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A short note on fusion proteins: An approach for insect-vector management

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Abstract
Management of the insect-vector is very much necessary, as they involved in the transmission of plant viruses which cause heavy loss in the cultivated crops by reducing both growth and yield. The effective management has been achieved through insecticides, but it has great environmental risk. Development of new strategies are gaining much importance over insecticide's application. One such mechanism is the use of fusion proteins viz., spider toxins, plant lectins and virus coat-proteins, as they act on ion channels in nervous system, midgut epithelial cells and as carrier protein respectively. Spider venoms are complex mixtures of neurotoxic peptides, proteins and low molecular mass organic molecules. Their neurotoxic activity is due to the interaction of the venom components with cellular receptors, in particular ion channels. A large number of disulfide-rich (SS) insecticidal peptides have been isolated from spider venoms. Many of these have desirable properties for development as bioinsecticides, including high potency, rapid speed of kill, lack of vertebrate toxicity, low production costs, and activity against a wide range of crop pests and disease vectors. Moreover, they should be stable in the field owing to their SS-rich molecular architecture, and their degradation is unlikely to produce toxic residues. Their major disadvantage relative to chemical insecticides is their lack of contact activity. However, they have a major advantage over chemical insecticides in that transgenes encoding these peptides can be used to engineer insect-resistant plants and enhanced entomopathogens. In the case of GM plants, these transgenes are likely to be synergistic with *Bt*.

Keywords: fusion proteins, toxin, lectins, vector

Introduction

Fusion or chimeric proteins are created through the joining of two or more genes that originally coded for separate proteins. Translation of this *fusion gene* results in a single or multiple polypeptides with functional properties derived from each of the original proteins. Recombinant fusion proteins are created artificially by recombinant DNA technology for use in biological research or therapeutics. Naturally occurring fusion proteins are commonly found in cancer cells, where they may function as onco-proteins [24]. The *bcr-abl* fusion protein is a well-known example of an oncogenic fusion protein, and is considered to be the primary oncogenic driver of chronic myelogenous leukaemia.

Some fusion proteins combine whole peptides and therefore contain all functional domains of the original proteins. However, other fusion proteins, especially those that are naturally occurring, combine only portions of coding sequences and therefore do not maintain the original functions of the parental genes that formed them [17]. A recombinant fusion protein is a protein created through genetic engineering of a fusion gene. This typically involves removing the stop codon from a cDNA sequence coding for the first protein, then appending the cDNA sequence of the second protein in frame through ligation or overlap extension PCR. That DNA sequence will then be expressed by a cell as a single protein. The protein can be engineered to include the full sequence of both original proteins, or only a portion of either. Fusion proteins can be manufactured with toxins or antibodies attached to them in order to study disease development [7]. Fusion proteins can also be manufactured with toxins or antibodies attached to them in order to study disease development.

Sources of fusion proteins
1. Spider toxin
2. Plant lectin

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I. Spider toxin: In recent years there has been renewed interest in natural products as a potential source of novel insecticides because many of the most successful second- and third-generation insecticides are natural products or their derivatives [22]. In particular, there is growing interest in the potential of insecticidal peptides derived from the venom of insect predators such as scorpions and spiders [12].

The ecological advantages conferred by the possession of a venom system are evident from the extraordinarily diverse phyla that have evolved venoms for predation, defence, and competitor deterrence. The extant suite of venomous taxa includes echinoderms, molluscs, vertebrates and arthropods (*e.g.,* ants, bees, centipedes, scorpions, spiders, and wasps) [18]. Spiders are the most successful venomous animals and, with the possible exception of predatory beetles, they are the most abundant terrestrial predators. The remarkable evolutionary success of spiders is due in large part to their ingenious exploitation of silk and the evolution of pharmacologically complex venom that ensures rapid subjugation of prey [1].

The envenomation apparatus of spiders acts like a pressurized hypodermic needle that is capable of delivering controlled microliter doses of venom. The primary purpose of spider venom is to rapidly subdue prey. Based on the biochemical and pharmacological complexity of spider venoms, focusing specifically on the disulfide-rich peptides are the major venom constituents [9].

