



Characteristics of protein variants in trichlorphon-resistant *Bactrocera dorsalis* (Diptera; Tephritidae) larvae

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ABSTRACT. Functional proteins in larvae of *Bactrocera dorsalis*, a major fruit pest, play a central role in their resistance to organophosphorus insecticides. Changes in proteins in *B. dorsalis* larvae after trichlorphon treatment may have a role in the resistance response to trichlorphon. We analyzed 14 protein spots of crude proteins from *B. dorsalis* larvae post-treatment with trichlorphon in two-dimensional gel electrophoresis through mass spectrometry and protein sequencing. We found functional proteins that are responsible for signal transduction (pkaap and dual specificity tyrosine-phosphorylation-regulated kinase), immunity (hemolectin), synthesis and decomposition (twinstar, cathepsin B,

RE66325p), oxidative stress metabolism (glutathione S transferase and CG7320), energy metabolism (Act57B), and cytoskeleton formation (actin). These proteins appear to be involved in the resistance response to trichlorphon.

Key words: Insecticide resistance; *Bactrocera dorsalis*; Trichlorphon; Two-dimensional gel electrophoresis; Mass spectrometry

INTRODUCTION

The resistance of insects to pesticides is one of the most serious problems in crop protection. Organophosphorus compounds are extensively used for pest control due to their broad-spectrum toxicity and potent eradication of the target insect pests in the past decades. In general, one function is to inhibit acetylcholinesterase with subsequent accumulation of acetylcholine at nerve endings. The specific change in the structure of acetylcholinesterase can lead to resistance. The mutations in the gene (*ace*) encoding the acetylcholinesterase are associated with resistance to organophosphorus pesticides (Fournier et al., 1992). Another function is to induce delayed polyneuropathy through organophosphorus pesticides, which is related to the inhibition of esterase activity of neuropathy targets (Johnson, 1982). In addition, the metabolic mechanisms of insect reaction to organophosphorus compounds are involved in antioxidant defense and lipid peroxidation in cytotoxicity (Ranson et al., 2002; Akhgari et al., 2003). However, the correlation between the resistance response of insects after organophosphorus compound treatment and the change in protein expression level is still limited.

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the most notorious pests of tropical and subtropical fruits and vegetables (Steiner, 1957; Ye and Liu, 2005). It was documented in Taiwan in 1912 for the first time, and later observed in most Asian-Pacific regions (Steiner, 1957). Due to internal feeding by the larvae and adult traits including high mobility and spread capacity, high fecundity, and strong flying behavior, *B. dorsalis* is considered as a fanged pest in Southeast Asia. Broad-spectrum insecticides have been used to control *B. dorsalis*, especially organophosphorus pesticides. On mainland China, at least 10 colonies of *B. dorsalis* collected from the field exhibited middle-level resistance to trichlorphon (organophosphorus insecticide) (Jin et al., 2011). The resistance rate of the laboratory strain to trichlorphon is equal or higher than that to cypermethrin (pyrethroid pesticide) and avermectin (microbial pesticide) (Pan et al., 2008). This result indicates that the susceptibility of *B. dorsalis* to organophosphorus compounds is unstable.

Two-dimensional (2-DE) gel electrophoresis and mass spectrometry (MS) can provide powerful characterization of the proteins of interest. Recently, the change in protein variants in insects induced by infections, such as parasitoid attack, and chemical compound toxicity, has been investigated by using the 2-DE method (Candas et al., 2003; Huang et al., 2004; Nguyen et al., 2008; Song et al., 2008). The identification of proteins in the oriental fruit fly at different periods after insecticide application will provide more useful information of biologically important molecules to explore trichlorphon toxicity. In the present study, our objective was to explore the response of *B. dorsalis* to trichlorphon

exposure at the protein level for comparing physiological and metabolic changes after different induction time. Fourteen specific proteins expressed in the larvae of *B. dorsalis* were separated and identified. Consequently, the identified proteins were classified into six functional groups. These results will extend the further understanding of the repertoire of functional molecules with the toxicity of organophosphorus pesticides.

