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Changes in Esterase Activity and Acetylcholinesterase Sensitivity of Insecticide-selected Strains of the Brown Planthopper (*Nilaparvata lugens* Stål)

저항성 벼멸구의 효소활성 변화에 관한 연구

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ABSTRACT Acetylcholinesterase(AChE) and esterase activities as mechanisms of resistance to fenobucarb, carbofuran and diazinon in the insecticide-selected brown planthopper strains were investigated. Although there was no significant difference in AChE activity from susceptible and resistant strains, AChE insensitivity was highly increased in the carbamate insecticide-selected strains. On the other hand, esterase activity was moderately increased in all the selected strains. It is concluded that the cross-resistance and the level of resistance in the brown planthopper can be explained by the combination of altered AChE and high esterase activity, although a possible involvement of other factor(s) can not be excluded.

KEY WORDS Brown planthopper, resistance, fenobucarb(BPMC), carbofuran, diazinon, acetylcholinesterase, esterase

초 록 벼멸구의 살충제 저항성 기구를 구명하고자 fenobucarb, carbofuran 및 diazinon으로 벼멸구를 14~18세대 누대 도태하여 얻어진 벼멸구를 대상으로 저항성 기구를 조사하였으며, 얻어진 결과중 acetylcholinesterase(AChE)와 esterase의 활성 변화에 대하여 보고하고자 한다. AChE활성은 fenobucarb선발 계통에서 1.6배 증가하였으나 타 계통에서는 차이가 없었고, 세포내 분포도 mitochondrial fraction에서 70% 이상으로 계통간 차이가 없었다. 반면, AChE감수성은 fenobucarb와 carbofuran선발 계통에서 각각의 공시 살충제에 대하여 12.2배, 5.6배 감소하였으나 diazinon선발 계통에서는 diazoxon에 대하여 1.7배 감소에 그쳤다. Esterase활성은 fenobucarb선발계통에서 5.6~6.8배, carbofuran선발계통에서 6.4~7.8배, diazinon선발계통에서 4.0~4.4배 증가하였다. 벼멸구의 저항성 증가에 다른 요인이 관련됨을 배제할 수 없으나, 본 실험 결과 esterase활성증가와 AChE감수성 저하라는 두 요인이 상승적으로 작용하여 벼멸구 저항성을 유발하였음을 확인하였다.

검 색 어 벼멸구, 저항성, fenobucarb(BPMC), carbofuran, diazinon, acetylcholinesterase, esterase

Since 'mutant aliesterase hypothesis' were reported by Oppenoorth and van Asperen in 1960, many researchers have investigated detoxication enzymes as factors of resistance in insects.

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Esterase, mixed function oxidase, glutathione S-transferase, dehydrochlorinase etc. were summarized as detoxication enzymes. Their role as resistance mechanism have been extensively reviewed by Plapp(1976) and Terriere(1984).

Increased esterase activity as a resistance factor was reported in the house fly, green rice leafhopper, mosquito, aphid and mite. Resistance to malathion was reported to be concerned with carboxylesterase(Nagata et al. 1979, Yeoh et al. 1979). On the other hand, insecticides without carboxyl ester bond was reported to be degraded by phosphorotriesterase (phosphatase) (Kao et al. 1984, Motoyama et al. 1984).

Decreased AChE sensitivity as a factor of resistance was revealed first in the two-spotted spider mite by Smissaert(1964), and followed by the cattle tick, mite, green rice leafhopper, house fly and mosquito. Mechanism of resistance to the organophosphorus and pyrethroid-resistant brown planthopper is mainly due to increase in esterase activity(Miyata et al. 1983, Dai & Sun 1984). However, resistance to the carbamate insecticides is attributible to increase of AChE insensitivity rather than increased esterase activity (Chung & Sun 1983, Hama & Hosoda 1983).

Information on resistance mechanism is very important to understand resistance itself as well as to develop tools for suppression of resistance problem. In this respect, we have carried out some experiments on the resistance mechanism of the BPH. Continued to the previous report (Park et al. 1991b) which was concerned with integument penetration and degradation of insecticides in the resistant BPH, esterase activity and AChE insensitivity in the insecticide-selected BPH strains is presented in this paper.

