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Proteomics in Insecticide Toxicology

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Abstract

Mechanisms of insecticide resistance found in insects may include three general categories. Modified behavioral mechanisms can let the insects avoid the exposure to toxic compounds. The second category is physiological mechanisms such as altered penetration, rapid excretion, lower rate transportation, or increased storage of insecticides by insects. The third category relies on biochemical mechanisms including the insensitivity of target sites to insecticides and enhanced detoxification rate by several detoxifying mechanisms. Insecticides metabolism usually results in the formation of more water-soluble and therefore more readily eliminated, and generally less toxic products to the host insects rather than the parent compounds. The representative detoxifying enzymes are general esterases and monooxygenases that catalyze the toxic compounds to be more water-soluble forms and then secondary metabolism is followed by conjugation reactions including those catalyzed by glutathione S-transferases (GSTs). However, a change in the resistant species is not easily determined and the levels of mRNAs do not necessarily predict the levels of the corresponding proteins in a cell. As genomics understands the expression of most of the genes in an organism after being stressed by toxic compounds, proteomics can determine the global protein changes in a cell. In this present review, it is suggested that the environmental proteomic application may be a good approach to understand the biochemical mechanisms of insecticide resistance in insects and to predict metabolomic changes leading to physiological changes of the resistant species.

Keywords: Insect pest, Resistance, Insecticides, Proteomics

The environmental toxicological studies show the biological effects by pollutants and supports to determine environmental quality. In recent, many biomarkers have been developed to detect the responses of organisms living in the pollutant-contaminated areas. Environmental pollutants from industrial, agricultural, and medical industries undergo anaerobic or aerobic biodegradation by microbes. However, many man-made pollutants are not susceptible to biodegradation and they are accumulated in the environment. Thus, their continued environmental stresses may produce chronic and rapid ecological changes and there is a need to develop the adequate diagnostic method to evaluate the complicated effects of toxicants to the organisms in the environment. Functional genomic studies using bacteria or other living organisms, especially *Drosophila melanogaster*, can evaluate the effects and predict genomic changes by the toxicant after the exposure. The development of molecular biological method for the analysis of *D. melanogaster* genomes has extended to the insects that have not been mapped of their whole genomic DNA sequences[1] (Peter *et al*., 2002). The molecular analytical data of *D. melanogaster* has supported the extensive genetic knowledge to understanding the genetic basis of all biological responses that are expressed after becoming resistant to insecticides.

However, the levels of mRNAs do not necessarily predict the levels of the corresponding proteins in a cell, even if gene microarrays offer the expression of many or all genes in a cell. As genomics understands the expression of most of the genes in an organism after being stressed by toxic compounds, proteomics can determine the global protein changes in a cell. Proteomics is a study to complement the genomic studies. Mass spectrometry has evolved and matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) techniques has been employed in analyzing biomolecules, especially proteins. Using protein separations and mass spectrometry the environmental proteomic studies investigate the relationship between the environmental agents and the proteome, and determine how environmental agents affect cellular proteomes. In this present review, we suggest that the environmental proteomic studies on insecticide resistance in insect pests may be a tool to understand the biochemical mechanisms and to predict metabolomic changes leading to physiological changes of the resistant species to insecti-

cides.

Insecticide Resistance

An insect may employ behavioral strategies or have particular physiological characteristics or modified biochemical mechanisms that enable it to survive in the environments which would be lethal to the normal population. These mechanisms include avoidance behavior; reduced rate of absorption through the exoculticle, increased levels of, and catalytic activity of, enzymes correlated with resistance to insecticides; and decreased sensitivity of the site of action of the insecticides.

Behavioral Resistance

Behavioral resistance is best known in mosquitoes and *Drosophila* and may be very widespread. However, it has received little attention because of the difficulties of its detection and quantification, and the lack of information about normal behavior[2]. Although it is not highlighted, this mechanism may be the sum of total of other mechanisms. Geourghiou[3] suggests that hypersensitive insects which can sense a toxicant before picking up a lethal dose are more likely to develop behavioral resistance, i.e. avoiding the toxicant, than hyposensitive insects from the same population that can tolerate the toxicant through biochemical and physiological adaptations. Thus, this mechanism allows hypersensitive or hyperirritable insects to respond to a much lower concentration of insecticide than normal insects. It may be that the insects which are resistant because they are repelled by the insecticide have receptors which can recognize very small amounts of insecticide better than normal insects[4].

