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GENETIC AND BIOCHEMICAL CHARACTERISTICS OF CYCLODIENE EPOXIDASE IN THE HOUSEFLY*

M. A. Q. KHAN†

Department of Entomology, Oregon State University, Corvallis, Ore., U.S.A.

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Abstract—Housefly strains resistant to insecticides by oxidative detoxication mechanisms possess higher levels of microsomal cyclodiene epoxidase activity as compared with two susceptible or one Dieldrin-resistant strain. The epoxidase system in strains with higher activity has apparently greater affinity for aldrin, isodrin and heptachlor, as judged by the Michaelis constants. The epoxidase activity in resistant strains increases after the emergence of adult flies and maximum activity occurs at slightly different ages in each strain. Male flies have about half of the activity of the females. The high epoxidase activity in an Isolan-resistant strain is inherited through a semidominant gene, *Ox*, on chromosome II.

THE EPOXIDATION of aldrin, isodrin, heptachlor and photoaldrin to corresponding epoxides is a common reaction occurring in soil,[1] micro-organisms,[2] plants,[3] vertebrates[4] and insects.[5–7] In mammals, this takes place in the microsomal NADPH-oxidase system.[8, 9] A similar microsomal NADPH-oxidase system is responsible for aldrin epoxidation in insects like the housefly,[6, 10] the southern armyworm[11] and other lepidopterous insects.[12, 13]

Activities of the microsomal NADPH-oxidase system to epoxidize aldrin and hydroxylate naphthalene are parallel in 14 different insecticide-resistant and susceptible strains of the housefly.[14] Differences in the levels of activity of the oxidase system in these strains could be due to differences in the characteristics of the microsomal oxidase system.

Oxidation of methylcarbamate insecticides by the microsomal oxidase system from abdomen of houseflies is controlled by gene(s) on chromosome II.[15] Degradation of DDT by a similar enzyme system in the housefly strain *Fc* is apparently controlled by a gene on chromosome V.[16, 17] Inheritance of aldrin epoxidase was therefore investigated in an Isolan-resistant strain of houseflies.

MATERIALS AND METHODS

Insecticide-resistance spectra and genetics of the housefly strains used in these studies have been reported.[18] Information about the activity of DDT-dehydrochlorinase and microsomal oxidase enzymes has also been published.[14, 19] The strains selected for study were those possessing either high microsomal oxidase

* Technical Paper no. 2642, Oregon Agriculture Experiment Station, Corvallis, Ore.
† Present address: Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Ill.

activity, like *Isolan-resistant*,* *Fc*, *kdr-O*,w^5, *malathion-resistant*,* or low epoxidase activity, like *cyw*, *Dld* and marker strains *stw* and *stw*; *bwb*; *ocra*.

The assay methods of Khan and Terriere[19] were used with the following modifications: The microsomal pellet from 10 flies was resuspended in 12·5 ml of 0·1 M Tris-HCl buffer, pH 8·2. Five ml of this suspension was added to the NADPH-generating system, followed by the addition of 60 μl methylcellusolve containing the desired concentration of the cyclodiene derivative. Incubation at 34° for 10 min was followed by the extraction of reaction products and their analysis by gas chromatography.[19] This procedure was used for determining K_m and V_{max} values and for the effect of age and sex on the epoxidase activity. For determining the epoxidase activity in larvae and pupae, two batches of 10 larvae were used to prepare microsomes from each stage, and enzyme assays were performed as described before.

For the time-course epoxidation of aldrin by different housefly strains, microsomes from fifty female flies at the age when maximum oxidase activity is attained, i.e. 8–11 days old, were resuspended in 25 ml buffer. Five ml of this suspension was incubated with 100 nmoles aldrin for 5–60 min.

For the assay of aldrin epoxidase in progenies of genetic crosses, microsomes from ten females, 4–5 days old, were suspended in 25 ml buffer. Five ml of this suspension was incubated with 100 nmoles aldrin for 1 hr. Flies of this age were chosen because male and female flies do not show differences in oxidase activity at this age.[20]

For each experiment, microsomes from two batches of insects were assayed for enzyme activity. Incubations were run in duplicate and the average values used. Enzyme activities of various preparations were expressed on a per insect basis. Comparisons were also made on the basis of total weight of the insect. The average weight of each housefly, determined by weighing several groups of ten to fifty insects, was 20 mg per female housefly. The weight (in milligrams) of each larva during the growth period was: 1 day old, 0·40; 2 days old, 7·5; 3 days old, 13·5; 5 days old, 19·5; 6 days old, 17·1; 7 days old, 16·8; fresh pupae, 13·0; 2-day-old pupae, 14·5. The average weight of 1000 eggs was 300 mg.

