Review

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Delivery of intrahemocoelic peptides for insect pest management

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The extensive use of chemical insecticides for insect pest management has resulted in insecticide resistance now being recorded in >500 species of insects and mites. Although gut-active toxins such as those derived from *Bacillus thuringiensis* (Bt) have been successfully used for insect pest management, a diverse range of insect-specific insecticidal peptides remains an untapped resource for pest management efforts. These toxins act within the insect hemocoel (body cavity) and hence require a delivery system to access their target site. Here, we summarize recent developments for appropriate delivery of such intrahemocoelic insect toxins, via fusion to a second protein such as a plant lectin or a luteovirus coat protein for transcytosis across the gut epithelium, or via entomopathogenic fungi.

Introduction

Current status of insect pest management

With the world population projected to increase to >9 billion by 2050 [1], production of food in a cost-effective and environmentally sustainable manner is a high priority. A doubling of current food production will be required to sustain the future population at projected levels. However, an estimated 10–20% of major crops worth billions of dollars are lost to herbivorous insects, representing a major constraint to achieving this goal. In addition, post-harvest losses resulting from insect and mite-associated damage of stored food, cause estimated losses of 30%, valued globally at >100 billion US dollars [2]. Not only do arthropods negatively affect agriculture, they also negatively affect human health and welfare through infliction of injury and transmission of diseases. Bed bugs are of significant public health importance with their recent resurgence attributed in part to increased international travel and resistance to multiple pesticides [3,4]. Mosquito-vectored dengue virus and malaria have spread rapidly during the past decade into highly populated urban areas resulting in a dramatic rise in the numbers of clinical cases [5,6]. There are some 50 million dengue hemorrhagic fever infections per year resulting in 500 000 hospitalizations [7], and 250 million cases of malaria per year, leading to some 1 million deaths worldwide [8,9]. An estimated 2 billion US dollars has been spent annually on malaria control in recent years and costs associated with morbidity are massive. Vector control is one of the most effective strategies used to prevent the spread of mosquito-borne diseases [8].

Driven primarily by the significant deleterious impact of arthropods on the production of food and fiber and the associated economic losses, multiple research entities focus on arthropod management and crop protection solutions. However, the management of arthropod pests for protection of both agriculture and public health remains reliant primarily on the application of chemical insecticides. There are a number of disadvantages associated with their use including development of resistance by pest populations, deleterious impacts on non-target organisms, environmental pollution, and potential effects on human health [10]. Hence, there is ongoing pressure to develop target-specific, environmentally friendly, and biodegradable pest management tools.

Pest-tolerant transgenic plants provide a more sustainable approach for crop protection. Toxins derived from Bt have been highly effective for the management of lepidopteran (moth) and coleopteran (beetle) pests when delivered by transgenic plants [11]. Indeed, since their initial introduction in the early 1990s, transgenic plants have been widely adopted with 67% of corn and 77% of cotton planted in the US in 2012 expressing Bt toxins [12]. As a result, insecticide use and crop production costs have both been reduced. However, resistance to Bt toxins has been documented [13,14] and Bt toxins are not sufficiently toxic for management of sap-sucking hemipteran pests [15–17] without modification [18], with a few notable exceptions [19]. In some cases, the reduced application of chemical insecticides on Bt crops has resulted in increased populations of hemipteran pests [20,21].

RNAi has the potential to be used for the development of target-specific management methods for insect pests and the practical application of this approach for arthropod control has been demonstrated [22–24]. However, the efficacy of RNAi following oral delivery of silencing RNA appears to be restricted to Coleoptera.

In this review, we outline recent work conducted towards exploitation of toxins that act within the hemocoel for insect pest management, including significant new advances.

Insecticidal peptides that lack oral toxicity

The venom from a wide range of predatory species (e.g., scorpions, wasps, predaceous mites, cone snails, anemones,

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lacewings, and parasitoids), provides an outstanding resource for isolation of insect-specific neurotoxins [25,26]. These insecticidal neurotoxins typically target sodium, potassium, calcium, or chloride channels. With few exceptions, these neurotoxins are not orally active and require appropriate delivery systems to access their target site, the nerves. Arachnid venoms, which are complex peptidic libraries, have received particular attention [27]. Based on the number of species and number of toxins present in the venom of those examined, there are an estimated 0.5–1.5 million arachnid-derived insecticidal peptides [25]. There are predicted to be at least 10 million bioactive spider-venom peptides [28]. Of the 800 peptides in the ArachnoServer 2.0 Database, 136 are insecticidal with 38 being insect selective, 34 nonselective, and 64 of unknown phyletic selectivity [25]. Arthropod-derived neuropeptides, enzymes, and hormones that function to regulate insect development and maintain homeostasis (e.g., diuretic hormones, and juvenile hormone esterase) also constitute peptides with potentially insecticidal effects when delivered outside their normal physiological timeframe. Although these endogenous regulators provide insect specificity, a major drawback is that high concentrations may be required to overcome natural regulatory mechanisms that restore appropriate physiological levels within the insect. Although a few peptides (e.g., proctolin and *Aedes aegypti* trypsin modulating oostatic factor, TMOF) are transported at low levels across the insect gut epithelium [29], the impact of misexpression of the majority of these insecticidal agents has been assessed through the use of recombinant baculoviruses as delivery vehicles (reviewed in [30,31]). The target specificity of these naturally occurring arthropod-derived proteins, peptides, and toxins is particularly appealing for the development of novel pest management technologies if appropriate delivery systems can be devised (Box 1).