MATERIAL AND METHODS

Oriental fruit fly strain

The insect species *B. dorsalis* was originally collected in 2004 from Guangzhou, China. Approximately 1500 larvae were collected from carambola and transported to the insect rearing room. A banana and maize-based artificial diet (containing 150 g corn gain, 150 g banana, 0.6 g sodium benzoate, 30 g yeast, 30 g sucrose, 30 g winding paper, 1.2 mL HCl and 300 mL H₂O) was used to feed the insects (Jin et al., 2011). The rearing conditions were 25° ± 1°C, a light period of 16L:8D and 70-80% humidity. In the experiment, trichlorphon was provided by Nantong Agrochemical Co. Inc. (Nantong City, Jiangsu Province, China).

The median lethal concentration (LC₅₀) was determined by the residual contact method. The powder of trichlorphon was diluted in acetone with seven different concentrations. The test solutions for each concentration was 5 mL and used to coat the inner wall of 250-mL conical flasks after rolling the flask until the acetone was evaporated completely. Ten adult flies, 3-5 days old, were placed in each flask in triplicate. Mortality was recorded after trichlorphon treatment for 24 h. The control group was subjected to the same treatment without trichlorphon. Raw bioassay data were analyzed through probit analysis by using Statistical Analysis System (SAS) to calculate LC₅₀ (Stark, 2005). To establish the resistant strain, the third instar larvae were soaked at the working dilutions at the LC₅₀ dose for 2 s and adult flies were treated at the same working dilutions by using residual contact method for 1 h. Surviving adult flies were used for rearing, and the LC₅₀ was then determined every 3 generations. The LC₅₀ was 89.2 mg/L for the F₃₉ generation of *B. dorsalis* after resistance selection for trichlorphon.

Insect treatment with insecticide for protein extraction

The larvae (approximately 100-110 mg body weight) were starved for 2-3 h, and then soaked at 89.2 mg/L trichlorphon solution (LC₅₀) for 2 s. The blank control was resistant strains and treated with pure acetone alone. The behavior of test insects was observed after treatment for 2 h. Surviving larvae were chosen and the insecticide-treated insects were collected at 3, 6 and 12 h. The controls were chosen after treatment for 3 and 6 h. Insect bodies with total weight of 0.15 g were collected from each group.

Sample preparation

For 2D-PAGE, insects were killed and frozen with liquid nitrogen. Insect bodies were homogenized in a lysis buffer containing 8 M urea, 2 M thiourea, 0.02 M Tris base, 30 mM DTT, 4% CHAPS, and 1% IPG buffer, pH 4-7. Chilled mortar and pestle were

used to extract whole proteins. The supernatant after centrifugation at 3000 rpm for 5 min was used as the crude protein sample. After precipitation in TCA/acetone, the protein concentrations were determined (Bradford, 1976). Bovine serum album (BSA) was used as the protein standard.

2-DE and gel staining

A 340- μ L sample (containing 150 μ g protein) was dehydrated on the 18 cm IPG strips (pH 4-7 from Amersham) for 12 h at 20°C and constant voltage of 50 V. Isoelectric focusing (IEF) was carried out at 200 V for 200 Vh, 500 V for 500 Vh, 1000 V for 1000 Vh, and 8000 V for 43,000 Vh at 20°C. Maximum current was set at 50 μ A/strip in an IEF unit from Multiphor (Amersham Biosciences). Following IEF, the IPG strip was equilibrated for 15 min in 375 mM Tris, pH 8.8, containing 6 M urea, 20% (v/v) glycerol, 2% (w/v) SDS, and 130 mM DTT. DTT was replaced with 260 mM iodoacetamide. The IPG strips were then sealed with 1% agarose in SDS running buffer at the top of slab gels (200 x 175 x 1 mm), which were polymerized with mixed acrylamide (10%) and N,N'-methylenebisacrylamide (0.01%). The second-dimensional electrophoresis was performed at 8°C in a vertical electrophoresis bath (at 14 μ A for 1 h, and then 28 μ A for 4 h).

