ACID PHOSPHATASE ACTIVITY IN THE DIGESTIVE SYSTEM OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (FORSKÅL)

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Summary

Acid phosphatase activity was determined biochemically in a homogenate of the digestive tract of the desert locust, *S. gregaria*. *p*-Nitrophenol was used as colorimetric standard and disodium *p*-nitrophenyl phosphate as substrate.

The enzyme was found to have a linear relationship between concentration and activity, maximum activity at pH 4·4, a Michaelis constant of $3{\cdot}0 \times 10^{-4}$M, and a temperature coefficient (Q_{10}) of 2·10 between 20 and 30°C. Zero-order kinetics were maintained for 35 min at 40°C. Magnesium, calcium, and potassium ions and magnesium and phenylmercuric acetates slightly activated the enzyme, while ferrous ion, sodium fluoride, disodium hydrogen phosphate, and disodium hydrogen arsenate inhibited the enzyme. The magnesium ion concentration required for maximum activation was found to be 0·025M.

I. Introduction

Phosphomonoesterases or phosphoric monoester hydrolases (3.1.3, Thompson 1962) are widely present in animal and plant tissues. During the last two decades work on biochemical characterization of phosphatases in insects has been done by Drilhon and Busnel (1945), Fitzgerald (1949), Rockstein and Herron (1951), Rockstein and Levine (1951), Denucé (1952), Rockstein (1956), Barker and Alexander (1958), Lambremont (1959), Ashrafi and Fisk (1961, 1962), Hodgson (1963), Sridhara and Bhat (1963), Gilbert and Huddleston (1965), McCaman, Smith, and Cock (1965), Raychaudri and Butz (1965), Hirano and Gilbert (1967), and Naqvi, Ashrafi, and Qadri (1967).

In general these authors have studied the phosphomonoesterases in homogenates of holometabolous insects, whereas the present work deals with the biochemical characterization of acid phosphatase in the alimentary canal of a hemimetabolous insect, the desert locust, *Schistocerca gregaria* (Forskål).

II. Materials and Methods

Enzyme Source

Adult locusts (28 days old) which had been fed on 6% glucose solution for 1 day prior to the experiment were selected as the source of enzymes. Locusts were dissected in cold double-distilled demineralized water and the alimentary canal was transferred to a test tube containing 5 ml chilled double-distilled demineralized water. It was ground for exactly 3 min in a Teflon–Pyrex tissue grinder. The homogenate was filtered through a 2-mm layer of glass fibre in a

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Gooch crucible under suction (moderate pressure), and the filtrate collected at 0°C. The filtrate was diluted to 10 ml by adding 5 ml of cold double-distilled demineralized water. The homogenate thus represented a 1:10 dilution of the enzyme originally present in the alimentary canal.

Enzyme Assay

Fresh homogenate (0·5 ml) was added to the reaction mixture containing 2 ml citric acid–sodium chloride buffer (pH 4·4, final concentration 0·036M), 2 ml double-distilled demineralized water, and 0·5 ml disodium *p*-nitrophenyl phosphate (Sigma substrate 104 R) at a final concentration of 1·43 mM. The mixture was incubated for 30 min at 38°C. The remainder of the procedure was as described by Naqvi, Ashrafi, and Qadri (1967). Preliminary experiments had shown that the rate of reaction was linear with respect to enzyme concentration and limited with respect to time. The reaction followed zero-order kinetics for 35 min after which it showed first-order kinetics. Each experiment was repeated five times.

For tests of activation or inhibition of the enzyme, 0·5 ml of both the enzyme solution and the ionic solution were used, and the reaction mixture was made up to a total volume of 5·0 ml by adding the appropriate volume of double-distilled cold demineralized water. The concentrations of the ionic solutions used are given in Table 1.

