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Existence of Acid and Alkaline Phosphohydrolase Activity in the Phytoflagellate *Ochromonas danica** **

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Abstract. Cell homogenates of light-grown *Ochromonas danica* contained distinct non-specific non-phosphate-repressible acid and alkaline phosphohydrolase activities. Acid phosphohydrolase activity had a broad pH range of 2.0–5.0 and the optimum for alkaline phosphohydrolase activity was pH 8.6. Acid phosphohydrolase (pH 3.6) activity had an optimum temperature of 55° C; the alkaline enzyme activity had an optimum temperature of 37–40° C.

Key words: Phosphohydrolase — Acid — Alkaline — *Ochromonas* — Phytoflagellate.

The phosphohydrolases of algae and protozoa have been rarely characterized biochemically (Muller, 1967; Aaronson, 1973; Eckhout, 1973). Earlier cytochemical evidence (Grusky and Aaronson, 1969) indicated that the phytoflagellate, *Ochromonas danica*, contained both acid (EC 3.1.3.2.) and alkaline (EC 3.1.3.1) phosphohydrolase activity, an unusual occurrence in photosynthetic organisms (Nagana *et al.*, 1955; Aaronson, 1973; Gahan, 1973) while common in fungi and metazoa. In this communication we present biochemical evidence for the existence of non-phosphate-repressible acid and alkaline phosphohydrolase activity in the exponentially light-grown phytoflagellate, *O. danica*.

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Materials and Methods

Ochromonas danica Pringsheim was maintained in a chemically defined medium (Aaronson and Baker, 1959) in 5 ml volumes in screw-cap test tubes in a refrigerated incubator at 25° C with 125—150 foot candles (f. c.) of white fluorescent light. Experimental cultures were grown in 500 ml volumes in 1 l screw-cap Erlenmeyer flasks at 25° C in 125—150 f. c. white fluorescent light in the same defined medium containing sufficient phosphate (30 mg-%) to repress inducible phosphohydrolases. Flasks were inoculated with 20,000 cells per ml from a 48—72 h culture and incubated for 5 days (log phase cells). Cells were harvested by centrifugation at 4340× g for 20 min. The cells were resuspended in 40 ml cold distilled water and homogenized with glass beads at 20,000 rev./min for 30 sec. in a Sorvall Omnimixer, immersed in an ice bath. Slurry was centrifuged at 4340× g for 20 min, and the cell-free homogenate was used immediately for enzyme assay or frozen (−10 to −15° C) and stored for later use.

Acid and alkaline phosphohydrolase activity was routinely assayed in duplicate by incubating the enzyme for 10—15 min at 37° C with *p*-nitrophenyl phosphate as substrate. The assay mixture (1.0 ml total volume) contained 0.5 ml, 15.0 mM *p*-nitrophenyl phosphate in buffer; 0.3 ml, 0.1 M acetate buffer, pH 3.6 or Tris-HCl buffer, pH 8.6; 0.2 ml homogenate which contained 20—30 μg or 0.1—0.15 mg total protein. The reaction was initiated by the addition of the substrate and was terminated by the addition of 5 ml of 0.1 M NaOH. The *p*-nitrophenol released by the enzyme was measured at 410 nm in a Hitachi-Perkin Elmer spectrophotometer model 139 by the method of Bessey *et al.* (1946). The rate of reaction was linear to time and enzyme concentration under these conditions. One unit of enzyme activity catalyses the release of 1 μmole of *p*-nitrophenol under the specified condition. Specific activity was defined as units of enzyme activity per mg of protein.

Enzyme activity towards a number of other phosphate esters was determined by the liberation of inorganic phosphate by the method of Ames and Dubin (1960) or Fiske and Subbarow (1925). Protein was determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as a standard. Buffers were prepared according to the procedures of Gomori (1955).

Results and Discussion

Examination of the phosphohydrolase activity of *Ochromonas danica* cell-free homogenate with *p*-nitrophenyl phosphate as substrate at various pH values indicated acidic enzyme activity with a broad pH range from 2.2—5.2 using acetate buffer and alkaline enzyme activity with a maximum at pH 8.6 using Tris-HCl buffer (Fig. 1). Only about 5% difference in activity was observed between pH 2.2 and 5.2. All the subsequent assays were done at pH 3.6 for acid and pH 8.6 for alkaline phosphohydrolase activity.

The substrate specificity of both acid and alkaline phosphohydrolase activity (Table 1) revealed marked differences in substrate specificities. The specificity of acid phosphohydrolase activity was broad and all the compounds tested as substrates were hydrolysed at varying rates. Alkaline phosphohydrolase was active on phenolphthalein diphosphate, fructose-1,6-diphosphate, ADP and inactive on glucose-1-phosphate and AMP. It is likely that the high activity with ATP indicates the presence

Table 1. Substrate specificity of acid and alkaline phosphohydrolase activity of *Ochromonas danica*

Substrate (7.5 mM)	Acid		Alkaline	
	Specific activity	Relative activity	Specific activity	Relative activity
p-Nitrophenyl phosphate	14.3	100.0	3.27	100.0
β-Glycerophosphate	15.2	106.6	0.04	2.25
Phenolphthalein-diphosphate	12.7	89.0	0.9	27.6
Glucose-1-phosphate	9.9	69.5	0	0
Fructose-1,6-diphosphate	7.2	50.5	0.54	16.5
AMP	16.3	114.0	0	0
ADP	6.9	48.5	1.29	39.5
ATP	1.3	9.1	8.75	268.0

All substrates were assayed at 37° C in 0.1 M acetate buffer at pH 3.6 for acid and 0.1 M Tris-HCl buffer at pH 8.6 for alkaline phosphohydrolase activity. Specific activity expressed as μmoles inorganic phosphate liberated per min per mg protein. Relative activity expressed as % of *p*-nitrophenyl phosphate activity.

