ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVII, nr 1—2
1978

Phosphatase activity of *Poa pratensis* seeds*

III. Effect of fluoride, citrate, urea and other substances on the activity of acid phosphatase Ia_2 and Ia_3

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(Received: November 3, 1977)

Abstract

Effects of fluoride, citrate, urea and other substances on the activity of acid phosphatase a_2 and a_3 toward p-nitrophenylphosphate and phenylphosphate were investigated. Both enzymes were inhibited by fluoride, p-chloromercuribenzoate and oxalate. Fluoride inhibited acid phosphatase a_2 noncompetitively with p-nitrophenylphosphate, whereas acid phosphatase a_3 showed inhibition of mixed type. Hydrolysis of phenylphosphate by both acid phosphatases was activated by citrate. Cytosine and uridine inhibited the activity of phosphatase a_2 toward p-nitrophenylphosphate and phenylphosphate, but no effect was observed in case of acid phosphatase a_3. After 30 min. incubation with 4 M urea both enzymes lost about 30% of activity.

INTRODUCTION

Molecular forms of acid phosphatases occuring in fungi and higher plants differ with respect to their location in cell structures (Wakao et al. 1975, Chen et al. 1975, Parish 1972), molecular weight (Kruzel 1977), and catalytic properties (Verjee 1969).

Isolation, purification, and preliminary characteristic of the two forms of acid phosphatase of *Poa pratensis* seeds have been given in the previous paper (Lorenc-Kubis et al. 1975). It has been shown that acid phosphatase a_2 and a_3 is characterized by low substrate specificity and inhibition of the type caused by zinc ions. The present work con-

* This work has been partly financed from the problem MR-II/15.

stitutes a continuation of studies on these enzymes. It was aimed at determining the type of inhibition caused by fluoride ions, and at studying the effect of citrate, urea and other substances on the activity of both enzymes.

MATERIAL and METHODS

Studies were carried out on acid phosphatase a_2 and a_3 isolated from seeds of meadow-gras *(Poa pratensis)*, according to the method described in the previous paper (Lorenc-Kubis et al. 1975).

Specific activity of each enzyme amounted to 10 units/mg of protein. Proteins were determined with turbidimetric tannin micromethod according to Mejbaum-Katzenellenbogen (1955). Activity of acid phosphatase was determined measuring p-nitrophenyl or inorganic phosphate liberated either from p-nitrophenylphosphate or from phenylphosphate after 10 min and 30 min in 37°C pH 5.1, according to the method described previously (Lorenc-Kubis et al. 1975).

One unit of phosphatase activity represents 1 μmol of p-nitrophenyl or inorganic phosphate liberated from the substrate during 1 min in 37°C in 0.1 M acetate buffer, at pH 5.1.

RESULTS and DISCUSSION

Table 1 presents the effect of various substances on the activity of acid phosphatase a_2 and a_3 toward the substrates p-nitrophenylphosphate and sodium phenylphosphate. Fluoride ions inhibited in 70% the activity of acid phosphatase a_2 and a_3 toward p-nitrophenylphosphate (Lorenc-Kubis et al. 1975), and in 80% the activity of each enzyme toward phenylphosphate. Fluoride ions constitute uncompetitive inhibitors for many acid phosphatases of plant origin (Yoshida and Tamiya 1971, Uehara et al. 1974).

Inhibition of the activity of acid phosphatase a_2 by fluoride ions was of a noncompetitive character, whereas in case of acid phosphatase a_3 — of the mixed type (Fig. 1 and 2). Estimated values of K_m and K_i for acid phosphatase a_2 amount to 1.66×10^{-3}, and for acid phosphatase a_3 to: K_m 6.66×10^{-4} and K_i 1.54×10^{-3}. Also p-chloromercuribenzoate and oxalate ions inhibted the enzymes under study (Tab. 1) In the presence of 2×10^{-5} M pCMB acid phosphatase a_2 retained 62-70% of its total activity, and acid phosphatase a_3 — 70-80%. Decrease of the activity of both enzymes under the effect of pCMB most probably shows that free sulfhydryl groups of the enzymes participate in the

hydrolysis of the substrates. Similar result of the inhibition caused by pCMB was found in case of two acid phosphatases E_{II} and E_{III} isolated from wheat germs (V e r j e e 1969), as also in case of acid phosphatase Ib and Ic of rice bran (I g a u e et al. 1975).

Cytosine, as well as uridine, decrease the activity of acid phosphatase a_2 toward both substrates under study. On the other hand guanine inhibits the activity of acid phosphatase a_2 toward p-nitrophenylphosphate, and of acid phosphatase a_3 toward phenylphosphate, in about 20%. Ribose activates the effect of acid phosphatase a_2 toward p-nitrophenylphosphate (Tab. 1).