Imaging analysis

Preparative gels were stained with a silver-stain compatible MS/MS protocol (Shevchenko et al., 1996) and scanned by ImageScanner™ (Amersham Biosciences). Three or four repetitions were performed. The ImageMaster™ 2D Platinum version 5.0 software (Amersham, Biosciences) was used for quantitative analysis. Proteins were analyzed in triplicate to confirm overall reproducibility of the protein spot pattern. After choosing one reference gel, the protein spots were matched automatically across gels, and the background was subtracted manually. The protein spots were normalized by using the mean volume of three unchanged spots under two conditions. The selective criteria for these gels were the absence of significant distortion. The confidence threshold for the up- and downregulated protein spots was set at 3- to 4-fold of the spot intensity in anyone treatment when compared to the control.

In-gel digestion and mass spectrometry

Protein spots of interest were excised from the gels. The gel slices were washed with 100 μ L double-distilled H₂O three times, and then destained with 25 μ L 30 mM K₃Fe(CN)₆ and 25 μ L 100 mM Na₂S₂O₃ for 1 min. The gel slices were dehydrated using 100% (w/v) acetonitrile at room temperature. The gel slices were then crushed and dried after 0.02 μ g/ μ L Trypsin (Promega, USA) digestion in 5 μ L 100 mM NH₄HCO₃ solution at 37°C for 16-18 h. The digested peptides were extracted from gel slices with 0.1% trifluoroacetic acid (TFA) in 50% (v/v) acetonitrile/water.

The identification of the isolated proteins was performed using a Finnigan LTQ mass spectrometer (ThermoQuest, San Jose, CA, USA) coupled with a Surveyor HPLC system (Thermo Quest). First, a Microcore RP column (C18: 0.15 x 120 mm; ThermoHypersil, San

Jose, CA, USA) was used to separate the digested proteins, where 0.1% (v/v) formic acid and 0.1% (v/v) formic acid in 100% (v/v) CAN were used as solvent A and B, respectively. The gradient was started at 2% solvent B for 15 min, and increased linearly up to 98% solvent B within 90 min. The peptides were eluted from the C18 microcapillary column at a flow rate of 150 μ L/min, and then electrosprayed directly into the mass spectrometer with the application of a spray voltage of 3.2 kV under the capillary temperature of 170°C. The full scanning range was M/Z 400 to 2000.

Database searching

Protein identification using MS/MS raw data was performed with the SEQUEST software (University of Washington, licensed to Thermo Finnigan) based on the SwissPort database (Tu et al., 2005; Zhou et al., 2007). A relative molecular mass of 57 Da was used for the average molecular mass of cisterns in MS/MS data searching. Both *b* and *y* ions were also included in the database searching. The results of protein identification were filtered with the Xcorr ($1+ \geq 1.9$, $2+ \geq 2.2$, $3+ \geq 3.75$) and DelCn (≥ 0.1).

Identification of protein functions

The searching results were used as a reference to explain the possible functions of proteins (<http://flybase.org> and <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The E-value was less than $1.00E-30$ as the significant hit for selecting an insect protein rather than an equally matched primate protein. The theoretical and experimental pI data were not always consistent and these discrepancies could be attributed to post-translational modifications, multiple protein isoforms, and different protein compositions.

RESULTS

2-DE profiles for protein variants from *B. dorsalis* larvae

Approximately 400 individual spots (corresponding to proteins with molecular sizes ranging from 14 to 100 kDa and isoelectric points from pI 4 to 7) were resolved by 2-DE PAGE, as shown in Figure 1. No significant change in protein expression level was observed in acetone treatment alone for 3 or 6 h. Four gels from the control group and post-treatment for 3, 6 and 12 h were analyzed by the ImageMaster™ 2D Platinum analysis software. Approximately 50 proteins revealed different expression levels between the treatment groups and the control group. Meanwhile, 26 proteins exhibited a significant difference. Among these 26 proteins, 9 proteins with a significantly higher expression level and 5 proteins with a significantly lower expression level were observed in the treatment groups.