MATERIALS AND METHODS

Brown planthopper strains

The brown planthopper was selected with fenobucarb(BPMC), carbofuran and diazinon for 14-18 generation. Each selected strain was designated R_f, R_c and R_d strain. LD_{50} value of the R_f strain for fenobucarb, R_c strain for carbofuran and R_d strain for diazinon was 60.82, 16.72 and 72.3μg/g female, respectively. LD_{50} values of the susceptible strain, a strain maintained without exposure to any insecticide, for fenobucarb, carbofuran and diazinon were 1.58, 0.41 and 12.48μg/g female, respectively.

Chemicals

Fenobucarb, carbofuran and diazoxon which donated by Chemistry Division of Agricultural Chemicals Research Institute, were used for AChE inhibition study. Purity of each insecticide was 98.2%, 99.3% and 99.5%, respectively.

Sources of reagents are as follows; acetylthiocholine iodide, 5.5-dithiobis-(2-nitroben-
zoic acid) and eserine sulfate from Sigma Chemical Company; diazo blue B salt and triton X-100 from Fluka Chemical Company; α- and β-naphthyl acetate from Tokyo Kasei Chemical Company; α- and β-naphthol from Kanto Chemical Company; sodium lauryl sulfate from Junsei Chemical Company.

Enzyme preparation

Twenty five and 250 mg of 3 to 5 day old female BPHs for esterase and AChE assay were homogenized with Elvejehm glass homogenizer in 15 ml of 0.067 M and 10 ml of 0.1 M phosphate buffer in ice-cold water, respectively. The

homogenate was filtered through cheese cloth. The nuclear plus debris(1,000 g × 20min. precipitate), mitochondrial (12,000 g × 30min. precipitate), microsomal (105,000 g × 2h. precipitate) and solubel(105,000 g × 2h. supernatant) fractions were obtained by differential centrifugation as previously described by Hama(1976) and Miyata et al. (1983) using a Hitachi SCF-55H ultracentrifuge. The precipitate were washed with the same buffer twice. These fractions were resuspended with the same buffer to make concentration of 1.67 mg/ml for esterase and 25 mg/ml for AChE assay, respectively.

Esterase assay

Non-specific esterase activity in the subcellular fractions from whole body homogenates was determined using α-and/or β-naphthyl acetate according to the method of van Asperen (1962). A subcellular fraction(0.05 ml) and 0.067 M phosphate buffer (3.95 ml; pHa 7.2) were mixed throughly and incubated in test tube (15ml volume)for 15 minutes at 30℃. After incubation, 0.05ml of 0.03M α- and/or β-naphthyl acetate in ethanol (final concentration; 3.7×10^{-4}M) were added to the mixture and re-incubated for 10 minutes at 30℃. One ml of mixed solution (1% of diazo blue B : 5% of sodium lauryl sulfate=2 : 5, v/v) was added to stop enzyme reaction. Absorbance for α-naphthyl acetate was determined at 600 nm and for β-naphthyl acetate at 550 nm by UV/VIS spectrophotometer (Gilford, Model 260 : single beam type, USA), and was converted to μmoles using calibration line for α- and β-naphthol. All the test were duplicated.

Acetylcholinesterase (AChE) assay

AChE activity in the subcellular fractions from whole body homogenate was measured according to the method of Ellman et al. (1961). 4.8 ml of 0.1 M phosphate buffer (pH 7.4), 0.2 ml of enzyme source and 0.2 ml of triton X-100 (in phosphate buffer) were mixed and incubated for 15 minutes at 30℃. After incubation, 0.2 ml of 0.01M 5,5'-dithiobis-(2-nitrobenzoic acid) (final concentration; 3.8×10^{-4}M) and 0.03 ml of 0.075 M acetylthiocholine iodide(final concentration; 4.3×10^{-4}M) were added to the incubated mixture. The mixture was re-incubated for 30 minutes at 30℃. After incubation, 0.05 ml of 0.07M eserine sulfate was added to stop enzyme reaction. Absorbance was determined at 412 nm by UV/VIS spectrophotometer. Absorbance of mixtures without insecticides was regarded as whole AChE activity. The in vitro AChE inhibition was determined by adding of various concentration of the test insecticides in acetone to the above incubation mixture. I_{50} values were calculated by probit analysis of AChE inhibition rate of each concetration of the test insecticides. All the tests were duplicated.