Box 1. Barriers to delivery of peptide toxins

Insect cuticle: The insect cuticle (an apolar lipid matrix), which covers the exterior of the insect as well as the fore- and hindgut, presents a major barrier to the direct application of insecticidal peptides for pest management. The development of neuropeptide analogs that can be directly delivered through the insect cuticle holds promise as a method for overcoming this obstacle [58], and the use of entomopathogenic fungi for toxin delivery via the cuticle has been demonstrated [57].

Peritrophic membrane (PM): The PM, composed of chitin and proteins, that lines the midgut of many insects serves to protect the midgut epithelium from mechanical damage and provides a barrier against pathogens, such as baculoviruses. Pores in the lepidopteran PM range from 21 to 29 nm and passage across the PM is driven primarily by hydrostatic forces. Although this membrane is not thought to present a significant barrier to the movement of most proteins and peptides from the gut lumen to the surface of the epithelial cells, coexpression of the *Aed. aegypti* TMOF with a baculovirus-derived chitinase that disrupts the PM had a significantly greater impact on larvae of the tobacco budworm, *Heliothis virescens*, compared to lines expressing the transgenes separately [29].

Stability in the gut: Although insect neuropeptides such as kinins, pheromone-biosynthesis-activating neuropeptide, and allatostatin, have potential for use in pest management, the rapid degradation of such peptides by proteases in the insect gut and hemolymph presents a major obstacle [59]. Peptidase-resistant analogs made through production of biostable analogs or polyethylene glycol polymer conjugates of the insect kinins have been developed to enhance peptide stability, and resulted in pyrokinin-mediated antifeedant activity and mortality in the pea aphid, *Acyrthosiphon pisum* [58].

Protein removal from the hemocoel: Once in the hemocoel, insecticidal peptides may be removed by the pericardial cells or degraded by proteolytic enzymes. The pericardial cells are specialized cells involved in regulation of hemolymph composition. These cells synthesize and secrete some hemolymph proteins while actively removing others via filtration and receptor-mediated endocytosis (e.g., lysozyme, horseradish peroxidase, hemoglobin, ferritin, and juvenile hormone esterase). Novel insecticidal peptide or toxin fusion proteins active within the hemocoel also risk clearance by pericardial cells from the hemolymph. The determinants for endocytosis into the pericardial cells are largely unknown, thus, the potential for clearance of any given fusion protein has to be tested empirically.

Potential carrier proteins: proteins that move from the insect gut into the hemocoel

Numerous papers describe the movement of a diverse range of proteins from the insect gut into the hemocoel in a broad range of arthropods (Table 1, Box 2) [32]. These proteins and peptides range widely in molecular mass and include bovine serum albumin (BSA), immunoglobulins (IgG), and teratocyte-secreted protein (TSP)14. Some of the proteins that transcytose across the gut epithelium of insects (e.g., IgG, albumin, and horse radish peroxidase), also transcytose across mammalian epithelial cells.

Analysis of the mechanisms underlying protein transepithelial transport in insects has been facilitated by use of isolated midgut epithelia of *Bombyx mori* in conventional Ussing chambers, along with the use of fluorescent probes and confocal microscopy to distinguish between transcellular and paracellular transport pathways [29]. These analyses confirm that the efficiency of transport of these proteins tends to be low. For example, about 1% of BSA is transcytosed with the majority targeted to lysosomes in the silkworm, *B. mori* [26].

Lectins as peptide transport vehicles

Lectins are carbohydrate-binding and protease-resistant proteins that are widely distributed in animals, plants, and microorganisms [33]. These proteins carry out various biological functions by binding reversibly to specific monosaccharides or complex glycans through noncatalytic domains. In plants, lectins play an important role in defense against insect herbivores and a broad spectrum of plant lectins has been tested for insecticidal activity against agriculturally important lepidopteran, coleopteran, dipteran, and hemipteran pests [34–36]. Lectins negatively affect multiple physiological processes by binding to glycoproteins in the gut membrane. Along with binding to the insect gut, certain plant lectins such as the snowdrop lectin, *Galanthus nivalis* agglutinin (GNA), can pass intact into the insect hemolymph following oral delivery [37]. GNA binds an insect gut membrane receptor glycoprotein, aminopeptidase N [38], which may mediate entry into the cell by receptor-mediated endocytosis, followed by transcytosis of a portion of the endocytosed lectin. In the insect circulatory system, GNA has been detected in hemolymph, Malpighian tubules, fat bodies, ovarioles, and the central nerve cord [39].

The movement of GNA from the gut into the hemocoel provides a mechanism for the effective oral delivery of toxins to their site of action, allowing for exploitation of