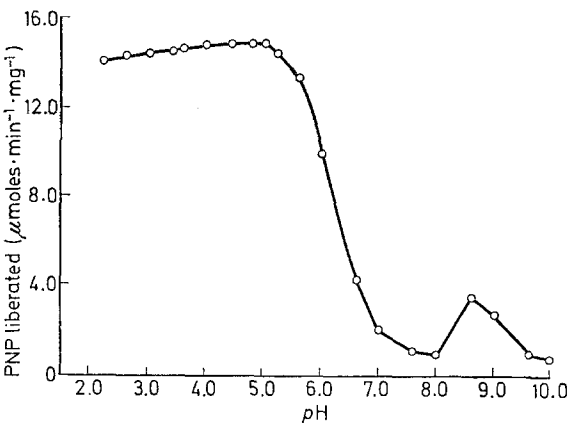

Fig. 1. Effect of pH on phosphohydrolase activity of *Ochromonas danica*. The units of activity are μmoles *p*-nitrophenyl phosphate hydrolysed per min per mg protein. Buffer used were glycine-HCl (pH 2.2—3.6), acetate (pH 3.6—5.6), Tris-maleate (pH 5.2—7.4), Tris-HCl (pH 7.2—9.0) and carbonate-bicarbonate (pH 9.0—10.0). The assay was performed by the standard assay conditions described in the text

of an ATPase in the homogenate. DNA or lecithin were not hydrolysed by *O. danica* cell homogenates.

Marked differences in acid and alkaline phosphohydrolase activities were noted in relation to temperature optima (Fig. 2) and temperature

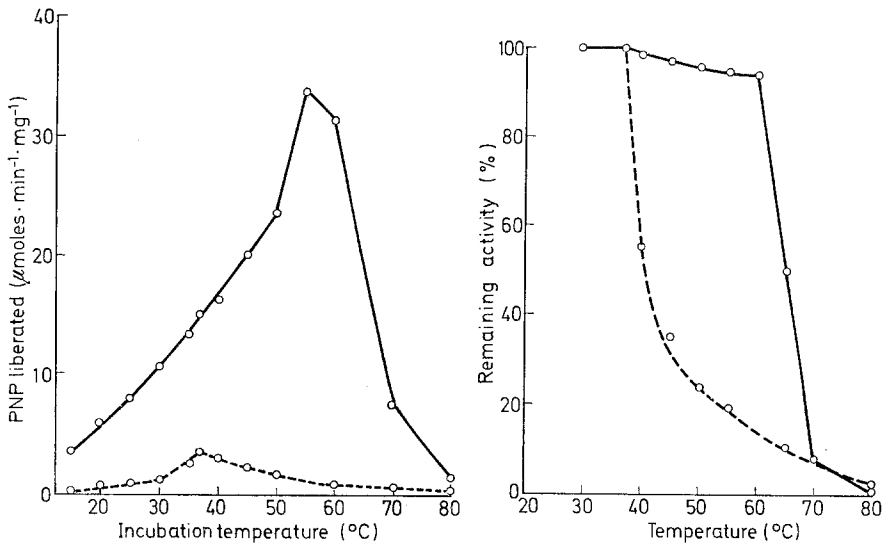

Fig. 2. Temperature optimum of the acid (solid line, pH 3.6) and alkaline (dashed line, pH 8.6) phosphohydrolase activity of *Ochromonas danica*. The units of activity are μmoles *p*-nitrophenyl phosphate hydrolysed per min per mg protein at different experimental temperature

Fig. 3. Thermal denaturation of the acid (solid line) and alkaline (dashed line) phosphohydrolase activity of *Ochromonas danica*: cell homogenate was heated for 20 min in acetate buffer (pH 3.6) or Tris-HCl buffer (pH 8.6) at different temperatures. Heating was terminated by transferring a sample into an icebath and remaining activity was assayed at 37° C as described under the standard assay conditions

stability (Fig. 3). Assay mixtures containing *p*-nitrophenyl phosphate as substrate, acetate buffer (pH 3.6) or Tris-HCl buffer (pH 8.6) and homogenate were incubated at different temperature for 10—15 min. The temperature maximum for acid phosphohydrolase activity was 55° C and a range of 37—40° C in several experiments for alkaline phosphohydrolase activity. Homogenate incubated for 20 min at the several temperatures showed that the acid enzyme activity was more stable than the alkaline enzyme activity.

Acid phosphohydrolase activity was not significantly affected by 5 mM Mg^{2+}, Ca^{2+}, Cu^{2+} and Mn^{2+} but was inhibited by Co^{2+}, Hg^{2+}, Zn^{2+} and Fe^{3+}. Alkaline phosphohydrolase activity was considerably stimulated by Mg^{2+}, Mn^{2+} and Fe^{3+} and inhibited by Co^{2+}, Hg^{2+}, Zn^{2+}, Ca^{2+} and Cu^{2+}. Various inhibitors were assayed on acid and alkaline phosphohydrolase activity at pH 3.6 and 8.6 with *p*-nitrophenyl phosphate as substrate. Cysteine-HCl, *p*-chloromercuribenzoate, citrate, iodoacetic

acid, iodoacetamide and urea inhibited alkaline phosphohydrolase activity only. Fluoride and tartrate inhibited both enzyme activities.

The results presented here demonstrate clearly that homogenates of log-phase light-grown *O. danica* contained distinct acid and alkaline phosphohydrolase activities. The distinctions are shown by the differences in pH optima, substrate specificities, temperature optima, response to several cationic activators and cationic and organic inhibitors.

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