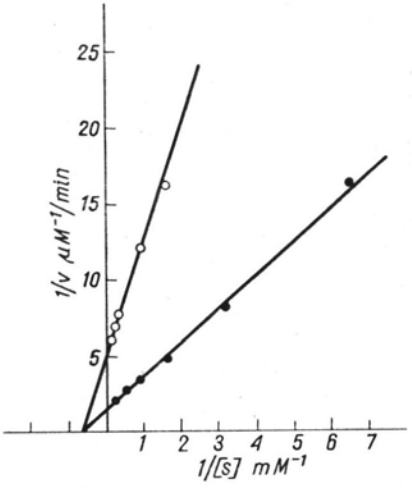

Fig. 1. Effect of fluoride ions on acid phosphatase a_2 activity, substrate — p--nitrophenylphosphate

The reaction mixture contained in a total volume of 2,0 ml 0,1 M acetate buffer, pH 5,1; 0,035 unit of phasphatase a_2, 1×10^{-3} M NaF and indicated amount of p-nitrophenylphosphate. Incubation was made for 10 min. at 37°. Reaction was stopped by addition of 10 ml O,1 N NaOH, and the absorbance of the solution was measured at 410 nm.

●——● — with no inhibitor; o——o — with inhibitor

Fig. 3 presents the effect of citrate on the activity of acid phosphatase a_2 and a_3 toward p-nitrophenylphosphate and phenylphosphate. Some differences were noted in the activity of both enzymes under study. In the presence of 10 — 40 mM citrate the activity of acid phosphatase a_2 and a_3 toward phenylphosphate increased, while the activity of these enzymes toward p-nitrophenylphosphate remained almost unchanged.

Table 1

Effect of various compounds on the activity of acid phosphatase a_2 and a_3 toward p-nitrophenylphosphate and phenylphosphate

Reaction mixture contained in a total volume of 2,0 ml 0,1 M acetate buffer, pH 5,1: 0,035 unit of enzyme, 10 μmoles of p-nitrophenylphosphate or phenylphosphate and indicated amount of various compounds. Incubation was made for 10 or 30 min. 37°. The reaction was stopped by addition of 2 ml of 5% trichloroacetic acid. The liberated inorganic phosphate was determined by the method of Fiske and Subbarow.

Compound added		Relative activity %			
		AcPh a_2		AcPh a_3	
	M	p-NPP	Phenyl-phosphate	p-NPP	Phenyl-phosphate
NaF	1×10^{-2}	27	18	31	25
p-nitrophenol	1×10^{-3}	100	100	100	100
Na-citrate	1×10^{-2}	105	117	100	113
K-oxalate	1×10^{-2}	65	80	93	94
p-CMB	2×10^{-5}	62	70	71	80
urea	1×1	100	100	100	100
adenine	1×10^{-3}	100	93	100	92
cytosine	1×10^{-3}	78	91	100	100
uridine	1×10^{-3}	86	89	100	92
guanine	1×10^{-3}	84	100	100	81
l-arabinose	1×10^{-3}	100	100	100	93
ribose	1×10^{-2}	107	92	120	100
none	—	100	100	100	100

AcPh a_2, AcPh a_3 — Acid phosphatase a_2 and a_3, p-NPP — p-nitrophenylphosphate. p-CMB — p-chloromercuribenzoate

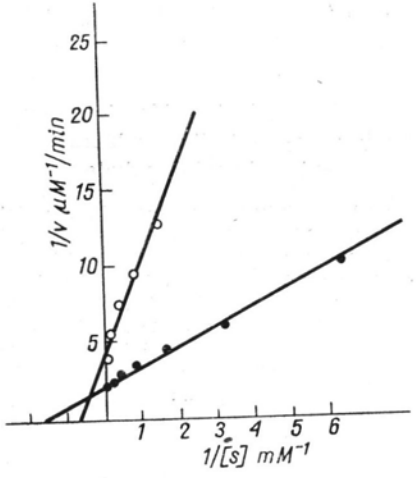

Fig. 2. Effect of fluoride ions on acid phosphatase a_3 activity, substrate — p-nitrophenylphosphate

The assays were carried out as described in the legend to Fig. 1

●——● — with no inhibitor; o——o — with inhibitor

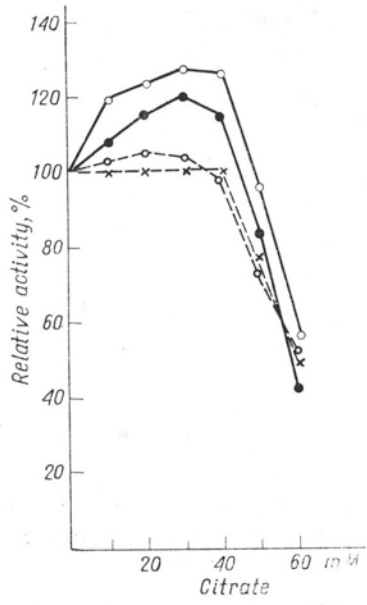

Fig. 3. Effect of Na-citrate on p-nitrophenyl-phosphate and phenylphosphate hydrolysis by acid phosphatase a_2 and a_3

Reaction mixture contained in a total volume of 2.0 ml 0,1 M acetate buffer pH 5,1 : 0,035 unit of enzyme, 10 μmoles of p-nitrophenyl phosphate or phenylphosphate and indicated amound of Na-citrate. Incubation was made for 30 min. 37°. The liberated inorganic phosphate was determined after Fiske and Subbarow.