Protein identification

To examine the homology of protein variants from different organisms, 14 protein spots were subjected to Q-TOF and MS analysis. The possible functions of these proteins including D3, D4, D5, U2, U3, U4, U7, and U8 were identified through BLAST searching (Table 1).

Table 1. Protein identification in the larvae of *Bactrocera dorsalis* after trichlorophen treatment through mass spectrometry analysis.

Spot No. ^a	NCBI accession	Protein identification ^b	Species origin ^c	Peptide sequence	Possible functions ^d	Theoretical		Observed		Similar protein searched by Blastp	
						MW (kDa)/PI	MW/pI	E-value	Protein name	Possible function	
U1	gi 19921398	Pkaap	<i>Drosophila melanogaster</i>	K.TAINHQSGNLNIDAR.Q K.TALNHQSGNLNIDAR.Q	Protein kinase A binding Protein localization Regulator of G protein signaling	69.7/5.6	22.5/5.4				
U2	gi 193601326	Predicted, similar to GA20020-PA	<i>Acyrtosiphon pisum</i>	R.AYIVVTPLEGG.T	Unknown	393.9/5.3	20.0/5.3	0	Hemolymph coagulation [<i>Drosophila melanogaster</i>]	Hemolymph coagulation Hemostasis	
U3	gi 195425594	GK10641	<i>Drosophila willistoni</i>	K.QIDVETVGDNR K.QIDVETVGDNRNAYDQF LEDIQK.C K.YIQATDLSEASR.E R.NAEYDQFLEDIQK.C R.YYVYRDEK.Q	Unknown	17.3/6.82	13.5/6.0	1E-81	Twinstar [<i>Drosophila melanogaster</i>]	Actin binding	
U4	gi 195135338	GII6624	<i>Drosophila mojavensis</i>	R.ALDDVPPEYK.I	Unknown	24.8/5.1	20.5/6.1	7E-87	Glutathione S transferase E2 [<i>Drosophila melanogaster</i>]	Glutathione transferase activity	
U5	gi 170048987	Dual specificity tyrosine-phosphorylation-regulated kinase	<i>Culex quinquefasciatus</i>	K.IVDFGSSCQLGQR.I	Tyrosine-phosphorylation-regulated kinase	63.5/9.4	23.5/5.9				
U6	gi 161084784	C67320	<i>Drosophila melanogaster</i>	K.GLAGDHISYELYQFVK.L	Oxygen transporter activity	71.8/5.6	24.5/6.1				
U7	gi 195581124	GDI0456	<i>Drosophila simulans</i>	R.ALQLAEGLAEEIRD.R.V	Unknown	30.0/9.9	28.5/6.0				
U8	gi 195438830	GK16235	<i>Drosophila willistoni</i>	R.QSEFTTSTTVAISTEKED TFSITSSPIR.L	Unknown	141.0/5.8	30.5/6.0				
U9	gi 161343859	TPA_inf. cathepsin B	<i>Myzus persicae</i>	R.MCYGNQDLDYR.E	TPA_inf. cathepsin B	29.4/6.3	60.0/6.1				
D1	gi 195480603	Act57B	<i>Drosophila yakuba</i>	K.AGFAGDDAPR.A K.IWHHTFYNELR.V K.LCYVALDFEQEMATAASS SSLEK.S K.SYELPDGQVITIGNERF R.VAPEEHPVLLTEAPLNPK.A	ATP binding Protein binding	41.8/5.3	40.5/5.3				
D2	gi 164451511	Actin	<i>Culex nigripalpus</i>	K.IWHHTFYNELR.V	Housekeeping protein	41.8/5.3	13.5/5.5				
D3	gi 195484806	GE12582	<i>Drosophila yakuba</i>	R.AAVGMLSHGAGGME PAMSR.P	Unknown	145.9/6.3	12.0/5.7	0	REF66325p [<i>Drosophila melanogaster</i>]	Presence of a polyA tail as integrity checks for sequence accuracy	
D4	gi 195474297	GE19106	<i>Drosophila yakuba</i>	R.ALQLAEGLAEEIRD.R.V	Unknown	23.6/9.7	21.5/5.7				
D5	gi 195024355	GH20859	<i>Drosophila grimshawi</i>	K.FPLYEDLVADVHRVLR.K	Unknown	159.5/6.2	31.0/5.7				