The venoms of spiders are less well studied than those from other venomous taxa such as marinecone snails, scorpions, and snakes. Venom components from only 174 ($\sim$0.4%) of the 43,244 extant species catalogued to date have been described. However, the taxonomic coverage is better than these numbers suggest, as these 174 spiders belong to 32 (30%) of the 109 extant families of venomous spiders [4]. Thus, despite the paucity of research on this successful group of animals, we can still draw some general conclusions about the chemical composition and pharmacological diversity of their venoms.

The venom compounds can be broadly grouped into five classes on the basis of their chemical structure and mechanism of action. Each of these toxin classes were studied in context of insecticide development [15].

1. a. Salts and Small Organic Compounds: a very low concentration of Na+ ($\sim$10 mM) and a high concentration of K+ (70–200 mM) which is the inverse of the Na+/K+ ratio in the hemolymph of most insects. The high K+ concentration may contribute to venom toxicity by causing depolarization of axonal fibers in the vicinity of the venom injection site, as proposed for the K+-rich prevenom in scorpions [3]. A wide range of small organic compounds ($<$1kDa) including aminoacids (e.g., GABA, glutamate, and taurine), acylpolyamines, biogenic amines (e.g., histamine and octopamine), neurotransmitters (e.g., acetylcholine), nucleosides (e.g., adenosine), and nucleotides (e.g., ATP) were present. None of these compounds have been seriously pursued as insecticide leads [2].

1. b. Cytolytic Peptides: Peptides (defined as proteins less than 10 kDa) are the dominant components of most spider venoms and the primary source of their pharmacological diversity. Proteomic analyses have revealed that some spider venoms contain more than 1,000 unique peptides. Thus, as a group spider venoms might contain as many as 20 million bioactive peptides based on a conservative estimate of 200 peptides per venom and 100,000 extant species [5]. This incredible chemical diversity is one reason why spider venoms are widely used as natural sources for drug and insecticide discovery programs. These peptides have not been used as insecticide leads owing to their intrinsically weak insecticidal activity and their lack of selectivity; most of them are hemolytic and broadly cytolytic in both vertebrates and invertebrates, with many having antimicrobial activity [2].

1. c. Disulfide-Rich Peptide Neurotoxins: these are the dominant compounds in most spider venoms and they are the major contributors to the venom's insecticidal activity [8]. Only a few atypical spider venoms, such as those from widow spiders (*Latrodectus* sp.), which contain a large proportion of large pre synaptic neurotoxins, and the hobo spider *Tegenaria agrestis*, in which sulfated nucleosides constitute approximately 50% of the venom dry weight [3]. However, even the venoms of sicariid spiders, which are better known for containing sphingomyelinase A (SMase A), the cause of dermonecrotic lesions in humans, are richly populated with SS-rich peptides. In terms of insecticidal activity, the SS-rich peptide neurotoxins are typically at least 10-folds more potent than most cytolytic venom peptides. Individually or in combination, these peptides can either deaden the insect nervous system and cause flaccid paralysis or over activate the nervous system and cause convulsive paralysis [13].

The range of pharmacologies exhibited by the SS-rich neurotoxins is extraordinary and includes lectins, protease inhibitors and modulators of transient receptor potential (TRP) channels, mechanosensitive channels, acid-sensing ion channels, ionotrophic glutamate receptors, glutamate transporters, calcium-activated potassium (KCa) channels, voltage-gated calcium (CaV) channels, voltage-gated sodium (NaV) channels, and voltage-gated potassium (KV) channels [7].

1. d. Enzymes: A number of enzymes, including collagenase, hyaluronidase, phospholipase A2, SMase A, andvarious proteases, are present in spider venoms. It has been proposed that their primary role is to degrade extracellular matrix (collagenase, hyaluronidase, proteases) and the underlying cell membrane (SMase A, phospholipaseA2) in envenomated prey to facilitate the spread of peptide neurotoxins [14]. Hyaluronidase is the most commonly reported enzyme in spider venom. It is used medically in conjunction with other drugs to speed their dispersion, consistent with its proposed role as a spreading factor in the venom of vertebrate predators such as snakes. However, because hyaluronan is found only in vertebrates and bacteria, hyaluronidase is unlikely to play a key role in predation of invertebrates. Rather, like its counterpart in hymenopteran venoms, spider venom hyaluronidase is probably used primarily for defensive envenomations against vertebrate predators, where it can act both as a spreading factor and as an allergen [8].