TABLE 1

INFLUENCE OF ACTIVATING AND INHIBITING IONS ON ACID PHOSPHATASE ACTIVITY

Compound	Final Concn. (moles/l)	Acid Phosphatase Activity*	Compound	Final Concn. (moles/l)	Acid Phosphatase Activity*
Activating ions			Inhibiting ions		
Magnesium chloride	0·01	32·8	Cadmium chloride	0·01	25·8
Potassium chloride	0·01	31·2	Cupric sulphate	0·100	25·8
Magnesium acetate	0·01	29·9	Zinc sulphate	0·001	24·8
Phenylmercuric acetate	0·001	29·9	Mercuric acetate	0·01	23·7
Calcium chloride	0·01	29·4	Sodium cyanide	0·100	23·7
Sodium chloride	0·01	28·0	Mercuric chloride	0·01	22·9
Barium chloride	0·01	28·0	Manganous chloride	0·01	20·7
			Aluminium chloride	0·001	19·9
Control		27·4	Calcium acetate	0·01	18·8
			Cobaltous chloride	0·01	16·4
			Cupric chloride	0·01	10·2
			Disodium hydrogen arsenate	0·100	7·8
			Disodium hydrogen phosphate	0·100	5·9
			Sodium fluoride	0·100	5·9
			Ferrous chloride	0·01	0·6

* Measured as number of μmoles *p*-nitrophenol liberated.

III. RESULTS

(*a*) *Substrate Effects*

During the preliminary studies four substrates were tested, and among them *p*-nitrophenyl phosphate was found to be hydrolysed three times as rapidly as β-glycerophosphate, and five to seven times as rapidly as glucose-1-phosphate and glucose-6-phosphate. *p*-Nitrophenyl phosphate was used as substrate in all further work reported here.

(*b*) *pH for Maximum Activity*

Maximum activity of acid phosphatase was recorded at pH 4·4 (Fig. 1) in homogenates from both male and female locusts.

(*c*) *Substrate Concentration*

The relationship between activity and substrate concentration was found to be of the classical Michaelis–Menten type. It is expressed by means of a double reciprocal plot (Lineweaver and Burk 1934) in Figure 2. The Michaelis constant (K_m) was calculated to be $3{\cdot}0 \times 10^{-4}$M for a 30-min incubation period.

(*d*) *Temperature Effects*

The temperature which gave maximum activity was found to be 40°C for a 30-min incubation period and the curve (Fig. 3) followed the Arrhenius law. The temperature coefficient (Q_{10}) was calculated from Figure 4 to be 2·10 between 20 and 30°C.

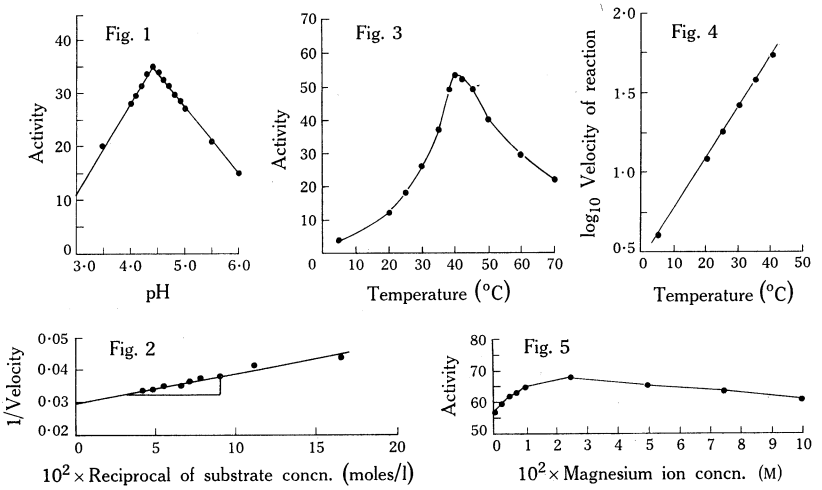

Fig. 1.—Optimum pH for acid phosphatase activity (expressed as μmoles of *p*-nitrophenol liberated). Citric acid buffer used.

Fig. 2.—Lineweaver–Burk plot showing calculation of K_m value for acid phosphatase.

Fig. 3.—Effect of temperature on acid phosphatase activity (expressed as μmoles of *p*-nitrophenol liberated).

Fig. 4.—Temperature coefficient curve for acid phosphatase.

Fig. 5.—Optimum magnesium ion concentration for maximum activation of acid phosphatase.