RESULTS AND DISCUSSION

Esterase activity

The R_f, R_c and R_d strains showed moderate increase of esterase activity (Tabel 1). The increase rates of esterase activity to hydrolyze α- and β-naphthyl acetate were 5.6-5.8, 6.4-7.8 and 4.0-4.4 in the R_f, R_c and R_d strains, respectively, suggesting that increased esterase activity might be involved in the resistance. It has been also reported that increased esterase activity was obviously concerned with the resistance of some insects to the organophosphorus insecticides (Oppenoorth & van Asperen 1960, Niwa et al. 1977, Georghiou & Pasteur 1978, Kuwahara et al. 1981). However,

Table 1. Non-specific esterase activity[a] in the resistant and susceptible female planthopper strains

strains	Substrates	
	α-naphthyl acetate	β-naphthyl acetate
R_f	2.41(5.5)[b]	3.06(6.8)
R_c	2.80(6.4)	3.51(7.8)
R_d	1.77(4.0)	1.98(4.4)
S	0.44(1)	0.45(1)

[a] Esterase activity was expressed as μmole of hydrolyzed naphthyl acetate/25 mg/min.
[b] The figures in parentheses mean the ratio of esterase acitvity between the resistant and susceptible strain.

Table 2. Subcellular distribution of non-specific esterase activity[a] in the resistant and susceptible female brown planthopper strains

Subcellular fraction	Esterase activity			
	R_f	R_c	R_d	S
Mitochondria	0.13(4.1)[b]	0.08(2.3)	0.04(2.0)	0.01(2.0)
Microsome	0.10(3.1)	0.06(1.8)	0.01(0.5)	0 (0)
Soluble	2.95(92.8)	3.08(90.3)	1.77(87.2)	0.45(88.2)

[a] Specific enzyme activity is expressed as μ mole of hydrolyzed β-naphthyl acetate/25 mg/min.
[b] The figures in parentheses are the percentages relative to the total activity in initial 1,000 g supernatant.

Chung and Sun(1983) and Hama and Hosoda (1983) reported that resistance of the BPH to the carbamate insecticides was not due to increased esterase activity but AChE insensitivity. In the present study, increased esterase acitivity is firmly related to resistance of R_f and R_c strain. This discrepancy may be caused by the BPH strains tested. We used laboratory-selected strains of the BPH, but field and /or field-origin strains were used in other's reports. Anyway, it might be the first report that increased esterase activity is concerned with resistance of the BPH to carbamate insecticides.

There was no noticeable inter-strain difference in esterase activity in any subcellular fractions or in the total activity(Table 2). Water-soluble fraction showed about 90% of total esterase activity. Miyata et al. (1983) also reported that 80-90% of esterase acitivity was present in water soluble fraction.

AChE studies

AChE activity was not varied except slight increment (1.6 times) in the R_f strain(Table 3). Smissaert et al (1970) reported that the organophosphorus insecticide-resistant two-spotted spider mite showed decreased AChE activity, but other researchers reported that there were no variation in AChE activity of the resistant house fly (Mengle & Caside 1960), green rice leafhopper (Hama & Iwata 1971, 1973) and Kanzawa spider mite (Kuwahara 1982) when compared to each susceptible strain.

Subcellular distribution of AChE activity was not different among the BPH strains tested. AChE activity was highest in mitochondrial fraction at the rate of 70-80%(Table 4). Hama(1976) also reported that subcellular distribution of AChE activity in the carbamate insecticide-resistant and susceptible green rice leafhopper strains was not different, and the highest portion was found in mitochondrial fraction(40-50%). Based upon our data and these earlier findings, AChE activity might not be related to resistance of the BPH.

AChE insensitivity was obvious in carbamate insecticide-selected strains, but scanty in diazinon-selected strain of the BPH (Table 5). The ratio of I_{50}

Table 3. Acetylcholinesterase activity of the resistant and susceptible female brown planthopper strains

Strains	Acetylcholinesterase activity[a]
R_f	3.15
R_c	1.80
R_d	2.05
S	2.00

[a] Specific enzyme activity was expressed as μmole of hydrolyzed acetylthiocholine/g/min.

Table 4. Subcellular distribution of acetylcholinesterase activity[a] in the resistant and susceptible female brown planthopper strains

Subcellular fraction	AChE activity			
	R_f	R_c	R_d	S
Mitochondria	1.85(78.9)[b]	1.12(73.2)	1.30(72.2)	1.17(72.2)
Microsome	0.17(7.3)	0.12(7.8)	0.13(7.2)	0.13(8.0)
Soluble	0.18(7.7)	0.13(8.5)	0.15(8.3)	0.16(9.9)

[a] Specific enzyme activity is expressed as μmole of hydrolyzed acetylthiocholine/g/min.

