ACID PHOSPHATASE ACTIVITY OF THE ALIMENTARY TRACT OF THE LARGE MILKWEED BUG, ONCOPELTUS FASCIATUS DALLAS (HEMIPTERA: LYGAEIDAE)

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A non-specific acid phosphatase from the alimentary tract of the milkweed bug, *Oncopeltus fasciatus* Dall., has an optimum pH range of 4.5 to 4.7, kinetics of zero order for 30 minutes incubation, and the maximum substrate concentration giving a zero order reaction is 0.00017 M (final). It has a Km value of 1.74×10^{-3} and an optimum temperature of 40°C. (30 minute incubation). It is activated by Mg++ and Mn++ ions.

Introduction

The objective of this investigation was to discover the optimum biochemical conditions for acid phosphatase activity in the digestive system of the large milkweed bug, *Oncopeltus fasciatus* Dall. Undoubtedly non-specific acid phosphatase occurs elsewhere in the milkweed bug as it does in other insects but for comparative purposes this study was limited to its presence in the alimentary canal.

Procedure

The matreials and methods as described by Ashrafi and Fisk [1,2] for stable fly phosphatase were employed. Briefly, 2 ml. of citrate buffer of pH 4.6, 0.5 ml. of 0.00143 M disodium *p*-nitrophenyl phosphate substrate and 2 ml. of distilled water were added to each of several tubes in an ice bath. A homogenate containing 6 alimentary tracts of adult milkweed bugs per 10 ml. was prepared and 0.5 ml. added per chilled tube. A 1-ml. aliquot was removed from each tube for a zero time sample, then the tubes were incubated 30 minutes at 40°C. Following the incubation, the *p*-nitrophenol resulting from phosphatase activity was made alkaline with sodium hydroxide and its optical density measured at 390 mμ with a Spectronic 20 photometer. Zero time readings were subtracted from incubated tube readings and the corrected optical densities were converted to micromoles *p*-nitrophenol using an experimentally established standard curve.

Results and Discussion

Effect of Varying pH.—Substrate and homogenate concentrations were kept constant. Citrate buffer solutions of pH 4.5, 4.2, 4.5, 4.6, 4.7, 4.8, 5.2, 5.5, and 6.0 were used. Data obtained is plotted in Fig. 1 which shows that the effective pH range lies between 4.5 and 4.7 with an optimum at pH 4.6. This pH was maintained throughout the rest of the investigation. Denuce[3] reported a pH optimum of 4.2 for silkworm acid phosphatase. Lambremont[4] found a pH range of 4.6 to 5.3 for the acid phosphatase of *Aedes aegypti* mosquitoes, and Ashrafi and Fisk[2] reported pH 4.4 as the optimum for stable fly acid phosphatase.

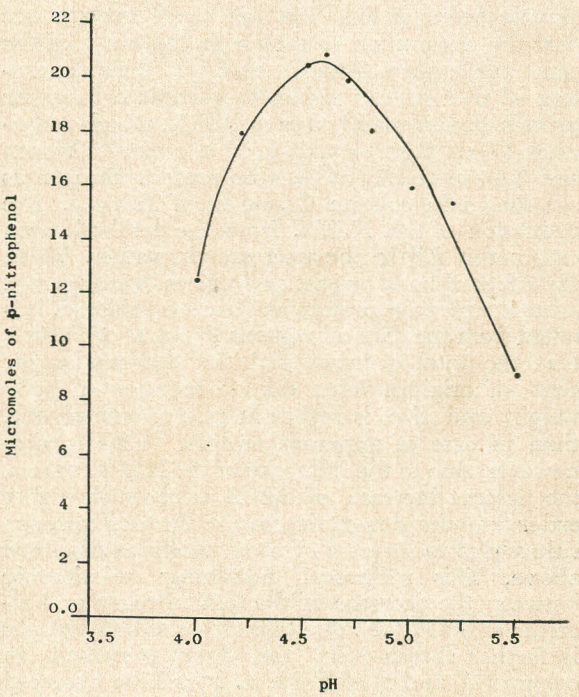

Fig. 1.—Curve for optimum pH range.

Effect of Varying Enzyme Concentrations.—Homogenate concentrations of 0.2, 0.4, 0.6, 0.8 and 1 ml. were employed. The volume of the basic reaction mixture was kept constant by adding appropriate amounts of water. A zero order reaction was maintained throughout this range of enzyme concentrations, cf. Fig. 2. Similar findings were reported by Lambremont[4] in the case of *Aedes*

