A COMPARISON OF SENSITIVITY TO INHIBITOR AMONG ACETYLCHOLINESTERASE (AChE) MOLECULAR FORMS OF RESISTANT AND SUSCEPTIBLE STRAINS IN *HELICOVERPA ARMIGERA* *

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Abstract The sensitivity of 2.8s and 8.7s acetylcholinesterase (AChE) to eserine sulfate is significantly lower in resistant (R) strain than in susceptible (S) strain in five AChE forms isolated by sucrose gradient centrifugation from cotton bollworm, *Helicoverpa armigera*. There are 186 and 85 times of difference in heads of adults and 10^{10} and 10^{5} times of difference in heads of larvae based on a comparison of I_{50} values for 2.8s and 8.7s forms respectively. The sensitivity of 5.3s form of AChE to eserine sulfate shows 123 times of difference between R and S strains in larvae, however no difference in adults. The above results indicate that insensitive 2.8s, 8.7s and 5.3s forms of AChE may play an important role in the resistance of cotton bollworm to organophosphate and carbamate insecticides.

Key words *Helicoverpa armigera*, acetylcholinesterase molecular forms

1 INTRODUCTION

Cotton bollworm, *Helicoverpa armigera* Hübner is a major pest of many agricultural crops throughout many parts of the old world and many kinds of insecticides were used for its control. Now, cotton bollworm has developed resistance to DDT, organophosphate, carbamate and pyrethroid insecticides (Lin *et al*. 1988, Mu and Wang al. 1993, Tang 1983, Glenn *et al*. 1994, McDonald *et al*. 1993, Daly 1988, Daly and Fitt 1990). Organophosphate and carbamate are two types of main inhibitors for AChE and the insensitivities of AChE (altered AChE) are one of the main resistant mechanisms to these two types of insecticides in insects. AChE from vertebrates and insects is known to exist in a number of molecular forms which have been divided into two classes, asymmetric and globular. The globular forms correspond to monomers (G1), dimers (G2), and tetramers (G4) of similar catalytic subunits. The asymmetric forms are characterized by the presence of a collagen-like tail associated with one, two, or three catalytic subunit tetramers (Massoulie *et al*. 1991). Globular forms can be further subdivided into two categories: soluble forms and detergent-binding forms, which contain a hydrophobic domain that presumably anchors the enzyme in phospholipid membranes (Massoulie *et al*. 1991, Lazar and Vigny 1980, Gnagey *et al*. 1987). The primary objective of this study is to compare the sensitivity of the different molecular forms of AChE to the various inhibitors

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in the resistant and susceptible strains of cotton bollworm to the organophosphate insecticides.

2 MATERIALS AND METHODS

2.1 Insects

Susceptible and resistant strains of cotton bollworm larvae were reared with artificial diet (Gao *et al*. 1996a). The susceptible strain had been maintained in the laboratory for >25 generations without exposure to any insecticides. Resistant strain was established from a resistant field population and had been selected repeatedly with parathion-methyl in the laboratory to increase its resistance (Gao *et al*. 1996a, b). Insect rearing was conducted at 26 ℃, a photoperiod of 16∶8h (L∶D) and relative humidity (RH) 70% - 80%. The resistant strain had developed 50 - 100 factors resistance to parathion-methyl, DDVP and methomyl.

2.2 Chemicals

S-Acetylthiocholine iodide was purchased from Fluka Co. 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) was purchased from Roth Co. Eserine sulfate, 99%, and Sepharose 4B were purchased from Sigma Co. Sephadex G-200 was purchased from Aldrich Co.

2.3 Analysis of the molecular forms of AChE by sucrose gradient centrifugation

Centrifugation of the extracts from heads of adults or larvae was performed using Spinco SW55Ti rotors at 105 000 *g* (34 000r/min) for 90min, at 4(C, in 5% - 20% sucrose gradients in 10^{-2} mol/L Tris-HCl, pH 7.0, 5×10^{-2} mol/L $MgCl_2$, with or without 0.5% Triton X-100 (Arpagaus and Toutant 1985, Gao *et al*. 1998).

