al*., 2014; Yang *et al*., 2017). The known serine protease inhibitors in honeybee and bumblebee venoms are cysteine-rich peptides consisting of 50–70 amino acids that inhibit enzymes like trypsin, chymotrypsin, or elastase. Bee venom serine protease inhibitors are known to inhibit the activity of microbial serine proteases as well as the activity of plasmin in mammals. Furthermore, bee venom serine protease inhibitors exhibit antimicrobial activity by binding to the cell walls of both Gram-negative and Gram-positive bacteria as well as fungi, causing structural damage (Fig. 4). In addition, they demonstrate the antifibrinolytic activity by inhibiting the activity of plasmin, which converts fibrin into fibrin degradation products in mammals (Fig. 5). Nevertheless, bee venom serine protease inhibitors do not show erythrocyte lytic activity in mammals (Kim *et al*., 2022). These compounds' antifibrinolytic and antimicrobial activities suggest their potential application as anticoagulants or antimicrobial agents. The anti-fibrinolytic

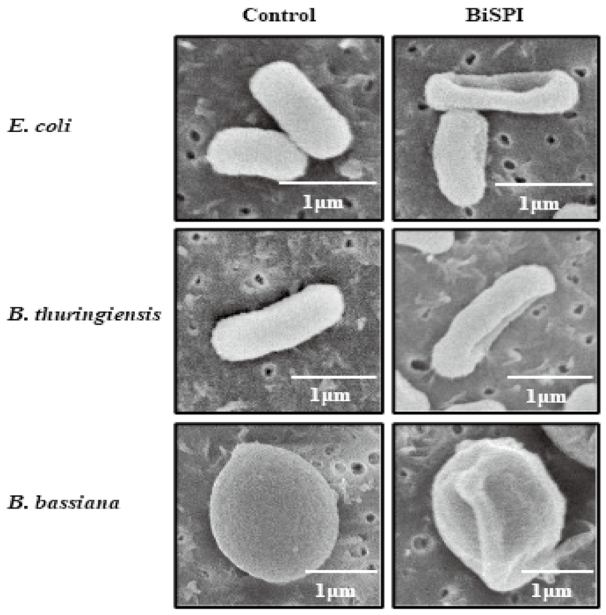

Fig. 4. Antimicrobial activity of bee venom serine protease inhibitors (Kim *et al*., 2022). BiSPI, *Bombus ignitus* venom serine protease inhibitor.

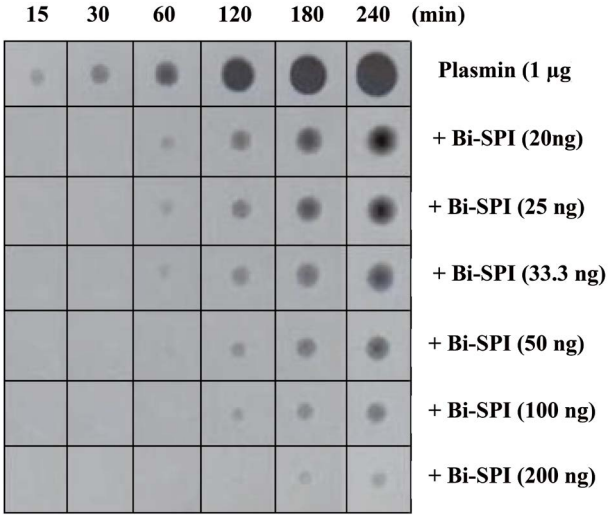

Fig. 5. Inhibitory activity of the plasmin of the bee venom serine protease inhibitor (Choo *et al*., 2012). Bi-SPI, *Bombus ignitus* venom serine protease inhibitor.

function of serine protease inhibitors is considered a means to reduce the venom's leakage by promoting blood clotting at the site of the sting, similar to what has been observed in snake venom serine protease inhibitors (Masci *et al*., 2000; Flight *et al*., 2009; Choo *et al*., 2012).

Bee venom is known to contain proteinase inhibitors besides serine protease inhibitors. Recently, a metalloprotease inhibitor that hinders plasmin activity and demonstrates antifibrinolytic activity was reported in the venom of *B. ignitus* bumblebees (Kim *et al*., 2021).

LOW-MOLECULAR-WEIGHT PEPTIDES IN BEE VENOMS

In addition to protease inhibitors with antifibrinolytic and antimicrobial activities, bee venom contains low-molecular-weight peptides with similar functions. The ICK peptide identified in the venom of the Asian honeybee (*Apis cerana*) exhibits insecticidal activity and functions as an antifungal peptide (Park *et al*., 2014, 2016). While the rapid and high insecticidal activity of bee venom ICK peptides has been demonstrated, the mechanism behind this activity has not yet been elucidated. The presence of the low-molecular-weight peptide secapin in honeybee venom has been known for some time (Gauldie *et al*., 1976), but research on its function is scarce. Recently, *A. cerana* secapin, consisting of 25 amino acid residues, was found to have various functions (Lee *et al*., 2016). Honeybee venom secapin not only exhibits serine protease inhibitory activity but also shows antifibrinolytic and anti-elastase activities. Moreover, secapin exhibits broad-spectrum antimicrobial activity against bacteria and fungi. The multifunctionality of secapin suggests its potential applications in blood clotting, anti-inflammatory, and antimicrobial functions.

Bee venom contains peptides with antimicrobial activity, as well as low-molecular-weight peptides. Recently, major royal jelly proteins (MRJPs) 8 and 9 in bee venom were identified as microbial serine proteases and antimicrobial agents (Lee *et al*., 2022). Amwaprin in bee venom was identified and characterized as a microbicidal and anti-elastolytic agent (Lee *et al*., 2023). Given that bee venom is a rich source of various peptides with toxicological and pharmacological effects (Son *et al*., 2007; Chen and Lariviere, 2010), the discovery of biologically active components in it will provide new insights into its properties.

CONCLUSION

Bee venom, as a defensive weapon used by bees, presents a fascinating avenue for future research with a wide

range of potential applications. Bee venom has a complex composition, including phospholipase A_2, serine proteases, serine protease inhibitors, melittin, and bombolitin, and has been historically used in folk medicine for various therapeutic purposes. Moreover, bee venom is applied in immunotherapy to treat allergies. As research continues to unveil the biological, toxicological, and pharmacological activities of bee venom components, they may emerge as the basis for the development of next-generation biological substances. These substances could find applications in medicine, biotechnology, and various industries, opening up new avenues for innovative treatments and therapies.

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