ACTIVATION OF PROPHENOLOXIDASE IN DROSOPHILA. VI. ANALYSIS OF ACTIVATING SYSTEM.

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In insect, phenoloxidase in hemolymph occurs as an inactive proenzyme and is activated upon bleeding. We have presented evidence showing that the activating enzyme (PPAE) is a serine protease.

Nature of the activation reaction has been analyzed with respect to effects of salts, pH dependency and other factors. Kinetic experiments revealed that the PPAE was rapidly inactivated during the activation reaction.

ACTIVATION OF PROPHENOLOXIDASE IN DROSOPHILA. VII. CHARACTERIZATION AND ACTIVATION OF A_1.

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Insect phenoloxidase exists as an inactive percursor (prophenoloxidase; proPO) and the proPO is converted to an active enzyme by an activating system. In Drosophila, it has been reported that proPOs are consisted of three A components: A_1, A_2 and A_3. We have so far confirmed the two isoforms: A_1 and A_3.

Procedure for the purification of A_1 was improved. It consisted of ammonium sulfate fractionation, DEAE-cellulose, chromatofocusing and phenyl Sepharose column chromatography. Purified A_1 migrated as a single band on SDS-PAGE. Using the homogeneous samples, properties of the protein were studied.

ACTIVATION OF PROPHENOLOXIDASE IN DROSOPHILA. VIII. PURIFICATION AND CHARACTERIZATION OF A_3.

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Insect phenoloxidase occurs as an inactive proenzyme (prophenoloxidase; proPO), which is converted to the active enzyme by the activating enzyme. In Drosophila, there are two molecular species of the proPO, designated as A_1 and A_3. They are distinguishable by ammonium sulfate fractionation and native-PAGE. A_3 was purified by ammonium sulfate fractionation, Sephacryl S-200, DEAE-cellulose and hydroxylapatite column chromatography. The properties of A_3 including molecular weight, isoelectric point, thermostability, pH stability and substrate specificity of the activated proPO were studied.

ACTIVATION OF PROPHENOLOXIDASE IN DROSOPHILA. IX. ACTIVATION OF A_1 WITH 2-PROPANOL.

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In D. melanogaster, A_1 component of the prophenoloxidase (proPO) could be activated with both an endogeneous activating system (AMM-1) and organic compounds including alcohols. In the activation of A_1 with alcohols, 2-propanol was the most and glycerol was the least effective among the alcohols tested. A_1 was activated within 2 min after the addition of 2-propanol. Rate of activation and final yield of the PO activity depended on the concentration of 2-propanol. When the concentration of 2-propanol was lowered by dilution, PO activity decreased gradually. Upon re-addition of 2-propanol to this diluted mixture, PO activity re-elevated. Thus the reversibility of the activation of A_1 in response to the alteration of the concentration of 2-propanol could be observed.

The maximum level of the PO activity, which had been activated with 2-propanol, was higher than that activated with AMM-1. Optimum concentration of 2-propanol for the rate of activation was 50 %.

The activated state of A_1 showed properties of a tyrosinase-type. The results indicate that the activation of A_1 with 2-propanol is caused by the reversible conformational change of the proPO molecule.