[b] The figures in parentheses are the percentages relative to the total activity in initial 1,000 g supernatant.

Table 5. Insensitivity of acetylcholinesterase of the resistant and susceptible female brown planthopper strains

Strains	I_{50}[a]		
	Fenobucarb	Carbofuran	Diazoxon
R_f	3.92×10^{-1}(12.2)[b]	5.18×10^{-2}(2.4)	1.24×10^{-1}(1.5)
R_c	1.00×10^{0}(31.1)	1.21×10^{-1}(5.6)	1.08×10^{-1}(1.3)
R_d	9.06×10^{-2}(2.8)	3.67×10^{-2}(1.7)	1.40×10^{-1}(1.7)
S	3.22×10^{-2}(1)	2.18×10^{-2}(1)	8.44×10^{-2}(1)

[a] Insecticide concentration which inhibit 50 percent of acetylcholinesterase activity(μmole at 30℃).

[b] The figures in parentheses mean the ratio of I_{50} between the resistant and susceptible strain.

values of R_f, R_c and R_d strains showed 12.3, 5.6 and 1.7, respectively.

The in vitro inhibition of AChE with various concentration of diazoxon as well as fenobucarb and carbofuran was studied using acetylthiocholine iodide as substrate (Table 5). AChE from both the R_f and R_c strains exhibited a marked decrease in sensitivity to inhibition by these compounds. The ratio of I_{50} values of the resistant and susceptible strains varied with the inhibitor. The order of inter-strain difference was fenobucarb>carbofuran>diazoxon. The results suggest that altered AChE in the R_f and R_c strains are an important factor in carbamate resistance. However, diazoxon inhibit AChE to a lesser extent in all the tested strains. The involvement of insensitive AChE in organophosphorus insecticide resistance has been reported with green rice leafhopper (Chung & Sun 1972, Miyata & Saito 1976), house fly (Yeoh et al. 1981, Devonshire & Moores 1984), mite(Smissaert et al. 1970) and tick (Roulston et al. 1968) as well as carbamate insecticide resistant strain of BPH (Chung & Sun 1983, Hama & Hosoda 1983). Based upon our results and these earlier results, increased AChE insensitivity may be concerned with resistance to carbamate and/or oxon-type organophosphorus insecticides.

Devonshire and Moores(1984) reported 3-40 times decrease in bimolecular rate constant(K_i) of AChE in the resistant house fly, and similar results were also found in the cattle tick (Roulston et al. 1968) and mites (Smissaert et al. 1970). These findings indicate decreases in affinity between AChE and insecticides, suggesting a certain variations in ionic site(binding site) of AChE. Increase in AChE insensitivity in the selected BPH may be due to similar mechanism as suggested above, although we have no data on the biochemical characteristics of AChE.

As illustrated in Table 6, multiplication of fig-

Table 6. Correlation between resistance ratio(R.R.) and two resistance factors

Strains	R.R.	Ratio[a]		A×B
		Esterase activity(A)[b]	Acetylcholinesterase insensitivity(B)	
R_f	50.3	6.2	12.2	75.6
R_c	49.2	7.1	5.6	39.8
R_d	5.8	4.2	1.7	7.1

[a] Ratio of resistant strain over susceptible strain.

[b] Average activity on α− and β−naphthyl acetate.

ures for increased esterase activity and AChE insensitivity are very similar to resistance level of all the selected strains. Resistance level and multiplication figures of two resistance factors are 50.3 and 75.6; 49.2 and 39.8; 5.8 and 7.1 in the R_f, R_c and R_d strains, respectively. In the green rice leafhopper (Miyata & Saito 1976) and house fly (Yeoh et al. 1981), two or more resistance factors were reported to act multiplicatively. So, we conclude that increased esterase activity and AChE insensitivity enlarge resistance level mutiplicatively, regardless of insecticide types used in selection of the BPH. More amount of absorbed insecticides are degraded by enhanced action of esterase, and remains which reach at synapses are deactivated by increased AChE insensitivity in the resistant strains of the BPH. But, there are some difficulties to make general conclusion, because of low level of resistance in the R_d strain, slight decrease of penetrated insecticide amount and 1.6 times increase in AChE activity in the R_f strain. Further investigation will be needed on the molecular or biochemical levels of the resistance.

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