^aRefer to the spot numbers in Figure 1. ^bProteins were identified by using MS/MS raw data and analyzed by the SEQUEST software based on the database of Swissport.^cFunctions as described in Flybase (October, 2011) for fully identified gene products or proposed function form others. MW = molecular weight; pI = isoelectric point.

Functional classification of different proteins

A total of 14 spots representing the candidate responsive proteins induced by trichlorophon were identified, as shown in Figure 1. All identified proteins could be classified into six functional groups including signaling, immunity, protein synthesis, oxidative metabolism, energy metabolism, and cytoskeleton. The upregulated and downregulated proteins are shown in Figure 2. However, some proteins, including U7, U8, D4, and D5, could not be identified.

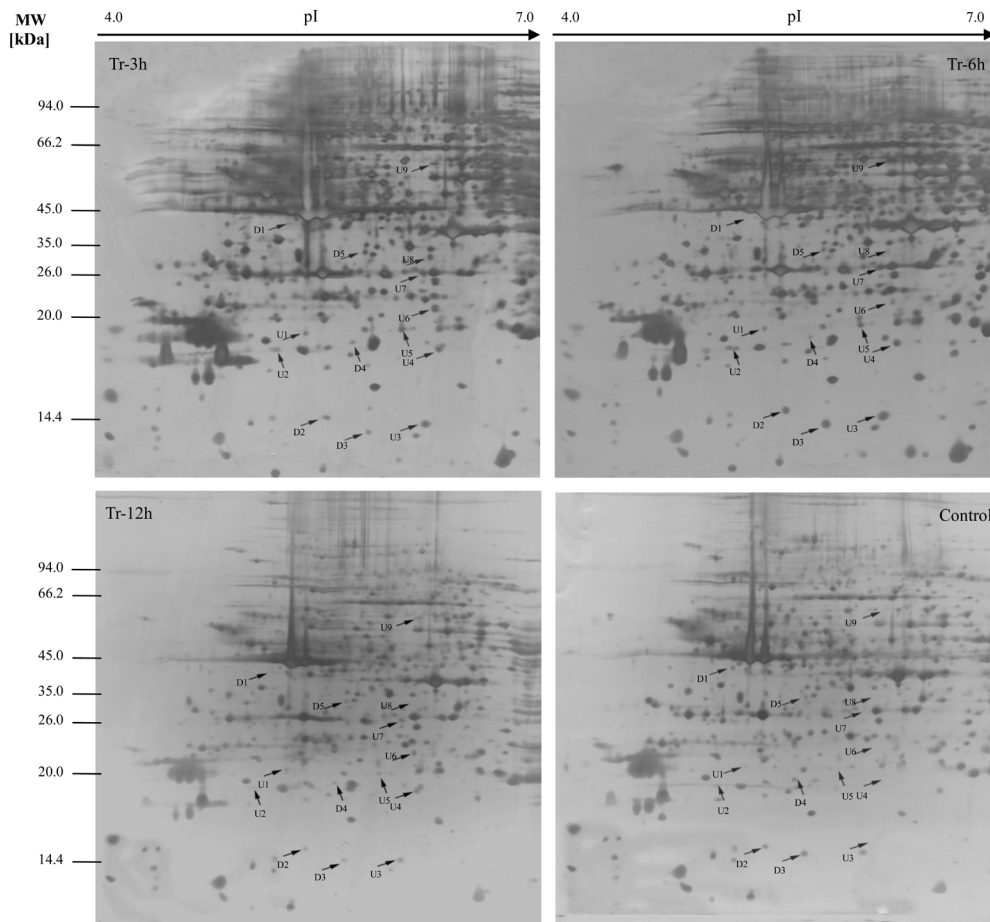


Figure 1. Representative two-dimensional gels revealed the separation of the proteins in *Bactrocera dorsalis* after trichlorophon treatment. The proteins were prepared after trichlorophon treatment at LC_{50} dose for 3 (Tr-3h), 