Genetics. Insecticide-resistant strains with high microsomal epoxidase activity were crossed[21] with the susceptible multimarker strain, *stw*; *bwb*; *ocra*, bearing visible mutant markers: stubby wing, *stw* (chromosome II); brown body, *bwb* (chromosome III); and ocra eye, *ocra* (chromosome V); and with the single marker strain, *stw*. Three resistant strains with high epoxidase activity (*Isolan-R*, *Fc*, and *kdr-O*,w^5), one dieldrin-resistant strain (*cyw*, *Dld*) and the *stw*; *bwb*; *ocra* strain with low epoxidase activity were used in initial crosses with the *stw* strain. The *Isolan-R* strain was crossed in separate experiments with both the *stw* and *stw*; *bwb*; *ocra* strains, and the F_1 heterozygotes were then backcrossed with the respective marker strain. Epoxidase activities were determined in various phenotypes resulting from these crosses.

RESULTS

Since the microsomal oxidase activity is affected by sex and age of houseflies,[14] relationships between age and stage of development and the level of epoxidase were studied. Results of an experiment with larvae and pupae of the *Isolan-R* strain for the microsomal aldrin epoxidase are shown in Table 1. Low levels of oxidase activity were

* *R* will be used throughout this paper as an abbreviation for *resistant* in the designation of housefly strains.

TABLE 1. ALDRIN EPOXIDASE ACTIVITY IN THE MICROSOMES PREPARED FROM EGGS, LARVAE AND PUPAE OF THE *ISOLAN-R* STRAIN OF HOUSEFLIES

Stage of development or growth	Dieldrin produced (nmoles/g wet wt.)*
Eggs	0·006†
Larvae (days old)	
1	0·25
2	0·112
3	0·097
5	0·025
6	0·028
7	0·030
Pupae	
just after pupation	0·043
2 days after pupation	0·006

* Microsomes from two batches of samples were combined and each value represents an average of three replicates.
† Microsomes were prepared from about 3000 eggs.

present in the eggs; this activity reached the highest level in the larvae 1 day after hatching. Epoxidase activity decreased as the larvae grew in size. There was a slight increase in activity in fresh pupae than the 7-day-old prepupate larvae. This activity decreased to the lowest level in older pupae and then started increasing after emergence of adult flies.

For the effect of age on epoxidase activity, female flies of the same generation were assayed at ages ranging from 1 to 21 days. The results showed that the maximum epoxidase activity occurred after 7–8 days (Fig. 1). The optima for female flies of the four strains were slightly different: about 12 days for *kdr-O,w*^{5}; 13 days for *Isolan-R*;

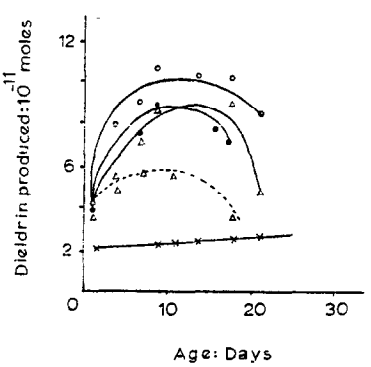

FIG. 1. Aldrin epoxidase activity (10^{-12} moles dieldrin produced/insect/min) of housefly strains at different ages. △ = *Isolan-R* females; ● = *kdr-O,w*^{5} females; ⊙ = *Fc* females; × = *stw*; *bwb*; *ocra* females; △ - - - △ = *Isolan-R* males. Microsomes were prepared from ten female flies. Aldrin epoxidase activity was determined for microsomes prepared from two batches of ten female flies. Each point represents an average of four replicates.

10 days for *Fc*; and with no change from 8 to 20 days for the *stw* strain. Differences in epoxidase activity between the sexes of the *Isolan-R* strain start after 4 days, the males showing lower activity than the females.

A study of the time-course epoxidation of aldrin by microsomes from *Isolan-R*, *Fc*, *kdr-O*,w^5 and *stw* strains showed that the rate of epoxidation was linear for 12–15 min of incubation (Fig. 2). The three resistant strains epoxidized aldrin at a rate ten times higher than did the *stw* strain, an observation reported earlier.[14, 19]

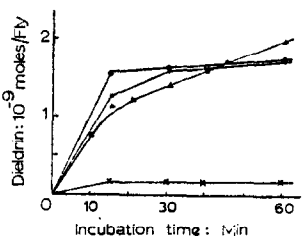

FIG. 2. Time course of epoxidation of aldrin (10^{-9} moles dieldrin/fly) by microsomes from different housefly strains. △ = *Isolan-R*; ● = *kdr-O*,w^5; ○ = *Fc*; × = *stw*. Microsomes were prepared from fifty female flies. Each point represents an average of three replicates.

