Comparative Studies on Esterase Zymograms among
Various Tissues in the Silkworm, *Bombyx mori* L.
(Lepidoptera : Bombycidae)

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By means of agar gel electrophoresis esterase zymograms of extracts from various tissues, singulary of individual larvae, were compared using a number of silkworm strains. Six major bands moving toward the anode were found with β -naphthyl acetate as the substrate and naphthanil diazoblue B as coupler. The fastest migrating intense bands A and B were detected in the mid-gut, and the less mobile esterase C was present in the silk gland. Esterases (E, F) detected in tracheas completely corresponded to integument esterase types. This phenomenon suggests that esterase bands in the integument and tracheas may be controlled by the same genes. The bands detected for the esterases in the integument correspond with the slow migrating bands of the Malpighian tubules. No similarity was noted among the esterase patterns obtained for the blood, mid-gut and silk gland and those of the integument or tracheae.

INTRODUCTION

Recently, several reports have been made on the existence of a number of electrophoretic variants and tissue specific patterns in zymograms of many organisms (SHAW, 1965). In the majority of genetic studies, however, whole body, serums or erythrocytes have been employed as an enzyme source, and the specificity of isozyme patterns in various tissues has been demonstrated by the mere comparison of zymograms in many cases.

We considered that the relationship of zymograms of extracts from various tissues will be made clearer by comparison of zymograms in different tissues of individual animals, providing some genetic knowledge.

In this report esterase zymograms of extracts from several tissues of silkworms are illustrated. The relationship among them is described together with substrate specificity of esterase bands and the possible significance of the results.

MATERIALS AND METHODS

Fifth instar larvae of 80 different strains of silkworm were used in the present study. These materials contained the F_2 from matings between strains which showed comparatively high variation in esterase types. After the blood was sampled by means of absorbing in cotton thread, the larval body was dissected. The internal organs and the integument were ground separately with an aliquot of distilled water in a mortar. Methods for preparation and electrophoresis of tissue homogenates and enzyme staining were essentially the same as those previously described (EGUCHI et al., 1965; EGUCHI and YOSHITAKE, 1966).

RESULTS AND DISCUSSION

Zymograms of extracts from several tissues of the silkworm, strain Keihaku, are shown in Fig. 1. For comparing zymograms of different tissue extracts

Fig. 1. Comparison of esterase zymograms of extracts from several tissues of silkworm larvae of the 6th day of 5th instar (strain: Keihaku) on the same agar gel using lyophilized samples. Bl: Blood, I: Integument, T: Trachea, Ma: Malpighian tubule, S: Silk gland, Mi: Mid-gut.

samples were placed on the same gel. The tissues investigated have one or more bands moving toward the anode. The blood has esterase with same migratory property to one of esterase components (E, F) present in the integument and the Malpighian tubules. Fast moving esterase bands (A, B) are found in the mid-gut. A single dense band (C) is present in the silk gland. The border of bands A and B is not clear in this photograph, but two distinct bands are detected when

the more diluted extract is employed.

Zymograms of extracts from different tissues in individuals were compared one by one using 360 larvae. As is apparent in Fig. 2, enzymes detected in trachea completely correspond to integument enzymes in esterase types. This phenomenon suggests that esterase bands in the integument and tracheas may be controlled by the same genes. Similar relationship is recognized between esterases in the integument and slow migrating bands in Malpighian tubules. On the contrary,

Fig. 2. Comparison of esterase zymograms of extracts from several tissues of individual larvae using raw materials. In each of six photographs the origin and cathode are at the right hand and the anode at the left. Abbreviations are the same as in Fig. 1.

correspondence in esterase types cannot be found among blood, mid-gut and silk gland, also between these organs and the integument or tracheas.

In view of the results described above it seems that the major esterase bands are closely related with the ontogenetic origin of tissues from which enzyme extracts were obtained. The zymogram from the extract of the silk gland differs with those from the integument, tracheas and Malpighian tubules, though this organ is of ectodermal origin as the others. This fact suggests that the silk gland may be the specially differentiated organ.

Lactate dehydrogenase was shown to exist as five isozymes within the tissues of the mouse, and it was described that tissues exhibit specificity in their patterns of isozymes, but this specificity is based upon the relative amounts, rather than upon the presence or absence of particular isozymes (MARKERT and MØLLER, 1959; MARKERT and URSPRUNG, 1962). Thus MARKERT and URSPRUNG (1962) pointed out that the basic control mechanism only regulates the relative rates of production of two polypeptides (A and B) within each cell in order to

generate all the isozyme patterns observed in mouse tissues. It appears that bands in different organs with similar migratory properties are considered to be controlled by same genes in the studies of lactate dehydrogenase isozymes.

In the silkworm, the mode of inheritance of isozymes detected from the integument esterase (EGUCHI and YOSHITAKE, 1966) is different from that of blood esterase (EGUCHI et al., 1965), though one band or two bands in the blood are similar to the integument enzymes in their positions in the agar gel.

Electrophoretic resolution of lactate dehydrogenase in mature testes from a variety of animals revealed one or more unusual isozymes in addition to the usual five forms, and it was indicated that lactate dehydrogenase synthesis in post-pubertal testis is controlled by more than two genes (ZINKHAM et al., 1963, 1964). Polymorphisms of phosphoglucose mutase (SPENCER et al., 1964) and glucose-6-phosphate dehydrogenase (SHAW and BARTO, 1965) in various organs of man or the deer mouse were reported, but these authors did not refer to the mode of inheritance of isozymes in different organs.

Esterase is known to have wide range substrate specificity. Therefore substrate and inhibitor specificities of silkworm esterases in various tissues were tested by the pH indicator method reported by OGITA and KASAI (1964). Phenyl acetate, methyl butyrate and tributyrin were hydrolysed by bands A, B and E, F. Band C split phenyl acetate and methyl butyrate, whereas band D could utilize only phenyl acetate.

Esterase activity of bands A, B, C and D was inhibited with 10^{-6} M paraoxon but was not sensitive to 10^{-3} M ethylenediamine tetraacetate or 10^{-4} M eserine sulfate. The activity of bands E and F was inhibited with 10^{-5} M eserine sulfate or 10^{-5} M paraoxon but was not sensitive to 10^{-3} M ethylenediamine tetraacetate. Bands E and F produced when acetylcholine was used as one substrate appeared to be weaker in intensity than those produced with methyl butyrate or tributyrin.

From the experiments described above and the results obtained by other workers (ASPEREN, 1959; AUGUSTINSSON, 1961; MENZEL et al., 1963), it may be concluded that bands A, B, C and D are aliesterase whereas bands E and F have properties of cholinesterase and aliesterase. These bands in each group are probably isozymes.

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