3 RESULTS

The molecular forms of 2.8s, 5.3s, 8.7s, 11.9s, and 15.6s AChEs were isolated by sucrose gradient centrifugation from cotton bollworm. Table 1 shows the median inhibition concentration (I_{50}) of AChE by eserine sulfate from the heads of adults. The sensitivity of 2.1s, 8.7s and 15.6s AChEs to eserine sulfate is much lower in resistant strain

Table 1 Comparison of sensitivity to eserine sulfate among the different molecular forms of AChEs from the heads of adults (I_{50}, M)

AChE Fraction	Without Triton X-100			With Triton X-100		
	R	S	R/S	R	S	R/S
2.1s	4.47×10^{-5}	2.40×10^{-7}	186.3	3.62×10^{-4}	2.06×10^{-6}	175.7
5.3s	1.02×10^{-5}	9.09×10^{-6}	1.1	2.07×10^{-4}	1.75×10^{-4}	1.2
8.7s	8.06×10^{-5}	9.5110^{-7}	84.8	1.09×10^{-5}	2.43×10^{-7}	44.9
11.9s	1.28×10^{-4}	1.08×10^{-4}	1.2	8.85×10^{-3}	1.15×10^{-3}	7.7
15.6s	3.05×10^{-3}	3.06×10^{-4}	10.0	3.62×10^{-4}	4.95×10^{-5}	6.3

than in the susceptible strain and the resistant strain are 186, 85, and 10 times in I_{50} values of 2.8s, 8.7s, and 15.6s AChEs inhibited by eserine sulfate as compared with the susceptible strain respectively. The sensitivity of 5.3s and 11.9s AChEs to eserine sulfate from the resistant strain is almost the same as that from the susceptible strain. The sensitivity difference of 8.7s AChE between R and S strains reduces one time in the presence of Triton X-100 compared to the absence of Triton X-100. However, the sensitivity difference of 2.8s AChE between the R and S strains in the presence of Triton X-100 is not significantly different from that in the absence of Triton X-100.

The sensitivity difference of 2.1s, 5.3s, and 8.7s AChEs to eserine sulfate between R and S strains is much more in larvae than in adults. I_{50} values of 2.1s, 5.3s, and 8.7s AChEs from R strain, inhibiting by eserine sulfate, are 10^{10}, 123 and 10^{5} times bigger than those from S strain in the absence of Triton X-100 and 10^{10}, 126 and 10^{9} times in the presence of Triton X-100 respectively. The sensitivity of 15.6s AChE from R strain to eserine sulfate is almost the same as S strain in the absence or presence of Triton X-100. However the sensitivity of 11.9s AChE to eserine sulfate is less in R strain than in S strain.

Table 3 shows the values of the dissociation constant (k_d), phosphorylation rate (k_2), and bimolecular velocity constant (k_i) of AChE with various inhibitors in the pres-

Table 2 Comparison of sensitivity to eserine sulfate among the different molecular forms of AChEs from the heads of larvae (I_{50}, M).

AChE Fraction	Without Triton X-100			With Triton X-100		
	R	S	R/S	R	S	R/S
2.1s	1.65×10^{-4}	2.88×10^{-15}	5.8×10^{10}	8.39×10^{-4}	5.16×10^{-14}	1.6×10^{10}
5.3s	6.36×10^{-5}	7.61×10^{-7}	123.0	3.25×10^{-4}	2.57×10^{-6}	126.5
8.7s	5.87×10^{-5}	1.56×10^{-10}	3.8×105	1.01×10^{-5}	6.97×10^{-15}	1.5×10^{9}
11.9s	3.23×10^{-2}	1.09×10^{-2}	3.0	3.87×10^{-2}	5.88×10^{-3}	6.6
15.6s	6.48×10^{-2}	3.38×10^{-2}	1.9	5.01×10^{-2}	4.16×10^{-2}	1.2

Table 3 Comparison of AChE sensitivity to various inhibitors from the adult head of cotton bollworms.

Inhibitors	$K_i\times10^4$ (min^{-1}, M^{-1})		K_2 (min^{-1})		$K_d\times10^{-6}$ (M^{-1})	
	Without Triton X-100	With Triton X-100	Without Triton X-100	With Triton X-100	Without Triton X-100	With Triton X-100
Eserine sulfate	53.334 ±11.200	1.098 ±0.290	0.326 ±0.024	0.266 ±0.002	0.611	6.488
Malaoxon	32.810 ±8.124	14.699 ±5.843	0.778 ±0.073	0.525 ±0.080	2.371	3.517
Thiodicarb	13.677 ±3.131	4.614 ±1.697	0.374 ±0.034	0.160 ±0.033	2.734	3.472
Carbofuran	13.090 ±2.066	7.814 ±4.717	0.576 ±0.132	0.159 ±0.143	4.400	2.035
Methamidophos	12.392 ±5.725	37.343 ±14.942	0.498 ±0.080	0.078 ±0.005	4.016	0.209
Methomyl	8.434 ±1.381	1.629 ±0.398	0.368 ±0.020	0.275 ±0.027	4.332	16.880
Paraoxon	5.999 ±1.955	3.970 ±0.551	1.504 ±0.996	0.606 ±0.055	25.071	15.264
DDVP	1.351 ±0.384	6.325 ±0.925	1.180 ±0.367	0.465 ±0.016	87.343	7.352
Monocrotophos	1.176 ±0.230	0.857 ±0.077	0.386 ±0.036	0.132 ±0.001	32.823	15.403