Sparks *et al*.[2] demonstrated that behavioral resistance is usually associated with biochemical and physiological mechanisms. Lockwood *et al*.[5] showed that behavioral resistance occurred in the absence of other biochemical and physiological mechanisms in only 8 percent of the studies reported.

Barson *et al*.[6] described an example where behavioral resistance was not related to biochemical and physiological resistance. They examined the behavioral responses of populations of a multi-insecticide resistant strain and a susceptible strain of *O. surinamensis* to pirimiphos-methyl, an organophosphate insecticide. The adult beetles were confined in pirimiphos-methyl treated or untreated arenas containing a refuge filled with insecticide-free whole or kibbled wheat for a period of 1-7 days. Mortality and the distribution of the beetles between the two range area and arenas were assessed every day. The results showed that when a favorable refuge diet of kibbled wheat was placed in refuge areas there was little difference in mortality between the two strains in areas treated with pirimiphos-methyl, and there was no evidence of a link between physiological resistance or biochemical resistance and behavioral resistance.

Physiological Mechanisms

Physiological mechanisms which can be important in resistance include changes in the rate of absorption of insecticides and altered patterns of insecticide excretion. An example of a decreased absorption mechanism has been described by Little *et al*.[7] in a pyrethroid-resistant strain of the tobacco budworm, *Heliothis virescens*. They examined the absorption rate of trans (1-[^{14}C]cyclopropyl)cypermethrin applied as a topical dose at the third instar of a resistant strain, PEG87, and a susceptible strain, BRC. They observed that it was absorbed at a markedly faster rate in the susceptible strain than in the resistant strain over a 48 h period.

Resistance resulting from a decreased penetration mechanism has been reported in the housefly *Musca domestica*. The *pen* gene on a recessive chromosome 3 controlled the reduction of penetration of insecticides, chlothion, diazinon, DDT, and dieldrin[8,9]. Apperson and Georghiou[10] studied mechanisms of resistance of parathion in *Culex tarsalis*. They suggested that slower penetration of parathion in resistant strains of *C. tarsalis* was one of the resistance mechanisms responsible for a 55.6-fold resistance factor to parathion in these insects. Patil and Guthrie[11] examined the composition of sclerotinized cuticle of three strains of the housefly *M. domestica* including a susceptible strain and two resistant strains, Rugers and Fc, and showed that the phospholipid component in the cuticle was about 2-fold higher in the resistant strains than in the susceptible one. Scott and Georghiou[12] also reported reduced penetration contributed to resistance to permethrin in the housefly, Learn-PyR strain, although detoxification mechanisms were the major contributors to resistance. This type of mechanism was also described in a strain of the German cockroach, *Blattella germinica*, Baygon-R, resistant to propoxur[13]. They detected a decreased penetration of [^{14}C] propoxur after topical application on the cockroach. As in the example of the Learn-PyR housefly strain[12] this reduced penetration is of only secondary or minor importance in the resistance compared with increased detoxification and altered target sites in the insect.

Increased excretion of pyrethroid metabolites has been reported by Little *et al*.[7] as a component of the resistance mechanism of the tobacco budworm, *H. virescens*. In their studies of the metabolism of trans

(1-[^{14}C]cyclopropyl)cypermethrin they detected an increased rate of excretion of radioactivity from resistant larvae in comparison with susceptible larvae. This increased radioactivity excreted was in the form of conjugated products and at 12 h after application of the pyrethroid, the resistant larvae excreted 20 times more of the conjugated products than the susceptible strain.

Biochemical Mechanisms

Biochemical mechanisms relating to resistance are resulted from changes to the target sites of insecticides which affect the interaction of the toxic compound with the site as well as altered metabolism of insecticides. Alterations to the acetylcholiesterase (AChE) enzymes of resistant insects and components of the sodium channels of the neuron system have been studied. Enzymes in detoxifying mechanisms of resistance generally include esterases, glutathione S-transferases, and P450-monooxygenases. However, the enzymes studies in relation to insecticide resistance have been well reviewed by Lee *et al*.[14]. Therefore, alterations in target sites will be considered in this present review for the biochemical mechanisms.