(*e*) *Enzyme Activation and Inhibition*

A number of ions were tested for activation or inhibition of acid phosphatase (Table 1). Magnesium chloride, potassium chloride, magnesium acetate, and phenylmercuric acetate slightly enhanced the activity. Sodium chloride and barium chloride had no effect. Ferrous chloride, sodium fluoride, disodium hydrogen phosphate, disodium hydrogen arsenate, and cupric chloride potentially inhibited the enzyme.

Cobaltous chloride, calcium acetate, aluminium chloride, and manganous chloride moderately inhibited the activity, while mercuric chloride, sodium cyanide, mercuric acetate, and zinc sulphate had a slight inhibitory effect. Cupric sulphate, cadmium chloride, and calcium chloride showed negligible effects.

(*f*) *Optimum Magnesium Ion Concentration*

The optimum magnesium ion concentration for maximum activation was found to be 0·025M, beyond which it started inhibiting the activity (Fig. 5).

IV. Discussion

This paper reports the first detailed biochemical characterization of acid phosphatase in a hemimetabolous insect, *S. gregaria*.

The optimum pH for activity of the enzyme is compared with values for acid phosphatases from other insects in the following tabulation:

Optimum pH	Source of Enzyme	Reference
4·4	Desert locust	Present study
4·2	Silkworm	Denucé (1952)
4·8–5·0	Housefly	Alexander, Barker, and Babers (1958)
4·6–5·3	*Aedes aegypti* (L.)	Lambremont (1959)
4·4	Stable fly	Ashrafi and Fisk (1961)
4·2	Silkworm (developing adult)	Huddleston and Gilbert (1961)

The relationship between activity and substrate concentration was similar to that reported by Lambremont (1959), Ashrafi and Fisk (1961), and Gilbert and Huddleston (1965). The K_m value ($3{\cdot}0\times10^{-4}$M) compares well with that of acid phosphatase from stable fly ($9{\cdot}7\times10^{-4}$M) and giant milkweed bug ($1{\cdot}74\times10^{-3}$M) reported by Ashrafi and Fisk but not with that of enzyme from *A. aegypti* ($8{\cdot}57\times10^{-2}$M). This is due either to the substrate used or to the nature of the enzyme.

Maximum hydrolysis of the substrate occurred at 40°C which agrees well with the values of 40°C reported by Ashrafi and Fisk (1961) and 37·5°C reported by Gilbert and Huddleston (1965). The Q_{10} value (2·1) between 20 and 30°C is also normal and close to the values reported by Lambremont (1959), Ashrafi and Fisk (1961), and Gilbert and Huddleston (1965).

In the present case magnesium chloride activated the enzyme slightly. Fitzgerald (1949), Rockstein and Herron (1951), Rockstein (1956), Lambremont (1959), Ashrafi and Fisk (1962), and Gilbert and Huddleston (1965) have also reported magnesium ion as an activator of acid phosphatases. The optimum concentration of magnesium ion for enzyme activation was found to be 0·025M. Fitzgerald (1949) reported an optimum magnesium ion concentration for acid phosphatase activation of 0·05M, Lambremont (1959) reported 0·08M, and Rockstein (1956) reported 0·16–0·20M. Thus the acid phosphatase of the desert locust required the least concentration for maximum activation. This is perhaps due to the substrate used.

The enzyme activity was greatly inhibited by ferrous chloride, sodium fluoride, disodium hydrogen phosphate, disodium hydrogen arsenate, and cupric chloride, and among them ferrous ion caused 100% inhibition. Lambremont (1959) reported inhibition by fluoride, and Macdonald (1961) by argentous, cupric, and ferrous ions. Gilbert and Huddleston (1965) also reported inhibition by fluoride, phosphate, and arsenate. But Ashrafi and Fisk (1961) found that ferrous and other ions had no effect on the activity of acid phosphatase from stable fly. This is surprising, as the same substrate was used in the present study. This indicates that the acid phosphatase of desert locust is not completely identical with that of stable fly. However, on the basis of pH for maximum activity (4·4), slight activation by magnesium ion, and other values characterized during the present investigation, acid phosphatase of desert locust can be placed in category 3.1.3.2 of Dixon and Webb (1964), or category III in the scheme of Roche (1950).

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