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SHORT COMMUNICATION

ACID PHOSPHATASE IN *ENTEROMORPHA*

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Key Word Index—*Enteromorpha linza*; Chlorophyceae; marine alga; non-specific acid phosphatase.

Abstract—Extracts of the alga *Enteromorpha linza* hydrolysed glucose-6-phosphate, *p*-nitrophenylphosphate 2′-, 3′-, and 5′-adenosine monophosphates with an optimum at pH 5. Cytidine and uridine-5′-nucleoside diphosphates, and 2′-, and 3′-adenosine monophosphates were relatively poorly hydrolysed. Zn^{2+} (10 mM) inhibited the hydrolysis of all substrates, whereas Mg^{2+} (10 mM) may be stimulatory. It is suggested that the hydrolysis of these phosphomonoesters was due to the activity of a non-specific acid phosphatase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.2).

INTRODUCTION

THE UBIQUITOUS nature of acid phosphatases in animal tissues and in some diverse non-animal sources such as yeasts, moulds, seedlings, citrus fruits and several micro-organisms is well established.[1,2] It has been suggested, for example, that wheat leaves contain two separate phosphatases; one which hydrolysed 5′-adenosine monophosphate and phenolphthalein diphosphate, and another which attacked α-glycerophosphate.[3,4] A non-specific acid phosphatase and a separate α-glycerophosphate-hydrolysing enzyme also occur in tobacco leaves.[5] By comparison, much less is known about phosphatase activities in algae, although *Euglena gracilis* possesses a phosphate-repressible acid phosphatase-synthesizing system.[6] The purpose of this study was to investigate the ability of algal extracts to hydrolyse a variety of phosphate esters, including sugar phosphates, nucleoside mono- and diphosphates, and *p*-nitrophenyl phosphate (*p*NPP).

RESULTS AND DISCUSSION

The pH activity profiles for *p*NPP, 2′-, 3′-, and 5′-AMP, and glucose-6-phosphate (G-6-P) were very similar, with the optimum at pH 5. This is consistent with an earlier report on marine phytoplanktonic algae[7] and for a variety of higher plant tissues.[8,9] On testing several possible substrates at pH 5 the most rapidly hydrolysed was found to be

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*p*NPP (Table 1). Nucleoside-5′-monophosphates were attacked at appreciable rates, particularly 5′-AMP, other nucleoside-5′-diphosphates that were tested were poorly hydrolysed. Phosphoserine and phosphothreonine were hardly attacked. Nucleoside-5′-monophosphates were generally better substrates than 2′- or 3′-AMP, but for phosphatases of tobacco leaves[5] and lupin seedlings,[10] the opposite has been reported.

TABLE 1. RATES OF HYDROLYSIS OF VARIOUS PHOSPHATE ESTERS BY A CELL-FREE EXTRACT OF *Enteromorpha linza*

Substrate	Units of activity at pH 5	Substrate	Units of activity at pH 5
p-Nitrophenyl phosphate	10·3	3′-AMP	2·6
		2′-AMP	2·0
5′-AMP	7·5	D-Glucose-6-phosphate	2·3
5′-GMP	5·2	2-Deoxy-glucose-6-phosphate	1·8
5′-CMP	4·0	D-Fructose-6-phosphate	2·4
5′-IMP	3·8	D-Fructose-1,6-diphosphate	4·6
5′-UMP	3·5	α-D-Glycerophosphate	1·1
5′-*d*GMP	6·6	β-D-Glycerophosphate	1·8
5′-*d*CMP	4·6	DL-Glyceraldehyde-3-phosphate	4·3
5′-*d*UMP	4·9	Phosphoserine	<0·4
5′-TMP	2·1	Phosphothreonine	<0·4
		6-Phosphogluconic acid	1·6
5′-CDP	0·5		
5′-UDP	0·4		

The standard assay, described in the text, was used. Each reaction mixture contained 10 μg protein.

Phosphatase activity in extracts of *E. linza* showed no requirement for divalent cations. Nevertheless, the effect of Ni^{2+}, Co^{2+}, Mg^{2+}, and Zn^{2+} upon the hydrolysis rates of several substrates at pH 5·0, 6·5, and 8·0 were examined. Zn^{2+} consistently inhibited the hydrolysis of all of the substrates tested, and at all pH values used (Table 2). On the other hand, Mg^{2+} (and, to a lesser degree, Co^{2+}) tended to be stimulatory, particularly at pH 5. The effect of Ni^{2+} appeared to be generally inhibitory, except at pH 8 where no inhibition occurred.