Alteration or Insensitivity of Target Sites

Acetylcholiesterase (AChE). Reduced sensitivity of AChE to organophosphates and carbamates which act by inhibiting AChE is the most common type of alteration of target site. The mechanisms described by Smissaert[15] in the spider mite, *Tetraychus urticae*, has been reported in many insect species including the Colorado potato beetle (*Leptinotarsa decemlinerata*), mosquitoes (*Culex pipiens*), armyworm (*Spodoptera frugiperda*), and the housefly (*M. domestica*)[16-19]. Altered AChE can contribute to the development of cross-resistance to a range of insecticides. Apparent decreased sensitivity of AChE may also occur as a result of an increase in the amount of AChE present in the insect.

Brown and Bryson[20] studied the AChE activity of a methyl-parathion resistant strain, Woodrow SC 1983, and a susceptible strain, Florence SC 1987, of the tobacco budworm, *H. virescens*. The AChE from adult resistant insects was 21-fold and 5-fold less sensitive to methyl paraoxon and ethyl paraoxon respectively than the AChE from the susceptible strain. On the other hand, th enzyme from the susceptible strain was 17 times less susceptible to monocrotophos than that from the resistant strain. The AChE the resistant strain recovered more quickly from inhibition by some compounds than the enzyme from the susceptible strain. It was more active with both acetylthiocholine and butyrylthiocholine and was activated (rather than inhibited) by high substrate concentration. It is possible that the AChE in the resistant strain may be present at higher levels in these insects.

Karunaratne and Plapp[18] studied the biochemistry and genetics of thiocarb resistance in the housefly, *M. domestica*. They found that a pyrethroid resistant strain (Ga) was 30-fold more resistant to thiodicarb than a susceptible strain (AABYS) and that this resistance was only partially relieved by the synergist piperonyl butoxide (PBO). Resistance to another carbamate, carbaryl, was several hundred-fold greater in the Ga strain in comparison with AABYS in the presence of PBO. For both thiodicarb and carbaryl the I50 for the inhibition of AChE was greater in the Ga in comparison with the AABYS ($\times 2.4$ for thiodicarb and $\times 9.1$ for carbaryl). These results suggest that a less sensitive AChE may be a factor in resistance to this carbamate in the Ga strain of houseflies.

Resistance to the organophosphate trichorfon in four strains of the bug, *Lygus hesperus*, was examined in comparison with a susceptible strain[21]. The resistant strains had elevated esterases levels and resistance was substantially reduced in the presence of the syndergist DEF. This indicates a role for esterase in resistance, but these strains were more tolerant to trichlorfon than the susceptible strain even in the presence of the synergist. The reduced sensitivity of the AChE activity from the resistant strains to 10^{-7} M paraoxon suggests that this is also a factor in resistance in these strains of *L. hesperus*.

Bisset *et al*.[22] described experiments with several strains of *C. quinquefasciatus* which suggested that insensitive AChE contributed to resistance to malathion and propoxur. Synergist studies with DEF, which reduced but did not eliminate resistance to malathion, and PBO, which had little effect on resistance to malathion, indicated that esterases were an important but not the only mechanism for resistance in the insects. Assays which demonstrated propoxur-insensitive AChE showed that target-site resistance was a component of the resistance mechanisms in these strains.

Ayad and Georghiou[23] examined parathion resistant (OP-R) and propoxur-resistant (Carb-R) strains of *Anopheles albimanus* and showed that in both strains the AChE was substantially less susceptible to paraoxon (about 400-fold) and propoxur (12,600-fold for the OP-R strain and 7,800-fold for the Carb-R strain). In addition the *Km* value for acetylthiocholine for the AChE from OP-R was 3 times that for the susceptible strain and the *Vmax* was 50 per cent of that in the S strain.

AChE is a membrane-bound enzyme and differences in the phospholipid environment in resistant and sus-

ceptible insects could be important in resistance mechanisms. Chialiang and Devonshire[24] examined an AChE preparation from susceptible and pyrethroid-resistant housflies. Arrhenius plots differed for the enzymes from susceptible (transition temperature 14 ˚C) and the resistant (transition temperature 19˚C, *kdr*, and for 21˚C, s*uper-kdr* strain) insects. After digestion by phospholipase A2, the transition temperature of the Arrhenius plots of the AChE from susceptible insects increased to resemble that of AChE from *kdr* strain, but the Arrhrnius plots of AChE from *super-kdr* strain were unaffected by the digestion. The relevance of these results to the mechanism of pyrehtroid resistance is not clear as it is now known that pyrethroids act at the sodium channel and not by interaction with AChE.