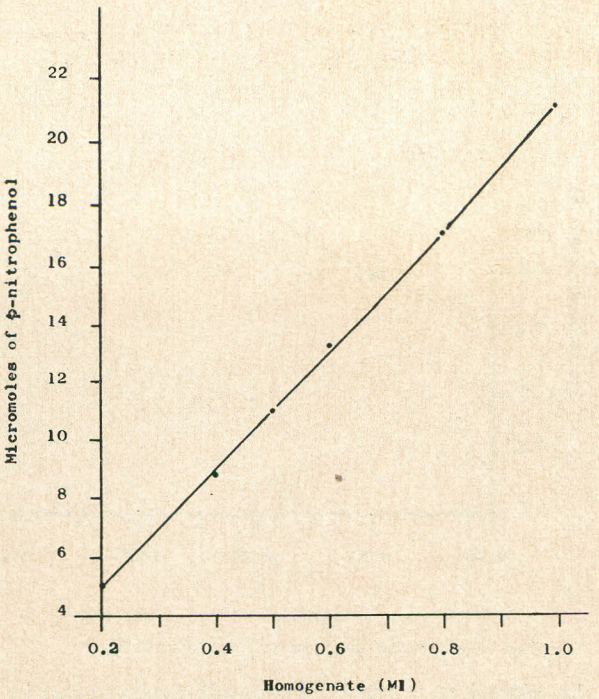

Fig. 2.—Effects of varying enzyme concentrations.

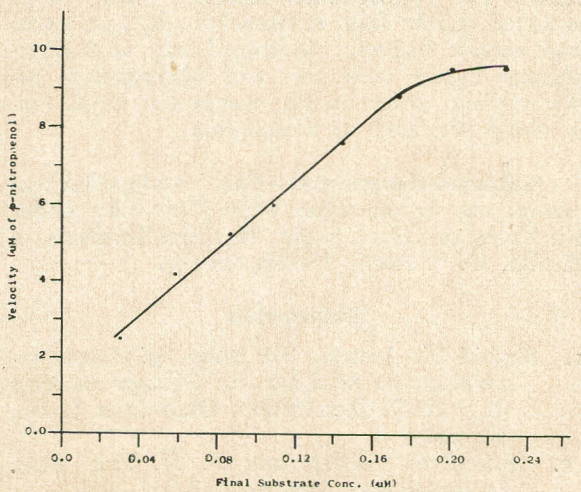

Fig. 3.—Effect of various substrate concentrations

aegypti and Ashrafi and Fisk[2] with *Stomoxys calcitrans*.

Effect of Varying Substrate Concentrations.—Eight different concentrations of substrate, disodium *p*-nitrophenyl phosphate, ranging from 0.0286 to 0.249 micromoles final concentration were used. The results as shown in Fig. 3 indicate that with substrate concentrations up to about 0.17 μM the reaction was of zero order, e.g., a linear response to increased substrate concentration, but beyond this value a first order reaction resulted.

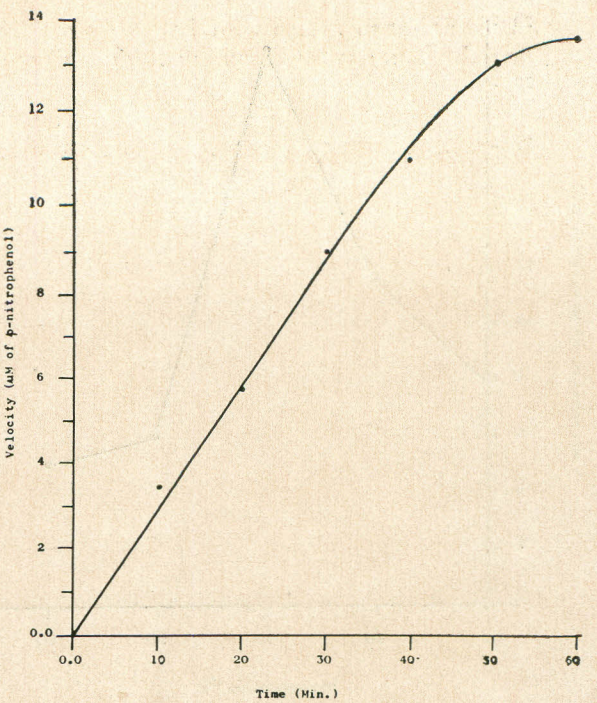

Fig. 4.—Effect of varying incubation timings.

Ashrafi and Fisk[2] using the same substrate reported a zero order reaction up to a final concentration of 0.9 μM with stable fly phosphatase. The K_m value, Michaelis constant, calculated by the method of Lineweaver and Burk,[5] was found to be 1.74×10^{-3} molar. Lambremont[4] reported a K_m of 8.57×10^{-2} for the yelllow fever mosquito and Ashrafi and Fisk[2] found a K_m of 9.77×10^{-4} for the acid phosphatase of the stable fly.