6 (Tr-6h), and 12 h (Tr-12h) as well as acetone treatment alone as the control. The proteins were separated by isoelectric points (pI) in first dimension and then by molecular weights (MW) in the second dimension. Four gels were run on the pH range of 4-7 and the resultant pattern was overlapped. Spots U1-U9 and D1-D5 represent the up-regulated and down-regulated proteins as the response to the toxicity of trichlorophon.

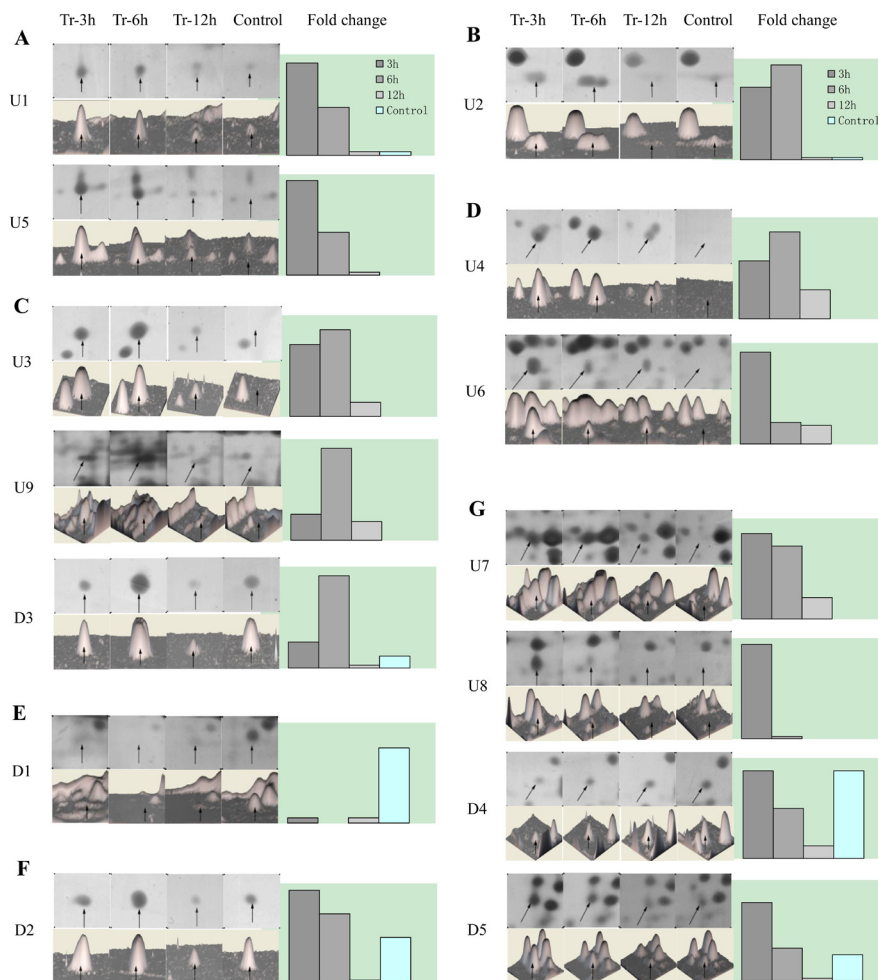


Figure 2. Selected areas of two-dimensional gels and identified spots corresponding to proteins in *Bactrocera dorsalis* after trichlorophon treatment. Image pairs and detection of protein spots with relative spot volumes were analyzed with the ImageMaster™ 2D Platinum software. The protein spots are classified as **A** (signal transduction); **B** (immunity); **C** (protein synthesis and decomposition); **D** (oxidative metabolism); **E** (energy metabolism); **F** (cytoskeleton protein), and **G** (other unknown functions) according to its possible functions or biological processes. For abbreviations, see legend to Figure 1.