Sodium channel. Knock-down resistance (*kdr*) can be related to changes in the sodium channels in the nervous system. This resistance may be related to a variation in the number of sodium channels or to altered binding capacity of the channels for insecticides.

A reduced density of normal sodium channels in the nerve membrane has been found in resistant strains of *Drosophila melanogster* (Meigen) and *Musca domestica* (L). Kasbekar and Hall[25] used a sub-lethal dose of tetradotoxin as a sodium channel blocker to show that a wild-type strain of *D. melanogaster* with a reduced number of sodium channels revealed resistance to pyrethroid. These data indicate that pyrethroid resistance in the *napts* strain of *D. melanogaster* may be related to the reduction in sodium channel density in the *napts* strain. Bull and Pryor[26] have shown that houseflies resistant to a range of insecticides including permethrin have only 65 percent of the saxitoxin binding sodium channels of the wild-type.

However, Pauron *et al*.[27] demonstrated that a reduced sodium channel density was not an obligatory component of the *kdr* resistance in the housefly. Using tritiated saxitoxin binding studies they have shown that the susceptible strains, OMS and NAIDM, and resistant strains, *super-kdr* and Learn-Pyr, have very similar sodium channel density. Pyrethroids have a synergisitic effect on the binding of the sodium channel toxin, batrachotoxin. When the binding of [^{3}H] batrachotoxin A 20α-benzoate ([^{3}H] BTX-B) was examined in the susceptible and resistant housefly strains in the presence of deltamethrin the binding of [^{3}H] BTX-B was much lower in the resistant strains. These results suggest that a modification of the pyrethroid binding site or a modification of its allosteric linkage to the batrachotoxin binding site on the sodium channel is responsible for the resistance mechanism in the pyrethroid-resistant flies. Amichot *et al*.[28] also examined the synergistic effect between [^{3}H] BTX-B and deltamethrin in pyrethroid resistant and susceptible *D. melanogaster* strains with the same number of sodium channels. They concluded that resistance resulted from a modification of the affinity of the pyrethroid for the receptor site on the sodium channel. They determined that the apparent affinity of deltamethrin with $K_{0.5app}$ (half-maximal stimulation) for [^{3}H] BTX-B binding on the sodium channel was 0.8 μM for the susceptible strain (Tub) and 5.5 μM for the resistant strain (TDDT).

Pepper and Osborne[29] showed that in comparison with susceptible strain larvae of two knockdown-resistant strains (*kdr, super-kdr*) of *M. domestica* were 10 times less sensitive to deltamethrin at segmental nerves, and 30 times (*kdr*) strain and 10,000 times (*super-kdr*) less sensitive at neuromuscular junctions. They suggested that insensitivity at a calcium-activated phosphorylation site may contribute to these differences.

Molecular biological studies support a relationship between an alteration of the binding site of pyrethroid on the sodium channel and the *kdr* resistant trait. Dong and Scott[30] isolated a 120 bp DNA fragment of the *para*-homologous sodium channel gene from German cockroaches. They used this fragment as a probe and identified a RFLP of the *para*-homologous sodium channel gene between a susceptible (CSMA) and a resistant (Ectiban-R) strain. RFLP analysis of F2 and backcross cockroach populations showed no recombination between the *kdr*-type (Ectiban-R) resistance locus and the CSMA sodium channel gene, suggesting that the modification of the *para*-homologous sodium channel is associated with the *kdr* mechanism in this species. Similar results have been reported for *M. domestica*[31] and *H. virescens*[32] to show that the *kdr* mechanism is associated with a mutation at or near a voltage-dependent sodium channel gene.

In *M. domestica* a single gene on the third chromosome was identified as responsible for permethrin resistance, and is probably allelic to the *kdr* gene[33]. Amichot *et al*.[28] found that in *D. melanogaster* the *kdr* resistance is linked to the second chromosome where a sodium channel gene, *sch*, is located. Analysis of this gene showed that an aspartic acid residue in the susceptible (Tub) strain gene product is replaced by an asparagine residue in the resistant (TDDT) strain gene product.