p-nitrophenyl phosphate: o——o acid phosphatase a_2; x— — —x acid phosphatase a_3

phenylphosphate: o——o acid phosphatase a_2

●——● acid phosphatase a_3

The effect of urea on the activity of acid phosphatase a_2 and a_3 is presented in Fig. 4. It was found that urea in concentrations of 4 — 6 M caused inactivation of both enzymes. In the presence of 4 M urea after 30 min incubation in pH 5.1 the activity of the enzymes lowered to about 70%, and in the presence of 6 M urea — to about 30%. Prolonged time of enzyme incubation in the presence of 4 M urea (Fig. 5) resulted in a significant decrease of the activity of phosphatase a_3, with simultaneous slight decrease of the activity of acid phosphatase a_2. Lowered activity in the presence of urea was noted also in case of acid phosphatase of yeasts (Wątorek and Morawiecka 1977), as also in case of three acid phosphatases of wheat germs (Verjee 1969) and acid phosphatase of potatoes (Kruzel 1977). It may be assumed that both forms of acid phosphatase (a_2 and a_3) of *Poa pratensis* seeds, similarly as acid phosphatase of potatoes, possess subunital structure or else they form a complex enzymatic system.

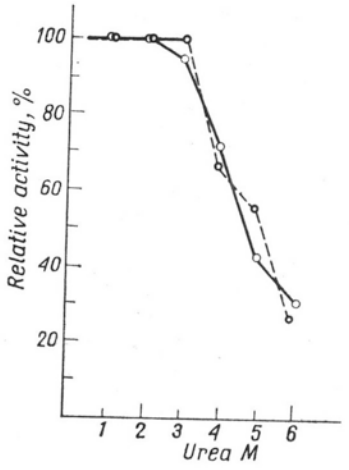

Fig. 4. Effect of urea on acid phosphatase a_2 and a_3 activity, substrate — p-nitrophenylphosphate

The acid phopshatase a_2 and a_3 (0,3 — 0,4 units) were incubated with 1 — 6 M urea in 0,1 M acetate buffer, pH 5,1, 37°. After 30 min. aliquots were withdrawn and activity toward p-nitrophenylphosphate was assayed under standard conditions

o——o phosphatase a_2; o— — —o phosphatase a_3

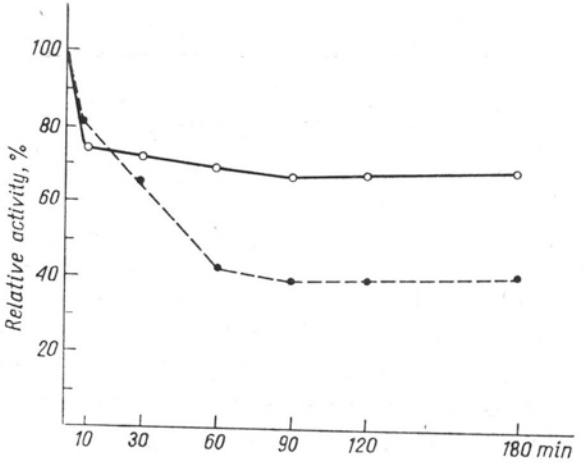

Fig. 5. Effect of 4 M urea on acid phosphatase a_2 and a_3 activity, substrate — p-nitrophenylphosphate

The acid phosphate a_2 and a_3 (0,3 — 0,4 unit) were incubated with 4 M urea in 0,1 M acetate buffer, pH 5,1 at 37°. After various times aliquots were withdrawn and the activity toward p-nitrophenyl phosphate was assayed under the standard conditions

o——o acid phosphatase a_2; acid phosphatase a_3 o— — —o

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Aktywność fosfatazowa nasion wiechliny łąkowej (Poa pratensis)

III. Wpływ fluorku, cytrynianu, mocznika oraz innych substancji na aktywność fosfatazy kwaśnej Ia_2 i Ia_3

Streszczenie

Zbadano wpływ fluorków, cytrynianu, mocznika i innych substancji na aktywność fosfatazy kwaśnej a_2 i a_3 nasion wiechliny łąkowej *(Poa pratensis)*. Badania przeprowadzono wobec dwóch substratów: p-nitrofenylofosforanu i fenylofosforanu sodu. Stwierdzono, że inhibitorami obu enzymów są fluorki, szczawian oraz p-chlororterciobenzoesan. Jony fluorkowe okazały się inhibitorami niekompetycyjnymi dla fosfatazy kwaśnej a_2 podczas gdy hamowanie aktywności fosfatazy kwaśnej a_3 ma charakter inhibicji mieszanej. Cytrynian aktywował oba enzymy wobec fenylofosforanu, nie wywierał natomiast wpływu na hydrolizę p-nitrofenylofosforanu. Cytozyna i urydyna obniżają aktywność fosfatazy kwaśnej a_2 wobec obu substratów, bez wpływu pozostają natomiast na aktywność fosfatazy kwaśnej a_3. Mocznik w stężeniu 4 M inaktywuje oba enzymy w około 30% po minutowej inkubacji.