DISCUSSION

Insecticide resistance is an inducible process rather than the function of a single enzyme or gene (Daborn et al., 2002; Soderlund and Knipple, 2003). The profiling of protein expression under trichlorophon stress in a time course mode has been recognized as a very useful approach. In the present study, 14 proteins were identified in *B. dorsalis* with metabolism challenge due to the application of organophosphorus pesticides. After trichlorophon treatment, at least six different functional proteins were found to be related to the resistance response.

Signal transduction proteins

The protein labeled U1, encoding pkaap protein, was more abundant in trichlorophon-induced larvae and its expression level was reduced approximately 10-fold 12 h post-treatment when compared to the blank control group. Protein pkaap is one of the signal transduction proteins that can affect G proteins (guanine nucleotide-binding proteins), a family of proteins involved in transmitting chemical signals outside the cells and causing chemical signal changes inside the cells. The signals are used for communication among hormones, neurotransmitters, and other signaling factors related to defensive reaction (Birnbaumer, 1990). The U5 protein is a dual-specificity tyrosine-phosphorylation-regulated kinase (DYRKs) belonging to an emerging family of protein kinases that are widely distributed in all eukaryotic organisms. The DYRK family members are involved in critical signal-regulated developmental and cellular processes, such as neurogenesis, cell proliferation, cytokinesis and cell differentiation (Lochhead et al., 2005; Gwack et al., 2006). DYRK is an antagonist to Ras/cAMP-dependent protein kinase (PKA) that can negatively regulate cell growth (Garrett et al., 1991). In *Drosophila*, the mutation of the DYRK family member, minibrain (*mbn*), can affect post-embryonic neurogenesis, thus resulting in specific reduction in the size of optic lobes and central brain hemispheres (Tejedor et al., 1995). The human orthologue of the minibrain gene, *Dyrk1A*, has been mapped to be a critical region of Down syndrome and is overexpressed in Down syndrome brains, implicating a similar role for this kinase in mammalian neurogenesis as well as signal transduction functions (Guimera et al., 1999). Similarly, our results indicate that pkaap and DYRK may play a signal transduction role in the resistant adaptation to organophosphorus pesticides.

Immunity-related proteins

Protein U2 is identified as a kind of immune protein, which is similar to hemocytin in *Drosophila melanogaster*. Hemocytin is an immune protein found in a variety of living organisms. Its expression level increased approximately 5-fold after trichlorophon treatment for 6 h, suggesting that immune metabolism is altered for detoxification. In addition, this protein may play an immunological role in reinforcing the importance of the clot in insect immunity (Dushay, 2009). *Drosophila* hemolectin gene is expressed in embryonic and larval hemocytes, and its knockdown can cause bleeding defects (Goto et al., 2003). Hemolectin double-stranded RNA exhibits a significant change in *Drosophila* prepupae stage after low-dose irradiation exposure to X-rays (Kanao and Miyachi, 2006). Our results confirmed that hemolectin is upregulated in the larvae of *B. dorsalis* after treatment with trichlorophon. Therefore, hemolectin also plays a role in immune responses through stopping the bleeding in internal organs or tissues with trichlorophon challenges.