TABLE 2. K_m AND V_{max} VALUES FOR MICROSOMAL EPOXIDASE OF DIFFERENT HOUSEFLY STRAINS WITH ALDRIN, ISODRIN AND HEPTACHLOR AS SUBSTRATES

Strain*	K_m (10^{-6} M)			V_{max} (10^{-12} moles/min/fly)†		
	Aldrin	Isodrin	Heptachlor	Aldrin	Isodrin	Heptachlor
Isolan-R (6)	13·56 ± 1·63	10·31	9·10	155·2 ± 4·6	200·0	113·7
Fc (6)	10·18 ± 1·73	9·26	7·95	167·6 ± 4·1	232·6	147·0
kdr-O,w^5 (1)	14·70			147·1		
Malathion-R (Grothe) (2)	13·33			140·85		
stw; *bwb*; *ocra* (6)	5·46 ± 0·40	5·75		13·4 ± 1·1	20·8	

* Values in parentheses show the number of experiments with aldrin as a substrate; with the rest of the substrates, the average of two experiments is used. Three replicates were used for each substrate concentration in each experiment.

† Microsomes were prepared from ten female flies.

A determination of K_m and V_{max} values[22] for the microsomal epoxidase with aldrin as a substrate showed that these values were, respectively, about two and ten times higher for the resistant strains than for the susceptible marker strain (Table 2). These differences in K_m and V_{max} values extended to other epoxidizable substrates, isodrin and heptachlor.

Three resistant strains with high epoxidase activity and the *cyw*, *Dld* and *stw*; *bwb*; *ocra* strains with low epoxidase activity were crossed with the marker strain *stw*. The females in the F_1 progenies were assayed for microsomal aldrin epoxidase activity and the values were compared with those of the parents. The F_1 hybrids with *cyw*, *Dld* and the *stw*; *bwb*; *ocra* strain possess low epoxidase activity like the parents (Table 3). Therefore the genes controlling epoxidase activity are not complementary in these strains. The hybrids between resistant and marker strains showed intermediate

TABLE 3. MICROSOMAL EPOXIDASE ACTIVITY OF INSECTICIDE-RESISTANT AND SUSCEPTIBLE STRAINS AND THEIR F_1 HYBRIDS

Strain	Dieldrin produced (10^{-9} moles/hr)*	
	Parent	F_1 (parent × *stw*)
Isolan-R	4·66	2·11
Fc	4·29	2·07
kdr-O,w^5	4·00	1·67
stw; bwb; ocra	0·44	0·50
cyw, Dld	0·38	0·46
stw	0·40	

* Incubation with two female equivalents of microsomes. Microsomes were prepared from ten female flies. Each experiment was replicated three times.

epoxidase activity, indicating the semidominant nature of the gene controlling high oxidase levels in resistant strains.[20]

When F_1 males from a cross between *Isolan-R* females and *stw; bwb; ocra* males were backcrossed with females of the *stw; bwb; ocra* strain, eight phenotype classes were obtained. Microsomal aldrin epoxidase activity in these phenotype classes was determined. The phenotypes which were heterozygous for the second chromosomal gene (*stw*$^+$/*stw*) contained the maximum epoxidase activity. Neither chromosome III nor V appears to contribute additional oxidase activity (Table 4).

When this experiment was repeated with the susceptible marker strain, *stw* (chromo-

TABLE 4. EFFECT OF DIFFERENT CHROMOSOMES OF THE *ISOLAN-R* STRAIN ON ALDRIN EPOXIDASE ACTIVITY IN A BACKCROSS BETWEEN FEMALES OF A SUSCEPTIBLE MARKER STRAIN

$\frac{stw;\ bwb;\ ocra}{stw;\ bwb;\ ocra}$ and males of the F_1 cross

$\left(\frac{stw^+\ bwb^+\ ocra^+}{stw^+\ bwb^+\ ocra^+}\ \text{Female} \times \frac{stw\ bwb\ ocra}{stw\ bwb\ ocra}\ \text{Male}\right)$

Phenotype chromosome			Dieldrin produced (10^{-9} moles/hr)*
II	III	V	
stw$^+$	*bwb*	*ocra*	2·11
stw	*bwb*	*ocra*$^+$	0·21
stw	*bwb*$^+$	*ocra*	0·24
stw	*bwb*	*ocra*	0·29
stw$^+$	*bwb*$^+$	*ocra*$^+$	2·29
stw^{++}	*bwb*	*ocra*$^+$	1·88
stw$^+$	*bwb*$^+$	*ocra*	1·98
stw	*bwb*$^+$	*ocra*$^+$	0·32

* Incubation with two female fly equivalents of microsomes. Microsomes were prepared from three different batches of ten female flies. The values shown are averages of three replicates for each experiment.