1. e. Large Presynaptic Neurotoxins: A notable exception occurs in the venoms of widow spiders (*Latrodectus* spp.), which contain a family of high molecular-weight proteins known as latrotoxins. These 110- to 140-kDa proteins have a similar domain architecture that consists of a unique N-terminal region and a C-terminal region composed of 13 to 22 ankyrin repeats [10]. For example, the venom of the European black widow spider, *Latrodectus tredecimguttatus*, contains a vertebrate-specific α-latrotoxin, a crustacean-specific α-latrocrustatoxin, and insect-specific α-,β-, γ-, δ-, and ε-

latroinsectotoxins.α-Latrotoxin is widely used as a pharmacological tool because of its ability to induce massive neurotransmitter release from vertebrate nerve terminals. The latro-insecto-toxins are the most potent insecticidal toxins isolated from spider venoms, with extremely low 50% lethal dose (LD50) values of less than 1 pmol g−1 in both lepidopterans and dipterans [10]. However, they have not been pursued as bio-insecticide leads because of their large size, complex mode of action and the difficulty in producing by using synthetic or recombinant methods.
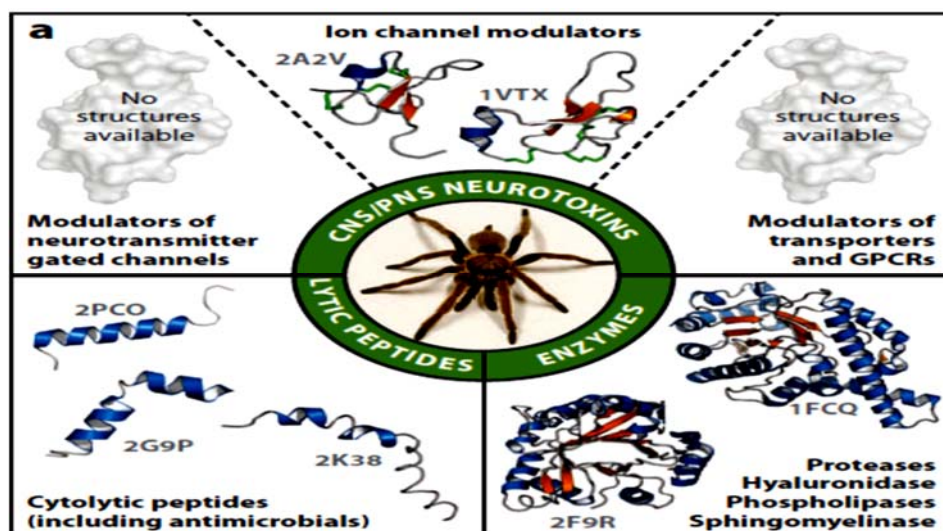

Fig 1: Mode of action of spider toxins

Toxin Cabals: The concept of toxin cabals was first introduced by Baldomero Olivera to explain how groups of venom peptides from marine cone snails could act synergistically to enhance venom potency. Spider venoms also contain toxin cabals that differ in their time and site of action. For example, both δ- and κ-toxins are present in the venom of the Chinese earth tiger tarantula (*Chilobrachys guangxiensis*) indicating that at least some spider venoms might contain lightning-strike cabals [9] Australian funnel webspiders (*Atrax* and *Hadronyche*) contain two different peptidic blockers of insect CaV channels, which ostensibly appear redundant. However, they have different sites and time courses of action. One of these toxins causes almost instantaneous paralysis. Moreover, certain venom components that induce pain such as ATP, histamine, serotonin, and SS-rich peptides that activate TRPV1 are likely to have a mainly defensive purpose (Fig. 1).