Protein synthesis and decomposition

The function of protein U3 is described as binding to actin. It is involved in the biological processes such as anatomical structure development, actin filament-based process, regulation of developmental process, cell projection organization and biogenesis. Cathepsin B (spot U9) is a typical cysteine protease that can cleave dietary proteins into peptides and amino acids. It is primarily expressed in the endosomal/lysosomal compartments, and its functions as

an endopeptidase or dipeptidyl carboxypeptidase are related to the protein degradation process (Koga et al., 1991, Linke et al., 2002). It is well known that insect cathepsin B also participates in many physiological processes. For example, *Bombyx mori* cathepsin B is involved in the programmed cell death of silk glands and results in the histolysis of silk glands during metamorphosis (Shiba et al., 2001). *Helicoverpa armigera* (Noctuidae, Lepidoptera) cathepsin B is involved in the degradation of yolk proteins during embryonic development (Zhao et al., 2005) and decomposition of adult fat bodies (Yang et al., 2006). The expression level of protein D3 is much higher than that expressed in 3 and 6 h after trichlorophon induction. It may be related to protein synthesis or decomposition during protein translation. The above three proteins reveal a lot of changes when compared to the blank control group. These results indicate that *B. dorsalis* can increase its protein synthesis and degradation after exposure to organophosphorus pesticides.

Oxidative metabolism proteins

Two protein spots (U4 and U6) were identified as GST and CG7320 in *D. melanogaster*. These proteins are specifically overexpressed in the larval period by the induction of trichlorophon. GSTs are a family of inducible enzymes that are important for detoxification reactions, thus performing protection for insect cells from insecticides (Hayes and Pulford, 1995). Higher expression levels of GSTs have been determined in chemical insecticide-resistant insect strains, and increased expression levels of carboxylesterases and cytochrome p450s are responsible for metabolic resistance to insecticides (Daborn et al., 2002). Our results proved that GSTs are involved in enhancing resistant metabolism. The protein CG7320, as an oxygen transporter, is involved in the biological process of expanding oxidative metabolism to fight against xenobiotics and pesticide toxicity. Increased oxidative metabolism can be an adaptive response of insects subjected to survival challenge, and can mediate detoxification that enhances the resistance of insects (Indian meal moth *Plodia interpunctella*) to bacteria (*Bacillus thuringiensis*) (Johnson, 1982). Therefore, it was clearly demonstrated that the oxidative metabolism-related proteins can be upregulated upon exposure to trichlorophon.

Energy metabolism-related proteins

Protein D1 is named as Act57B that is down-regulated under the trichlorophon stress. The possible functions of Act57B are ATP activation and protein binding. ATP is activated and then provides organisms energy produced in mitochondria of all living things. ATP binding means energy production, and energy metabolism level is related to resistance response.

Cytoskeleton proteins

The protein actin (spot D2) was detected in *B. dorsalis*. However, it was unstably expressed under trichlorophon stress. Actin is a highly conserved protein that is involved in the generation of contractile force in muscle as well as cell division, motility, and endocytosis and exocytosis in non-muscle eukaryotic cells (Mounier and Sparrow, 1993). Actin is also the component of the cytoskeletal system that allows for the movement of cells and cellular processes. By using a proteomics approach, insect resistance to xenobiotics and pesticides, physical wound,

parasitoid, and microorganism attack can readily cause a change in actin expression (Candas et al., 2003; Huang et al., 2004; Nguyen et al., 2008). Taken together, an increased expression of actin can reinforce a physical barrier to better resistance from further injury.

Other proteins

The protein spots U7, U8, D4, and D5 were identified as GD10456, GK16235, GE19106, and GH20859 in the database. However, these proteins are only confirmed by conceptual translation, molecular functions and biological processes. Their functions are still unknown. However, in the present study, these proteins are regulated in resistance response to trichlorophon exposure, which is of benefit for developing strategies to combat trichlorophon toxicity.

CONCLUSION

Our experimental data demonstrate that proteins related to signaling, immunity, oxidative metabolism, protein synthesis, energy metabolism, and cytoskeleton are regulated in the resistant strains of *B. dorsalis* under conditions of organophosphorus pesticide challenge. Proteomics tools are sensitive enough to untangle proteomic alteration linked to toxic compounds at different time periods. Therefore, the metabolism of oriental fruit flies may be stimulated to generate an adaptation to trichlorophon.

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