Effect of Different Incubation Periods.—One ml. aliquots were removed from the incubating reaction mixture at 10 minute intervals up to one hour and the amounts of resulting *p*-nitrophenol were measured. As shown in Fig. 4, the data shows that the reaction is essentially of zero order for the first 30 minutes. The results beyond 40 minutes indicate a shift to a first order reaction. Rockstein[6,7] found the kinetics of acid phosphatase in the house fly to be quite similar, that is a zero order reaction through 30 minutes, but shifting to first order beginning at about 40 minutes. A similar pattern was reported by Ashrafi and Fisk[2]. However, Fitzgerald[8] working with an alkaline phosphatase in the eggs of the grass-hopper *Melanoplus differentialis* stated that the zero order reaction was maintained for more than an hour.

Effect of Temperature Variation.—Water baths were set up to maintain temperatures of 20, 30, 40, 50 and 60 °C. and phosphatase activity was determined after 30 minutes incubation at these

Fig. 5.—Effect of temperature variation.

temperatures. The results presented in Fig. 5 show an increase in activity with increasing temperature up to 40°C., followed by a sharp decrease. Therefore 30°C. was the apparent optimum temperature under our conditions of test. This figure agrees with the results of Rockstein[6,7] and Ashrafi and Fisk.[2] Lambremont[4] found an optimum of 45°C., while Fitzgerald[8] employing a longer incubation period and alkaline pH reported a temperature optimum of 35°C.

Activation by Metal Ions.—To each separate 4.5 ml. portions of standard reaction mixture were added 0.5 ml. of 0.01 M concentration of each of the following ions: Ca^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, and Fe^{++}. Activation of the phosphatase enzyme was shown by Mg^{++} and Mn^{++}. With various concentrations of added magnesium ion the data shown in Fig. 6 was obtained. A concentration of 0.07 M added Mg^{++} (or 0.007M final concentration) gave the maximum activation of acid phosphatase enzyme from the alimentary tract of the large milkweed bug. Lambremont[4] found the maximum activation of *Aedes aegypti* acid phosphatase at 0.08 M added Mg^{++}. Ashrafi and Fisk[2] found no activation with magnesium or any of the other metal ions tested with stable fly acid phosphatase. However, with stable fly alkaline phosphatase Ashrafi[1] reported activation with Mg^{++} and Fe^{++}, the optimum concentration for Mg^{++} being 0.01 molar.

On the basis of pH profile and magnesium activation, the non-specific acid phosphatase may

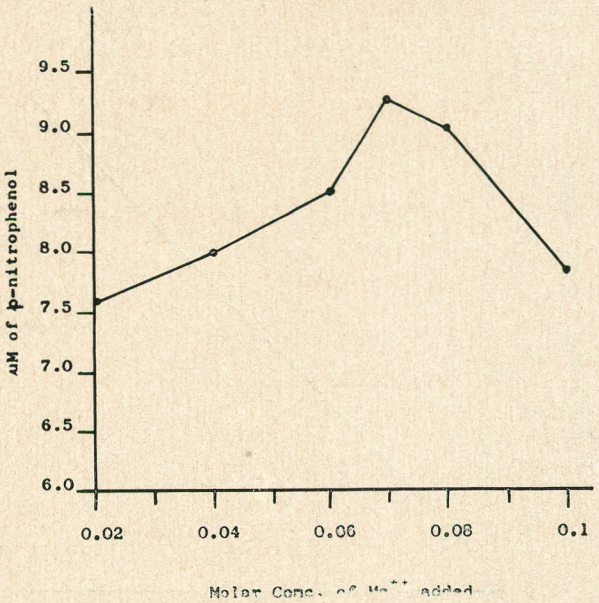

Fig. 6.—Curve for optimum concentration of Mg^{++}.

be placed as a class IV phosphomonoesterase in the classification of Roche,[9] although Roche's class IV phosphomonoesterase has been reported chiefly from erythrocytes, bottom yeasts and certain bacteria. Milkweed bug acid phosphatase agrees with the acid phosphatases reported from other insects, except the stable fly, in being activated by magnesium.

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References

1. Shahid H. Ashrafi, *The Study of Phosphomonoesterases in the Stable Fly, Stomoxys calcitrans* (L.), Ph.D. Dissertation, Ohio State Univ., 101 pp.
2. Shahid H. Ashrafi and F. W. Fisk, Ann. Entomol. Soc. Am., **54**, 598-602 (1961).
3. J. M. Denuce, Experientia, **8**, 64,65 (1952).
4. E. D. Lambremont, J. Insect Physiol., **3**, 325-334 (1959).
5. H. Lineweaver and D. Burk, J. Am. Chem. Soc., **56**, 658-666 (1934).
6. M. Rockstein, Bull. Brooklyn Ent. Soc., **51**, 8-17 (1956).
7. M. Rockstein and P. W. Herron, J. Cellular Comp. Physiol., **38**, 351-368 (1951).
8. L. R. Fitzgerald, J. Exp. Zool., **110**, 461-487 (1949).
9. J. B. Summer and K. Myrback (editors), *The Enzymes* (Academic Press Inc., N. Y., 1950), Vol. 1, Chapter II: *Phosphatases* by J. Roche.