Esposito, V.M. and V. Ulrich. West Virginia University, Morgantown, West Virginia. Characterization of D. melanogaster acid phosphatase.

Non-specific acid phosphatases have been found in many tissues and in many organisms (Schmidt, 1961). Although evidence for their existence has been accumulating since 1907, real interest did not develop in them until the discovery of bone enzyme by Robinson in 1923. Walker, Lemon, Davison and Schwartz (1954) concluded on the basis of an extensive literature survey, that acid phosphatase consisted of an inclusive group of enzymes with different substrate specificities. Electrophoretic variants or isoenzymes of acid phosphatase, their hybrid nature and their genetics have been presented by MacIntyre (1966).

Adult Drosophila
↓
Macerate in 0.45 sucrose
Centrifuge at 650 X g for 5 min.
↓
Pellet — Supernatant
Treat with 40% $(NH_4)_2SO_4$ saturation
↓
Stir at 4°C for 1 hour
Centrifuge at 12,000 X g for 20 min.
↓
Pellet — Supernatant
Add $(NH_4)_2SO_4$ to 70% saturation
↓
Stir at 4°C for 1 hour
Centrifuge at 12,000 X g for 20 min.
↓
Pellet — Supernatant (discard)
↓
Wash with 70% $(NH_4)_2SO_4$ - Centrifuge at 12,000 X g
Repeat 3 times for 20 min.
↓
Pellet
↓
Put into solution with 0.45 M sucrose
Dialyze at 4°C for 8 hours
↓
Protein Solution
↓
Acid Phosphatase preparation
Disc electrophoresis
Section containing acid phosphatase activity cut out and eluted with 0.45 M sucrose
Centrifuged at 12.000 X g for 20 min.
↓
Electrophoretically purified acid phosphatase

Fig. 1 Isolation and purification of acid phosphatase.

Cultures of D. melanogaster were reared and maintained on a standard cornmeal-agar medium that had been seeded with live yeast. Crude enzyme preparations were obtained in two ways. Individual larva were macerated in 0.05 ml of 0.45 M sucrose buffer with a micro-mortar and pestle and centrifuged at 650 X g for 2 minutes at 4°C. These were used for all experimental assays. Larger quantities of material were prepared by macerating 10 grams of adult flies in 20 ml of 0.45 sucrose buffer containing 0.5 grams Norit A. This mixture was then centrifuged at 650 X g for 5 minutes. The resulting sediment was discarded, and the supernatant, after filtering through glass wool, was used either directly as a crude preparation for the assays or further purified by $(NH_4)_2SO_4$ precipitation and electrophoresis (Figure 1). The enzyme was assayed by a modified method originally described by Lawrence, Melnick, and Weiner (1960).

Polyacrylamide gel columns, as described by Ornstein and Davis (1964), were used for electrophoretic purification of enzyme. Glass cylinders 65 mm in length and 7 mm inner diameter were used. To avoid heat inactivation of the enzyme electrophoresis was performed in a cold room (4°+1°C) with 3 milliampers per column. The running time was 90 minutes.

The principal criteria employed in separating and classifying phosphatases is their specificity to substrates, pH optimum and activity response to divalent cations (Schmidt, 1961). The pH optimum was determined on sodium alpha-napthyl acid phosphate. The buffers used to cover the pH range from 3.8 to 10.0 were acetate (pH 3.8-5.8), phosphate (pH 5.6-8.0)

and tris (pH 7.0-10.0). A pH of 5.0 was found to be optimum for this system.

Since various phosphatases require metal ions for activity, assays were conducted to determine the necessity of these as cofactors. Mg^{++} (magnesium chloride), Mn^{++} (manganous chloride) and Zn^{++} (zinc chloride) were introduced separately and in various combinations to the reaction mixture in concentrations of 1 to 10 mM. The divalent cations neither activated nor inhibited this system when either crude or purified enzyme preparations were used.

The substrate specificity of both crude and electrophoretically purified enzyme preparations was tested by use of the coupling technique (Burstone, 1958) against eight substrates, i.e., sodium alpha-naphthyl acid phosphate, naphthol As, naphthol AS-An, naphthol AS-E, naphthol AS-GR, napthol AS-Mx, naphthol AS-TR and naphthol AS-Bl. No substrate preference could be found. The enzyme system also reacted in the same way in every test when Mg^{++}, Mn^{++}, Zn^{++}, sodium fluoride and tartaric acid were added either individually or in combination. Demonstration of enzyme activity on all eight substrates indicates that this system is non-specific. A comparable acid phosphatase system, which also displayed non-specific activity, has been reported in the slime mold Dictyostelium discoideum (Gezelius, 1966). Therefore, on the basis of a pH optimum of 5, a general substrate specificity, and a lack of inhibition and activation by divalent cations, this enzyme system is classified as a phosphomonoesterase.

Inhibitors are the most characteristic modifying factors of phosphatases and sodium fluoride and tartaric acid are most commonly used to distinguish various phosphomonoesterases. These were used in concentrations of 10 mM with crude and with $(NH_4)_2SO_4$ and electrophoretically purified enzyme preparations. The inhibitors were added directly to the reaction mixture in one series of experiments, but in another series the enzyme preparations were incubated with the inhibitors prior to the addition of the reaction mixture. Sodium fluoride and tartaric acid caused complete inhibition in every experiment. The addition of the divalent cations Mg^{++}, Mn^{++} and Zn^{++} did not alter this inhibition. Therefore, on the basis of a pH optimum of 5, general substrate specificity and complete inhibition by sodium fluoride and tartaric acid this enzyme system is classified as a phosphomonoesterase II (E.C. 3.1.3.2).

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Minamori, S. and K. Ito, Hiroshima University, Japan. Mutagenic action of extrachromosomal element delta in D. melanogaster.

It was found that an extrachromosomal element denoted by delta in D. melanogaster may induce frequent lethal and semi-lethal mutations on the second chromosome. The average mutation rate induced on the chromosome carrying Dmb gene (allows the multiplication of delta) was about 9 percent when combining lethals and semi-lethals, and ignoring the clustering of lethals. While the rate in the chromosome without the Dmb gene was clearly not so high, even in the presence of delta.

Several instances of mutation cluster were observed. In four clusters, all lethals recovered from a single parent were allelic with each other. These findings lead to the conclusion that the mutation induced by delta may occur at the pre-meiotic cell stage, possibly in an early embryonal stage of the carrier.

A total of 113 lethal genes originating independently were found to locate at 27 different sites on the chromosome. The locations of these sites were determined and it was found that the distribution pattern of lethals along the chromosome was unique in contrast to the pattern reported by Ytterborn (1968) in the lethals induced by X-ray. The lethals were strongly concentrated at 0-10 (13.3%), 55-65 (30.0%) and 70-85 (50.4%) of the genetic map-unit, and none were located in the left part of centromere.

Reference: Ytterborn, K.H., 1968, Hereditas 59: 49-62.