The Protein Components of a Latent Phenoloxidase System in the Prepupae of Housefly

Sir:

As reported previously, phenoloxidase undergoes a sharp fluctuation in the process of metamorphosis of the housefly, Musca vicina Maquart, from larvae to pupae. The high activity observed at the final stage of larval development disappears completely in the prepupae. However, a homogenate of prepupae, which exhibits no phenoloxidase activity, displays enzyme activity on the addition of an extract of aged pupae. From this fact, it was inferred that the phenoloxidase survived as a proenzyme in the prepupae and its activator was contained in the aged pupae. The activation of the proenzyme was also brought about either by treating the homogenate with anionic detergents or by dialyzing it against water.

Attempts have now been made to purify the prophenoloxidase. A homogenate of prepupae in 0.05 m phosphate buffer (pH 6.8) containing 0.9 per cent NaCl centrifuged and the supernatant aqueous solution containing the proenzyme was separated. The proenzyme was precipitated by saturation of the solution with ammonium sulfate, dissolved in 0.2 M phosphate buffer at pH 6.0 and subsequently dialyzed in the same buffer. The solution obtained was employed as crude prophenoloxidase (prophenoloxidase N) solution. By 0.4 saturation of the solution with ammonium sulfate a rather labile proenzyme (prophenoloxidase A) was precipitated leaving a factor in the supernatant, which can inhibit the activation of the proenzyme by its activator, presumably by stabilizing it. The inhibitory effect of this factor, which was tentatively designated as factor N, was lost on heating above 60°C. Fig. 1 shows the inhibitory effect of factor N on the activation of the proenzyme by the activator and the loss of inhibitory activity of factor N caused by heating. Factor N was non-dialyzable and assumed to be a protein from its behavior in the purification process, and because of its sensitivity to heat.

1) M. Funatsu and T. Inaba, This Journal, 26, 535 (1926).
4) unpublished. cf 17th Symposium on Enzyme Chemistry (Japan) 1965, p. 218.
As for the prophenoloxidase A, although it was fairly unstable, it was in an inactive state in 0.2 M phosphate buffer at pH 6.0 and low temperature unless any activating agent was present. This means that the prophenoloxidase A solution does not contain free activator. In addition, the proenzyme A exhibited only a single protein peak in its gel filtration pattern on Sephadex G-50. However, two protein peaks were separated when the prophenoloxidase A solution was dialyzed against water and subjected to a gel filtration through Sephadex G-50. Phenoloxidase was found in the second peak (F-2), while the first peak (F-1) exhibited no apparent phenoloxidase activity but was found to contain a protein which has the ability to activate the proenzyme as can be seen in Fig. 2. In Fig. 2, curve 1 shows the time course of activation of the prophenoloxidase with the activator obtained from the aged pupae. When a small amount of F-1 was added to the reaction mixture of the proenzyme with the activator, a remarkable acceleration of the rate of activation was observed (curve 2). This acceleration is not due to the presence of pro-enzyme in the F-1, since a theoretical curve (curve 4), which was calculated by adding curve 1 to curve 3, gave a considerably lower rate than that actually found in curve 2. In addition, an attempt was made successfully to activate the prophenoloxidase with fraction F-1 (curve 6). Thus, it is believed that an activator exists in the proenzyme system but in an inactive form. It is, however, still not known whether this activator is identical with that contained in the aged pupae.

Consequently, it appears that three types of protein participate in the organization of an inactive (latent or dormant) phenoloxidase system in the prepupae of housefly. It is already known that a metamorphosis hormone, ecdysone, is secreted increasingly at the final stage of larval development, resulting in the acceleration of the synthesis of an activator of prophenoloxidase. From this fact, and those reported here, it is possible to postulate that the activator formed at the end of the larval stage combines with unidentified form of prophenoloxidase forming an enzymatically inactive complex which is stabilized by binding to a certain protein, factor N. Such a latent phenoloxidase system might be passed along to the prepupae and stored as a source of phenoloxidase at the initial step of the metamorphosis of the insect. If this is correct, the possibility exists that such a system can participate in the regulation of the enzyme activity of living organisms.

Masaru Funatsu
Katsuya Hayashi
Gentatsu Namihira

Laboratory of Biochemistry,
Faculty of Agriculture,
Kyushu University