Variations of Insect Haemolymph α -Mannosidase, β -Galactosidase, and β -N-Acetylglucosaminidase in the Silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)

Shigeru KIMURA

Sericultural Experiment Station, Yatabe, Ibaraki 305, Japan

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Activities of α -mannosidase, β -galactosidase and β -N-acetylglucosaminidase of silkworm haemolymph were examined for quantitative differences among four geographically different races and strains of *Bombyx mori*. Statistical analysis on a correlation of strains with the ratios of enzyme activities revealed that all strains were distinguishable by the index. α -Mannosidase levels were bimodally distributed in the four races, whereas β -galactosidase and β -N-acetylglucosaminidase showed a normal unimodal pattern. A difference in activity among the four races was found only for α -mannosidase which was significantly higher in the European race. No enzyme-deficient silkworm was found. Silkworms with extremely high and low activities were seen, suggesting that they were in a heterozygous state for glycosidases.

INTRODUCTION

The silkworm, *Bombyx mori*, the most domesticated of all insects, is in a homozygous state for morphological characters such as body markings, cuticular and cocoon colors and so on due to an arrangement by breeders for many generations, but such condition is not true for isozymes such as acid- and alkaline phosphatases (YOSHITAKE and AKIYAMA, 1964, 1965). Recently, insect haemolymph β -N-acetylglucosaminidase (EC 3.2.1.30) was found to be controlled by a recessive autosomal alleli. Neither an enzyme-deficient insect nor isozyme for β -N-acetylglucosaminidase was found, though homozygotes for high or low activities were separated with activities varying up to ten-fold between isolates only at the fourth rates by selection (KIMURA, 1981 a). Thus, the same situation may be suggested in insect α -mannosidase (EC 3.2.1.24) and β -galactosidase (EC 3.2.1.23) as already found in haemolymph (KIMURA, 1981 a). The present work describes geographic variation of three glycosidases in haemolymph and their heterogeneity in a population.

MATERIALS AND METHODS

The larvae of the silkworm, *Bombyx mori* were kindly supplied from the Branch Laboratory of the Sericultural Experiment Station in Kobuchizawa, Yamanashi, and were selected to avoid hybrids by reference to the data as reported by GAMO and OHZUKA (1980). Silkworm strains used were as follows: Japanese races; Aojuku (Ichinomiya) (101), Koishimaru (115), Kokin (117), Seihaku

Fig. 1. Variation of α -mannosidase, β -galactosidase, and β -N-acetylglucosaminidase activities among forty-nine aboriginal silkworm strains. Five larvae from each strain were used for enzyme assay and the mean value was recorded. Enzyme activity was expressed as units/ml haemolymph. A: α -Mannosidase, B: β -Galactosidase, C: β -N-Acetylglucosaminidase.

(120), Dainyorai (123), Datenishiki (125), Ryukyutasanken (Kiaya) (132), Wako (134), Okusa (138), Konjiki (Matsumoto) (141), Nihon nishiki (155), Japanese No. 1 (407), Japanese No. 115 (new) (449), Haku (462).

Chinese races; Kaijo (205), Kansen (208), Kinko (210), Kohoku (213), Sekko (221), Benishina (231), Yoko (232), Renshin (233), Kinkomaru (235), Kuroshima (236), Daizo (Nagano) (243), Hakuyoen (245), 210 (251), Chinese No. 108 (old) (545), Chinese No. 108 (546).

European races; Ascoli (302), Var (305), St. Julien (311), Spain (312), Szegsard (313), Blanc des Cévennes (314), Toruko koken (Turkish yellow) (316), No. 500 (Blanc pur indigène) (317), Bagdad (318), 91 Bagdad (319), Bione (320), Merche Yellow (322), Rosa (324), European No. 1 (601), European No. 5 (602), European No. 16 (old) (607).

Tropical races; Cambodia (fixed race) (351), Annam (352), Pure Mysore (354), Mysore (355).

Parentheses means a stock number as described in the monograph mentioned below. The characteristics in the strains are described in "Genes and Genetical Stocks of the Silkworm" edited by CHIKUSHI (1972).

Silkworms were reared routinely on mulberry leaves at 25°C. Haemolymph was collected individually from five larvae 4–5 days after the final larval ecdysis and dealt with as reported previously (KIMURA, 1981 a). At this time these larvae showed a constant value of enzyme activities for α -mannosidase (KIMURA, 1981 b), for β -galactosidase (KIMURA, unpublished data), and for β -N-acetylglucosaminidase (KIMURA, 1977).

Glycosidase activities were assayed as described previously (KIMURA, 1981 a). One unit is expressed in terms of the amount which is capable of liberating 1 μ mole of *p*-nitrophenol from *p*-nitrophenyl derivatives of glycosides for 1 min at 35°C, and

Table 1. Correlation between silkworm strains and three glycosidases
Analysis of variance table

Source	<i>f</i>	<i>S</i>	<i>V</i>	<i>F</i> ₀
Total	734	21.5718		
A	48	4.3533	0.0906	18.90*
B	2	9.8115	4.9057	1022.40*
A * B	96	4.5856	0.0477	9.95*
Error	588	2.8213	0.0047	

A: Silkworm strains, B: Glycosidases.

f: Degree of freedom, *S*: Sum of square, *V*: Variance, *F*₀: Variance ratio.