Spider venoms contain hundreds of related peptides (paralogs) that evolved by a process of massive gene duplication and divergence [14] Hence, spider venoms can be viewed as combinatorial peptide libraries that have been optimized for prey capture over the course of hundreds of millions of years of evolution. Spider-venom peptides strictly adhere to a one gene-one peptide paradigm [23] There is no evidence for expansion of their toxin repertoire by alternative splicing or production of multitoxin transcripts, in striking contrast to the tandem genetic architecture employed by arthropods to produce families of endogenous neuropeptides [6]

II. Plant lectins: Lectins are carbohydrate-binding proteins that bind glycans of glycoproteins, glycolipids, or polysaccharides with high affinity. Most of the plant lectins are secretory proteins, meaning that they enter the secretory system and subsequently accumulate either in vacuoles or in the cell wall and intercellular spaces. Although many roles have been proposed for plant lectins, the most likely function for vacuolar lectins is plant defence. As far as insects are concerned, toxic effects appear to be mediated through binding of the lectins to the midgut epithelial cells with consequent disruption of the cell function. The bound lectins may inhibit nutrient absorption or disrupt midgut cells by stimulating endocytosis of the lectins, and possibly other toxic metabolites present in the midgut. Various lectins have been proved toxic towards members of the Coleoptera, Lepidoptera and Diptera.

Most importantly, lectins can be used to control sap-sucking insects belonging to the order Homoptera, which includes some of the most devastating pests worldwide. Crop damage caused by these insects is not only due to feeding, but also to their role as vectors of plant viruses. [19] By insect feeding trials, significant anti-metabolic effects of the lectins *Galanthus nivalis* agglutinin (GNA) and wheat germ agglutinin (WGA) to sucking insect pest *Nilaparvata lugens* of rice. Studies of the antimetabolic effect of GNA were extended to the aphids *Myzus persicae* and *Aulacorthum solani*.

Transgenic plants expressing insect resistance genes 148 *Sitobion avenae*. Transgenic plants expressing GNA (tobacco, potato and wheat) caused significantly reduced parthenogenetic fecundity, but only marginal or none decrease in aphid survival. Lectins from species other than *Galanthus nivalis* also possess insecticidal activity. [16] Pea lectin in oilseed rape and obtained a significant reduction in pollen beetle larval weight, and small but significant reduction of larval survival. [11] Onion leaf lectin gene expressed in Indian mustard offers protection against aphid colonization. All these results show that lectins from different origins have a potential to be exploited in crop protection as transgenic resistance factors against various insect pests.

Effects of lectins on insect behavior
For a lot of insecticidal compounds, insects have evolved to start avoiding these compounds either at first encounter (based on a direct gustatory or olfactory response) or after some degree of intoxication. Since plant lectins can cause

increased mortality or adverse effects on development or fecundity of insects, their effects on feeding behaviour have received some attention over the past ten years. Another argument for the investigation of the effect of lectins on feeding behaviour is that taste and olfactory receptors, being integral membrane spanning proteins, are most likely glycosylated (Fig. 2).

II. a. Lectins can alter insect feeding behaviour: Effects of plant lectins on food-choice behaviour have specifically been observed in several studies. [20] Sharp drop in feeding activity (measured by honeydew production) of *Nilaparvata lugens* adults when fed GNA through artificial diet, but no effect was seen for PSA. *L. oleracea* larvae have been reported to consume less when fed an artificial diet containing GNA. When put on transgenic potato plants expressing GNA, this decrease in consumption (leading to less leaf damage) persisted. However, in an assay using excised leaf material, larvae tended to eat more of the GNA containing leaf. Since lectin concentrations in the excised leaves in this study were rather low (0.07% of total soluble protein), larvae might still be able to counteract any adverse effect of the lectin on their growth or development through compensatory feeding.

II. b. Lectins can alter oviposition choice: [21] Oviposition choice of the pea bruchid *Callosobruchus maculatus* was influenced by coating normal chickpeas with different lectins with varying specificities. All lectins tested decreased the number of eggs deposited on the seeds in a no-choice experiment. In a dual-choice experiment between lectin-coated and BSA-coated seeds, all lectins were reported to have a deterrent effect on oviposition regardless of their carbohydrate specificity. It was clearly shown that carbohydrate-binding capacity was required for this deterrent effect. No significant effect was seen on egg hatching or adult emergence from lectin-coated seeds. This could be due to the fact that the seeds were only coated with the lectin, meaning that once inside, larvae did not encounter any lectin and could develop normally.