A DEAE-cellulose column was charged with algal extract and eluted with a linear gradient of sodium chloride at pH 7. Although a good separation of the enzyme-rich fraction from the non-active fraction was obtained, the activities against *p*NPP, 3′-AMP, 5′-AMP and α-glycerophosphate (α-GP) were not separated. However, 5′-AMP and α-GP were hydrolysed by the enzyme-rich eluate at significantly faster rates relative to the hydrolysis of *p*NPP, after the chromatographic treatment. Further studies are necessary to determine whether the latter observation indicates the existence of substrate-specific phosphatases in *E. linza*.

EXPERIMENTAL

Plant material. The alga, *Enteromorpha linza*, was obtained locally; washed with cold H_2O and stored at −30°.

Enzyme solutions and substrates. Approx. 850 g (wet wt) was homogenized in 2 vol. H_2O in a Waring blendor and centrifuged at 8 000 *g* for 20 min (0–4°). The supernate was freeze dried and stored at −30°.

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TABLE 2. EFFECT OF SOME DIVALENT IONS ON HYDROLYSIS RATES OF VARIOUS PHOSPHATE ESTERS

Substrate	pH	Relative activity (control* = 1·0) in presence of			
		Ni^{2+}	Co^{2+}	Mg^{2+}	Zn^{2+}
*p*NPP	5·0	0·7	1·0	1·2	0·7
	6·5	1·1	1·2	1·6	0·7
	8·0	1·2	1·0	1·0	0·7
5′-AMP	5·0	0·8	1·2	1·4	0·1
	6·5	0·8	1·4	1·6	0·1
	8·0	1·2	0·9	1·1	0·3
3′-AMP	5·0	0·8	1·7	2·4	0·4
	6·5	0·8	1·1	1·7	0·4
	8·0	1·0	0·8	0·9	0·2
2′-AMP	5·0	0·9	1·1	1·9	0·5
	6·5	0·9	1·2	1·5	0·2
	8·0	1·2	1·1	1·1	0·1
α-Glycerophosphate	5·0	0·6	1·0	1·2	0·3
	6·5	0·7	1·0	1·0	0·3
	8·0	1·2	0·7	1·0	<0·1
β-Glycerophosphate	5·0	0·8	1·3	1·8	0·5
	6·5	0·9	1·4	1·6	<0·1
Glucose-6-phosphate	5·0	1·2	2·3	3·9	0·6
	6·5	1·0	1·6	2·6	0·5
	8·0	1·1	0·5	0·8	0·5
Glyceraldehyde-3-P	5·0	0·9	1·3	2·1	0·8
	6·5	1·2	1·4	1·8	0·5

* Activity in presence of buffer, substrate and extract only.

The standard assay was employed, except that the divalent cations were added (as chloride salts) to give a final concentration of 10 mM. Assays performed at pH 5·0 and at 6·5 contained 10 μg protein. Those performed at pH 8·0 contained 580 μg protein.

Portions (1–2 g) of the powder were suspended in 15 vol. H_2O. After centrifugation at 30 000 *g* for 30 min, $(NH_4)_2SO_4$ (0·53 g/ml) was added to the supernate, the ppt. taken up in 5–10 ml H_2O, and dialysed against H_2O for 16 hr. The enzyme lost *ca.* 20% of its activity in 3 weeks at −30°, when either 5′-AMP or pNPP served as substrate. The protein content ranged from 5 to 10 mg/ml as determined by the method of Lowry *et al.*,[11] with bovine serum albumin as standard.

Enzyme assay. The reaction mixture contained 1 μmol substrate, 40 μmol of either NaOAc buffer (pH 5·0 or 6·5) or Tris-maleate buffer (pH 8·0 or 8·5) and algal extract in a total vol. of 0·5 ml. After 30 min incubation at 30°, the reaction was terminated by the addition of 1 ml of the phosphate colour reagent described by Chen *et al.*,[12] modified by Walters and Loring.[13] A suitable blank containing substrate and buffer was incubated with each experimental tube, and the algal extract added to the controls after addition of the phosphate reagent. One unit of phosphatase activity is defined as that amount which released 1 μg inorganic phosphate under standard assay conditions.

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