Table 2. Glycosidases in geographically different races

Race	No. of variants	Enzyme activity (units/ml haemolymph)		
		α -Mannosidase	β -Galactosidase	β -N-Acetylglucosaminidase
Japanese	14	0.246 $\pm$ 0.111	0.215 $\pm$ 0.056	8.742 $\pm$ 3.149
Chinese	15	0.239 $\pm$ 0.132	0.186 $\pm$ 0.042	7.331 $\pm$ 2.443
European	16	0.331 $\pm$ 0.130 ^a	0.174 $\pm$ 0.035	7.385 $\pm$ 2.968
Tropical	4	0.158 $\pm$ 0.023 ^a	0.181 $\pm$ 0.016	7.168 $\pm$ 2.679

^a 1% level of significance.

for convenience sake the enzyme activity was defined as that corresponding to an increase of optical density at 420 nm per 20 μ l haemolymph from each strain.

The enzyme activity in each silkworm strain was described as the mean value and standard deviation of units per milliliter haemolymph instead of per mg protein for the reason previously reported (KIMURA, 1981 a).

Substrates were purchased from Sigma Chem. Co., St. Louis, U.S.A. and were dissolved in distilled water at a fixed concentration just before use.

RESULTS AND DISCUSSION

Based on the previous data (KIMURA, 1981 a), the enzyme activities from five larvae of each strain were individually determined and the mean and standard deviation recorded.

By measuring the enzyme activities from the forty-nine geographically different strains, the mean values of haemolymph glycosidases were found to be 0.26 $\pm$ 0.13 units/ml for α -mannosidase, 0.19 $\pm$ 0.04 units/ml for β -galactosidase, and 7.75 $\pm$ 2.82 units/ml for β -N-acetylglucosaminidase (Fig. 1). Distribution of β -galactosidase activity was the narrowest, and about 82 percent of the strains ranged from 0.12 to 0.24 units/ml. α -Mannosidase levels showed a marked bimodal distribution, while a normal distribution was seen for β -galactosidase and β -N-acetylglucosaminidase.

Table 1 represents the statistical analysis of a correlation between strains and the three glycosidases. Each strain showed a different activity ratio in the presence of the three glycosidases in haemolymph, suggesting that definite levels of the three enzymes in haemolymph were very characteristic for each strain.

Table 3. Extremely low or high activities of glycosidase in the silkworm

Strain	Enzyme activity (units/ml haemolymph)		
	α -Mannosidase	β -Galactosidase	β -N-Acetylglucosaminidase
Kuroshima (236)	0.103$\pm$0.053 (51.4)	0.162 $\pm$ 0.016 (9.9)	9.05 $\pm$ 1.75 (19.3)
Spain (312)	0.653$\pm$0.074 (11.3)	0.235 $\pm$ 0.030 (12.8)	10.86 $\pm$ 3.11 (28.6)
Yoko (232)	0.204 $\pm$ 0.112 (54.9)	0.118$\pm$0.016 (13.6)	5.41 $\pm$ 0.91 (16.8)
Haku (462)	0.443 $\pm$ 0.053 (12.0)	0.315$\pm$0.043 (13.7)	9.47 $\pm$ 0.51 (5.4)
No. 500 (317)	0.194 $\pm$ 0.065 (33.5)	0.119 $\pm$ 0.011 (9.2)	2.15$\pm$1.00 (46.5)
Wako (134)	0.368 $\pm$ 0.076 (20.7)	0.298 $\pm$ 0.025 (8.4)	16.98$\pm$1.85 (10.9)

Parentheses indicate coefficient variation (%).

When the geographic distribution of these glycosidases was recorded in detail, trends were observed only with α -mannosidase (Table 2). In general, the European race was higher in activity than Japanese, Chinese and Tropical races. Significant differences in the activity were especially seen between the European and Tropicals, though the number of Tropical strains examined was only four and the others fourteen, fifteen, or sixteen, respectively. It should be noted that the European race had been cultured at a considerably lower temperature than the Tropicals.

Strains with high or low glycosidase activities are shown in Table 3. Except for β -galactosidase differences in activity were about five to eight-fold between high- and low-enzyme silkworms. In light of the data on the selection of the silkworms showing either high or low activities of β -N-acetylglucosaminidase from a No. 500 strain (KIMURA, 1981 a), silkworms with enzyme activity at high levels of coefficient variation (>30%) may be in a heterozygous state for the enzyme concerned.

When three glycosidases were assayed from 130 strains including naturally or artificially induced mutants of *B. mori*, I could not find any enzyme-deficient silkworms. Judging from these results and the previous data to clarify the biological function of β -N-acetylglucosaminidase and α -mannosidase (KIMURA, 1977, 1981 a, 1981 b, 1982), it seemed that the insect glycosidases were not similar in physio-genetic character to those of mammals as reported by NISHIZAWA and HASHIMOTO (1970).

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