some II), the high epoxidase activity was associated with the heterozygotes *stw*$^+$/*stw* (Table 5). This showed that the epoxidase gene segregates with the second chromosomal markers. There is some evidence of crossing-over in the backcross progenies resulting from F_1 females and *stw* males. This crossing-over is known to occur in dipteran females.[19, 20]

TABLE 5. MICROSOMAL EPOXIDASE ACTIVITY IN THE PROGENIES OF BACKCROSSES BETWEEN THE SUSCEPTIBLE *stw* STRAIN AND THE F_1 HYBRIDS BETWEEN IT AND *ISOLAN-R* STRAIN

Backcross	Phenotype	Dieldrin produced (nmoles/hr)*
$\left(\frac{stw^+}{stw^+} \sigma \times \frac{stw}{stw} \varphi\right) \sigma \times \left(\frac{stw}{stw} \varphi\right)$	*stw*$^+$/*stw*	2·52
	stw/*stw*	0·32
$\left(\frac{stw^+}{stw^+} \sigma \times \frac{stw}{stw} \varphi\right) \varphi \times \left(\frac{stw}{stw} \sigma\right)$	*stw*/*stw*	0·53
	stw$^+$/*stw*	1·66
$\left(\frac{stw^+}{stw^+} \varphi \times \frac{stw}{stw} \sigma\right) \sigma \times \left(\frac{stw}{stw} \varphi\right)$	*stw*/*stw*	0·27
	stw$^+$/*stw*	2·36

* Incubation with two female fly equivalents of microsomes prepared from ten flies. Each value is an average of three different experiments, each with three replicates.

DISCUSSION

The present investigation further confirms that housefly strains can be grouped into two classes in regard to microsomal oxidase activity.[14] These two classes of housefly strains show as much as 10-fold differences in the activity of this enzyme system. This difference is not due to slower initiation of epoxidation by strains with low levels of activity, since the differences between the two classes prevail even when the time of incubation is increased to 1 hr. The affinity of the epoxidase system for aldrin, isodrin and heptachlor in the two classes of strains appears to be different, as judged by the K_m and V_{max} values and by the reaction rate, K_m/V_{max}.

The affinity or overall activity of the epoxidase system can be affected by a deficiency or change in any component of the electron transport system. Since the enzyme system involves whole microsomal membranes,[23] containing phospholipids,[24] neutral lipids and proteins, the differences in K_m values cannot be given any great significance.

In mammals, the increase in mixed-function oxidase activity caused by inducers is generally due to an increase in the P-450 content.[25] In guinea pigs, a strain homozygous for high rate of cortisol hydroxylation contains a higher amount of P-450 than does the strain with low cortisol hydroxylation.[26] In some insecticide-resistant and susceptible strains which differ in their oxidase level,[14] the high oxidase activity has been correlated with higher P-450 content.[27] The differences in the epoxidase levels in these strains could still be due to qualitative differences in the P-450, which is both a substrate-binding and oxygen-activating component, or in some other rate-limiting component.

In susceptible strains, the electron transport system or the P-450 alone could have undergone conformational changes due to the binding of some endogenous substrate

whose metabolism may be taking place at a higher rate than in the resistant strain. The mammalian mixed-function oxidase system, in addition to oxidation of xenobiotics, performs several other functions, like sterol hydroxylation,[25, 28, 29] fatty acid ω-hydroxylation[30] and lipid peroxidation.[31, 32] The latter does not take place in the housefly,[31] and the presence of the former two has not yet been reported in insects.

A characteristic of the insect microsomal oxidase system is the presence of natural inhibitors.[33, 34] It also may be possible that the two susceptible strains and the *cyw*, *Dld* strain possess these inhibitors. However, the cause of differences in the epoxidase level in these strains could be found only if the whole electron transport system involved in these reactions is constituted from these groups of strains and compared for the effects of the purified inhibitor(s).

The two classes of housefly strains behaved as homozygotes for the aldrin epoxidase system. The *Isolan-R*, *Fc* and *kdr-O*,w^5 strains possess the dominant allele, *Ox*, the gene for oxidase. The F_1 heterozygotes between the low epoxidase and high epoxidase strains are intermediate in the activity. The F_1 hybrids between the *cyw*, *Dld* and *stw*; *bwb*; *ocra* strains did not show any enhanced epoxidase activity, indicating that the same gene was missing in both strains and that these were not complementary genes. The gene, *Ox*, which is responsible for the high epoxidase activity in the *Isolan-R* strain, is located on chromosome II. The gene(s) responsible for carbamate resistance and microsomal oxidation of methylcarbamate insecticides is also located on this chromosome.[15]

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