γ-aminobutyric acid (GABA) receptors. Lindane and cyclodiene resistance is generally related to modification of the binding site on the GABA receptor of the neuron membrane. Studies on mechanisms of resistance in lindane-cyclodiene resistant laboratory

strains of the rust red flour beetle *Tribolium castaneum* (Herbst) showed that differences in nerve sensitivity were the main factors contributing to resistance. In a strain ninety times more resistant to dieldrin than a susceptible strain, there was much less response by nerve tissue to dieldrin treatment than in the susceptible strain[34].

Proteomics in insecticide toxicology. A crucial determinant in proteomics is the accurate quantification of differences in protein expression levels. For the determination of quantificational differences, several methods have been developed as the target proteins are labeled with stable isotopes using thiol-reactive ICAT reagents. To tag the proteins two comparable protein samples are preserved and leave to react with the regents. The tags are chemically identical, except one has heavy isotopes and the other has light isotopes. Then, samples are under digestion with digestive proteases and the productive peptides are analyzed by MS instrumentation. Analysis of the MS -MS data allows measurement of the ratio of the light- and heavy-isotope tagged peptides. This credit technique can be applied to the comparative quantification of the different strains of one insect species resistant to insecticides. Recently, it is reported that an easy, interesting quantitative analysis of proteins using stable isotopes by Krijgsveld *et al*.[35]. They reported that the quantitative ^{15}N metabolic labeling of *D. melanogaster* achieved by feeding them on ^{15}N-labeled *Escherichia coli* and yeast, respectively. The relative abundance of individual proteins obtained from different samples were determined by MS. This methodology provides tools for accurate quantitative proteomic studies in these model organisms.

Another possible application of proteomics is on the mapping of protein modification such as phosphorylation and deletion or insertion of amino acid. For the detection of protein modification, two methods including antibodies and site-directed mutagenesis have been widely used. However, these methods can not determine the proper site of modification and can never be avoided from the possibilities that the amino acid substitutions used in site-directed mutagenesis change some other aspect of the system. The MS instrumentation contributes to characterize the modification of proteins and the proper site in the peptides produced. However, if we use MALDI-TOF to obtain MS/MS spectra for the peptides, it is not possible to find the proper site of modification. Liquid Chromatograpy-MS/MS (LC-MS/MS) can deduce the exact characterization of the modification in the proteins. In insecticide resistance, some of target sites of insecticides are point-mutated. For example, resistance to insecticides among mosquitoes as vectors for malaria (*Anopheles gambiae*) and West Nile virus (*Culex pipiens*) is frequently due to a loss of sensitivity of the insect's acetylcholinesterase to organophosphates and carbamates, resulting from a single amino-acid substitution in the enzyme[36]. Therefore, the proteomic technique can apply on the insecticide toxicology and then figure out what happens in the insect cell after being resistant to insecticides or which insect proteins interact to the insecticides as their insecticidial mode of action.

Recently, few studies in insecticide toxicology employed proteomic techniques and they identified some proteins that involved in the resistance mechanisms in the insects. Sharma *et al*.[37] investigated toxicity in the brown planthopper to *o-sec*-butylphenyl methylcarbamate compound (BPMC), using a differential proteomics approach of identifying proteins on two dimensional-polyacrylamide gel electrophoresis (2D-PAGE). The brown planthopper *Nilaparvata lugens* is a serious pest of rice crop in the temperate and tropical regions of Asia and Australia[38]. BPMC is one of the most popular carbamate compound used commercially to control planthoppers. After BPMC treatment, the modulation of 22 proteins at the expression level was found. Compared to control samples, the BPMC-treated brown planthopper showed ten elevated proteins expression, eight decreased proteins expression, and four specific proteins that were observed only after BPMC treatment. Further proteomic studies were undertaken to identify the changed proteins with N-terminal and internal sequence validation and, then, the exposure of BPMC to the brown planthopper caused the increased expression of putative serine/threonine protein kinase, paramyosin, HSP 90, β-tubuline, calreticulin, ATP synthase, actin and tropomyosin, while the expression of β-mitochondrial processing peptidase, dihydrolipoamide dehydrogenase, enolase and acyl-coA dehydrogenase was reduced, which reflects the overall change in cellular structure and metabolism after insecticide treatment.