II. c. Changes in behaviour do not seem to be sensory mediated: Although an effect of plant lectins on behaviour has been observed in many studies, it is difficult to demonstrate and quantify this effect. If an effect of plant lectins on insect behaviour is observed, it is usually deterrent. In some cases, compensatory feeding can be observed. Most studies agree that the effects of plant lectins on insect behaviour are a consequence of intoxication rather than a direct sensory-mediated response of the insect. The slow response of the insect and the lack of conditioned aversion or preference are the main arguments for this. Also, lectin binding to the insect's digestive tract seems essential for every effect of lectins on insect behaviour.
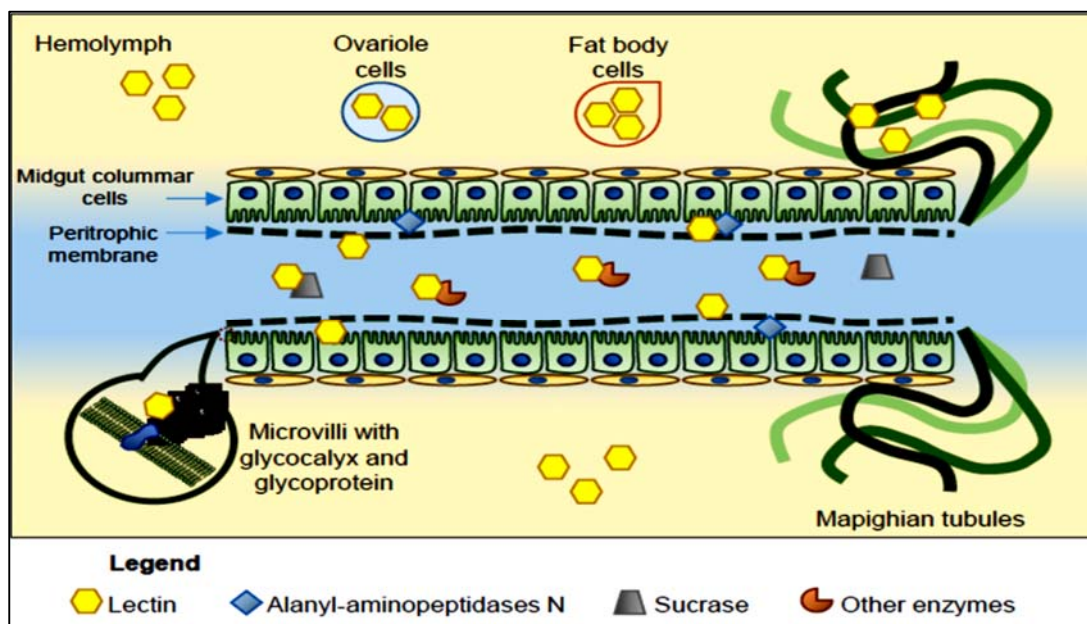

Fig 2: Binding sites of the lectins in insect body

Conclusion
Developing new methods is both an art and the concept for the successful management of insect vectors of plant viruses. Tailoring of some neurotoxic proteins emerging as novel approach for the management of insect vectors. The characterization and understanding of insect neurotoxins obtained from different sources not clearly defined due to their chemical complexity. The presence of a diversified pharmacopoeia in single venom represents an evolutionary advantage for a predator, resulting in the highly efficient capture of diverse prey. For the biologist, spider venoms thus represent an incredibly rich and diversified source of novel molecular tools for the exploration of the physiology of excitable cells, leading to a better understanding of ligand-receptor interactions at the molecular level, and the development of novel therapeutic strategies following nature's lead. So, proper characterization and understanding of these proteins could helpful in the development of transgenic plants which confer higher level of resistance to insect vectors without causing any environmental hazard.

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