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Review

Serpins in arthropod biology

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ABSTRACT

Serpins are the largest known family of serine proteinase inhibitors and perform a variety of physiological functions in arthropods. Herein, we review the field of serpins in arthropod biology, providing an overview of current knowledge and topics of interest. Serpins regulate insect innate immunity via inhibition of serine proteinase cascades that initiate immune responses such as melanization and antimicrobial peptide production. In addition, several serpins with anti-pathogen activity are expressed as acute-phase serpins in insects upon infection. Parasitoid wasps can downregulate host serpin expression to modulate the host immune system. In addition, examples of serpin activity in development and reproduction in *Drosophila* have also been discovered. Serpins also function in host-pathogen interactions beyond immunity as constituents of venom in parasitoid wasps and saliva of blood-feeding ticks and mosquitoes. These serpins have distinct effects on immunosuppression and anticoagulation and are of interest for vaccine development. Lastly, the known structures of arthropod serpins are discussed, which represent the serpin inhibitory mechanism and provide a detailed overview of the process.

Contents

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1. Introduction

Serpins are a superfamily of proteins, typically around 45 kDa, which generally function as serine proteinase inhibitors and participate in a suicide inhibitory mechanism in which both serpin and proteinase are permanently inactivated. The serpin reactive site that interacts with the target proteinase is part of an exposed loop near the carboxyl-terminal end of the serpin sequence. In an inhibitory reaction between a serpin and proteinase, the reactive center loop (RCL) of the serpin occupies the proteinase active site, and a specific peptide bond in the loop is cleaved (the scissile bond), resulting in a large conformational change in the serpin. However, the hydrolysis reaction is not completed, and the serpin and proteinase are trapped in a covalent complex [1–4]. The scissile bond is defined as the peptide bond between two amino acid residues named P1 and P1′. Residues on the amino-terminal side of the scissile bond are numbered in the C→N direction, and residues on the carboxyl-terminal side of the scissile bond (the "prime" side) are numbered in the N→C direction: (. . ..P5-P4-P3-P2-P1-↓-P1′-P2′-P3′-P4′-P5′ . . ..). The sequence of the reactive center loop determines the inhibitory selectivity of a serpin. Detailed information about serpin structure and mechanism from studies of arthropods is provided in Section 5 of this review.

Arthropod serpin sequences are not sufficiently similar to vertebrate serpins to permit assignment of orthology with mammalian serpins. Therefore, physiological proteinase targets of arthropod serpins must be determined experimentally, and not surprisingly, the majority of arthropod serpins are therefore orphans. The known roles of serpins in arthropod biology are summarized in Fig. 1 and Table 1, and discussed in detail in the following sections.

The first arthropod serpins characterized biochemically were from hemolymph of the silkworm, *Bombyx mori*. Proteins of ~45 kDa purified from larval plasma as inhibitors of trypsin and chymotrypsin were cleaved near their carboxyl-termini and formed SDS-stable complexes with proteinases [5–7] and were speculated to be serpins. Similar inhibitors were isolated from another lepidopteran insect, *Manduca sexta* [8,9], and amino acid sequences confirmed that the *M. sexta* and *B. mori* inhibitors were serpins [8,10]. Serpin sequences have now been identified in many arthropod transcriptomes and genomes, with 30–40 serpin genes in many species, including 34 in *B. mori* [11], 32 in *M. sexta* (M. Kanost, unpubished data), 31 in a beetle, *Tribolium castaneum* [12], 29 in *Drosophila melanogaster*, and a similar number in other *Drosophila* species [13]. Other species have significantly fewer serpin genes, including just 7 in the honeybee, *Apis mellifera* [14] and 10 in the tsetse *Glossina morsitans* [15]. Mosquito species vary from 18 serpin genes in *Anopheles gambiae*, 23 in *Aedes aegypti*, to 31 in *Culex quinquefasciatus* [16]. Ticks and mites also have considerable variation in the serpin gene content of their genomes, with 45 serpin genes in the blacklegged tick *Ixodes scapularis* [17], 22 in the cattle tick *Rhipicephalus microplus* [18], and only 10 in the scabies mite, *Sarcoptes scabiei* [19].