Azadirachtin that is widely used as biorational botanical insecticide is a mixture of several structurally related tetranortriterpenoids isolated from the seeds of the neem tree (Azadirachta indica)[39-41]. Azadirachtin can affect more than 200 species of insect pests due to its anti-properties including reduction of feeding, suspension of molting, death of larvae and pupae and, sterility of emerged adults in a dose-dependent manner[42,43]. It has been reported that protein expression can be lowered as insect exposes to diets containing azadirachtin or injecting larvae with azadirachtin[44,45]. *Spodoptera litura* (F.) is regarded as a very good target for the applications of azadirachtin during

the seedling stage, especially in upland rice[46]. It is polyphagous and has about 150 host species[47]. Huang *et al.*[42] applied proteomic techniques to study changes in protein metabolism of the pupae of *Spodoptera litura* (F.) induced by azadirachtin of 1 ppm. According to the separation of proteins from females by 2D-PAGE, 10 proteins of females were found to be significantly affected by azadirachtin treatment. Of these, 7 proteins were not present or non-detectable in azadirachtin treated female pupae. Three new proteins, however, appeared as a result of treatment with azadirachtin. They also identified six of these proteins successfully by MALDI-TOF-MS on the basis of peptide mass matching. These proteins are involved in various cellular functions. One identified protein that was not detected after azadirachtin treatment may function as an ecdyson receptor. It indicates a potential interaction between azadirachtin and the ecdysteroid hormonal system, which can cause a major disruption to the growth and development of an insect. Many researchers have been noted that a delay or a permanent block of molting by exposing insects to azadirachtin might be ascribed to the reduction of ecdysteroid titer associated with regulation of metamorphosis, reproduction, and molting[48-52].

The toxocity of Bt insecticides are highly specific to lepidopteran larvae[53] and they are water-soluble and enzymatically processed to be active toxins that bind to a protein (s) located in *Manduca sexta* midgut brush border epithelium[53], ultimately a toxin-induced lysis of susceptible midgut epithelial cells[54]. Aminopeptidase N purified from *Manduca sexta* has been identified as a Cry1Ac toxin biding protein[55-57]. BT-R1 and BtR175 that are cadherin-like proteins were also identified as a Cry1 biding proteins in *M. sexta*[58] and in *Bombyx mori* brush border membrane vesicle (BBMV), respectively[59]. Expression of BT-R_1 in vertebrate COS-7 cells resulted in Cry1Ab binding, leading to the gross morphological changes when cells were exposed to Cry1Ab[60]. Expression of BtR 175 resulted in Cry1Aa induced osmotic flux across the membrane, entailing dramatic changes in membrane currents[59]. In addition, when BtR 175 was expressed in Sf9 cells, gross cell morphology and cell swelling occurred due to exposure to Cry Aa toxin[59].

Candas *et al.*[61] investigated differences between *B. thuringiensis-susceptible and -resistant* Indianmeal moth, *Plodia interpunctella* by using 2DE. They reported that the levels of glutathione transferase, cytochrome c oxidase subunit 1, and NADH dehydrogenase subunit 5 were higher in resistant insects than in susceptible ones. It indicates that there is a shift in the redox state of the midgut epithelial cells. On the other hand, chymotrypsin activity decreased in resistant animals. It may affect processing of these altered proteins, processing of toxin, or both. They also observed a shift in both charge and size of an ortholog of mitochondria F_1F_0-ATPase subunit δ between Bt insecticide-susceptible and -resistant *P. interpunctella*.

McNall and Adang[62] used a combination of mass spectrometry and blot analyses in conjunction with 2D-PAGE to investigate two subsets of *M. sexta* BBMV proteins: *B. thuringiensis* Cry1Ac binding proteins and glycosylphosphatidyl inositol (GPI)-anchored proteins. In their study, alkaline phosphatase and actin were identified and confirmed through western blots as novel proteins that bind Cry1Ac in addition to the previously reported aminopeptidase N. Aminopeptidase N was the only GIP-anchored protein identified. According to Mooseker[63], actin interaction with *B. thuringiensis* toxin may result in the distruption of its normal function in the cytoskeleton. In *M. sexta* skeletal muscle, a reduction in myosin and actin expression affected the time of cell death[64].

It is suggested that the new analytical approaches such as proteomics are very useful to identify interacting proteins with insecticide and characterize modifications of the target-site proteins in insecticide -resistant insects, and the other amenable application of proteomics is to characterize changes in protein expression of the insects in response to insecticide toxicology.

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