ence or absence of Triton X-100. The sensitivity of AChE to eserine sulfate, malaoxon, thiodicarb, carbofuran, methomyl, and paraoxon reduces, but the sensitivity of AChE to methamidophos and DDVP evidently increases in the presence of Triton X-100. The k_i values of methamidophos and DDVP with AChE increase 3 and 5 times whereas the k_i values of malaoxon, paraoxon and monocrotophos reduce 2.23, 1.51 and 1.37 times respectively when the presence of Triton X-100. The k_i values of carbamate inhibitors with AChE significantly reduce in the presence of Triton X-100. It suggests that the aggregation of AChE monomers may affect the interaction of AChE with inhibitor.

4 DISCUSSION

Five fractions of AChE were obtained by sucrose gradient centrifugation from cotton bollworm. The sensitivity of 2.1s and 8.7s AChEs from the heads of larvae or adults to eserine sulfate are significantly different between the R and S strains in the absence or presence of Triton X-100, indicating that 2.1s and 8.7s AChEs may be responsible for cotton bollworm resistance to organophosphate and carbamate insecticides. Smissaert first described a modified AChE with a decreased sensitivity to acaricide inhibition in the two-spotted spider mite, *Tetranychus urticae* (Smissaert 1964). Lee and Batham observed the similar phenomenon in the cattle tick, *Boophilus microplus* (Lee and Batham 1966). Schuntner and Roulston first observed that the time for 50 % inhibition by diazinon was longer in a *Lucilia cuprina* resistant strain (Schuntner and Roulston 1968). Afterwards, the modified AChEs were found in the resistant strains of many insects. AChE is a type of enzyme whose monomer and polymer all possess the activity hydrolyzing acetylcholine. In the altered (modified) AChE, the k_d usually increases, reflecting a reduction of the affinity of AChE for the insecticides (Devonshire 1975). The k_2 may sometimes decrease, but the changing extent of k_2 is much less than that of k_d. There is a good negative correlation between the degree of resistance and the k_i in many resistant insects with an altered AChE. However, this directly proportional correlation is not to be expected when the altered AChEs are associated with other mechanisms of resistance, for example with slower penetration of the insecticide or with a better degradation due to oxidases, esterases or glutathione transferases (Fournier and Mutero 1994, Taylor *et al*. 1993). The different AChE molecular forms possess the different sensitivity profiles to inhibitors. There is a bigger difference of sensitivity to inhibitor between AChEs of R and S strains. The difference of AChE monomer sensitivity to inhibitor is bigger than the polymer between AChEs of R and S strains. It suggests that the interaction among monomers of polymer can partly recover AChE's sensitivity to inhibitor.

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棉铃虫 AChE 不同分子型对抑制剂的敏感度及其与抗药性的关系

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在分离到的棉铃虫 AChE 五种不同的分子型中,2.1s 和 8.7s AChE 抗性品系对毒扁豆碱的敏感度明显低于敏感品系,成虫头部 I_{50}值分别相差 186.3 和 84.8 倍,幼虫 I_{50}值分别相差 10^{10}倍和 10^{5} 倍。幼虫 5.3s AChE 对毒扁豆碱的敏感度抗性品系和敏感品系相差达 123 倍,而成虫则没有差异。研究结果表明 2.1s、5.3s 和 8.7s AChE 敏感度降低可能是造成棉铃虫对有机磷和氨基甲酸酯类杀虫药剂产生抗性的主要原因。

关键词　　棉铃虫　乙酰胆碱酯酶分子型　抗药性

中国昆虫学会 2001 年活动计划表

序号	项目名称	时间	规模	地点	联系人及电话
1	昆虫与环境—2001 年中国昆虫学会西部大开发学术讨论会	10 月	400 人	成都	龚富生 010 - 62565687
2	西部森林害虫综合防治学术研讨会	10 月	120 人	成都	杨忠岐 010 - 62889502 吴　坚 010 - 64214808 陈树椿 010 - 62338930
3	经济作物病虫害综合治理学术研讨会	10 月	100 人	待定	郭　荣 010 - 64194542
4	第六届全国青年工作者昆虫学术讨论会	10 月	100 人	昆虫	陈晓峰 010 - 68597428 吴孔明 010 - 62815929
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