Besides gene duplication, the number of unique serpins encoded by a given arthropod genome can also be increased post-transcriptionally. Some insect serpin genes have a unique structure, in which mutually exclusive alternate splicing of an exon that encodes the RCL results in production of several inhibitors with different inhibitory activities. This phenomenon was first observed in the gene for *M. sexta* serpin-1, which contains 14 copies of its 9th exon [20] (M. Kanost, unpublished data). Each version of exon 9 encodes a different sequence for the carboxyl-terminal 39–46 residues, including the RCL (Fig. 2), and the resulting serpin variants inhibit a different spectrum of proteinases [21,22]. Orthologous serpin-1 genes from other lepidopteran species, with alternate exons in the same position as in *M. sexta* serpin-1, have been identified [23–25]. The serpin-1 gene of *B. mori*, in the same superfamily as *M. sexta*, has only four alternate versions of exon 9 [11,26], indicating considerable genetic flexibility and relatively recent expansions and divergence of these alternate exons in lepidopteran evolution [24]. Alternative splicing at the same position, to produce serpins with differing RCLs also occurs in *An. gambiae* SRPN10 [27] and in spn4 orthologs in multiple *Drosophila* species [28] (discussed more in Section 2.3).

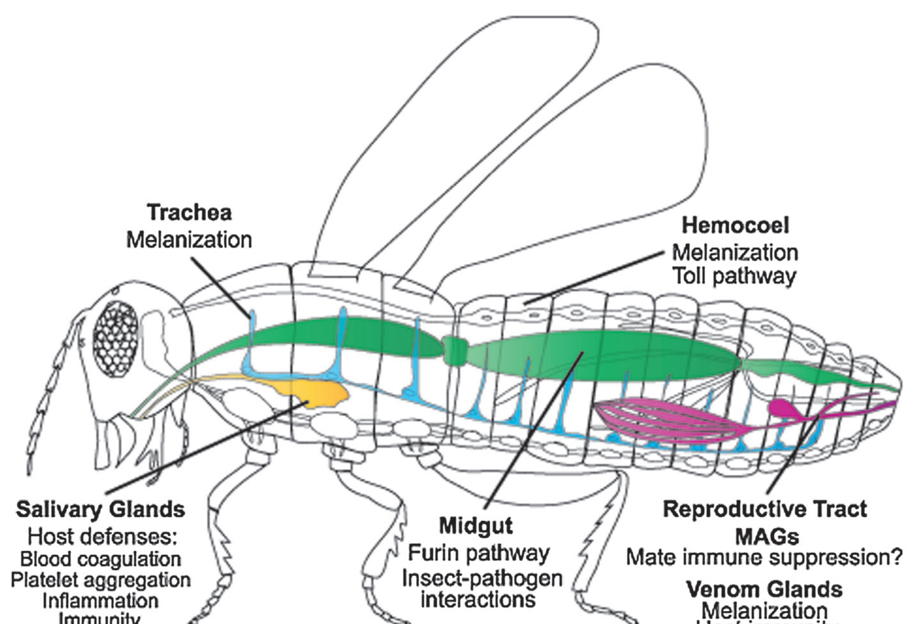

Fig. 1. The known physiological functions of serpins in arthropod physiology. The majority of insect serpins are produced in fat body and hemocytes and are then secreted into the hemocoel. In addition, serpins are expressed in a number of additional tissues, including tick and mosquito salivary glands (orange), midgut (green), trachea (blue), and in male accessory glands (MAGs), as well as the venom glands of parasitoid wasps (pink). Major known functions are listed and discussed in detail in the different sections of the manuscript.