INVOLVEMENT OF D-GALACTOKINASE IN THE OSMOREGULATION OF *POTERIOOCHROMONAS MALHAMENSIS*

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1. Introduction

In *Poterioochromonas malhamensis* (syn: *Ochromonas malhamensis* Pringsheim) which are unicellular, wall-less algae, osmotic regulation occurs through the changes in the pool size of IF[*O*-α-D-galactopyranosyl(1→1)-glycerol] [1,2]. Increasing the external osmotic pressure causes the cell to shrink rapidly, however, it regains its original volume during the following 1–2 h period. This recovery is accompanied by a sharp increase in the internal level of IF [3–5]; a rapid turnover of IF takes place at all times [6]. On the other hand, decreasing the external solute concentration causes a rapid fall in the IF level with a concomitant rise in the level of a reserve β-(1→3)-glucan in the cell [3,5]. Carbon from both, galactose and glycerol moieties of IF is incorporated into the polysaccharide.

The increase in the pool size of IF is due to an increase in the level of IFP-synthase (UDP-galactose: *sn*-glycerol-3-phosphoric acid 1-α-D-galactosyl transferase) [7]. The key factor in the osmotic control of this enzyme lies in the proteolytic activation of its inactive form [8–10]. The resulting IFP is subsequently dephosphorylated [1,11]. The presence of an α-galactosidase which catalyses the hydrolytic degradation of IF in the alga has been shown [12,13]. Here, a possible involvement of galactokinase in the osmoregulation of IF degradation is discussed.

Abbreviations: BSA, bovine serum albumin; IF, isofloridoside; IFP, isofloridoside phosphate; ME, mercaptoethanol

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2. Materials and methods

2.1. *Cell extracts*

Cells of *P. malhamensis* were grown as in [13]. The final suspension of the 3-day old cells was such that a 1:50 dilution with water gave an A_{510} of 0.5 (lightpath 10 mm). A known volume of cell suspension was centrifuged and the pellet was resuspended in the same volume of ice-cold 0.1 M phosphate buffer (pH 7.3) containing 5 mM ME and 1 mM EDTA and disintegrated using a French-press at 1600 lb/in^2. The homogenate was centrifuged at 50 000 × *g* for 30 min and the supernatant used as the crude enzyme extract.

2.2. *Assay*

The incubation mixture (100 μl) for the assay of galactokinase consisted of 10 μl crude extract + 10 μl [^{14}C]galactose (0.2 μCi; 60 μCi/μmol) + 5 μl ATP (50 mM, adjusted to pH 7.3) + 45 μl water + 30 μl 0.1 M phosphate buffer (pH 7.3) containing 25 mg/ml BSA, 10 mM ME, 5 mM $MgCl_2$ and 12.5 mM NaF. This was incubated at 25°C and 20 μl aliquots were withdrawn at various time intervals and applied to DEAE-cellulose discs. A further 60 μl water was then applied to each disc to ensure that it was fully wet. The discs were then dropped into a beaker containing 200 ml distilled water and washed twice with the same volume of water by gently shaking on a rotary shaker. The discs were finally dried and radioactivity counted using a PPO–toluene cocktail; the counting efficiency was 50%.

2.3. *Equilibrium density centrifugation*

Enzyme extracts were prepared from cells that were subjected to osmotic stimulus with 0.1 M NaCl for 3 h in normal growth medium and also in medium

containing ^2H_2O. The final concentration of ^2H_2O in the latter was 70% (v/v). For centrifugation, enzyme extracts (4 ml) were prepared from the treated cells and α-amylase (as a density marker) and cesium formate stock solution were added to obtain a final density of 1.29 g/cm^3. The extracts were then centrifuged using a SW-50L rotor in a Beckman Ultracentrifuge for ~65 h at 40 000 rev./min. One drop fractions were collected and every fifth fraction was assayed for galactokinase activity. α-Amylase was assayed as in [14]. The densities of the fractions were determined according to [15].

3. Results and discussion

A crude enzyme extract obtained from *P. malhamensis* was capable of high rates of ATP-dependent phosphorylation of D-galactose (1.6 μmol Gal-1-P formed . min^{-1} . mg protein^{-1}). This galactokinase activity had not been demonstrated in this organism. The galactose-1-phosphate formed in the enzyme reaction mixture was separated by high-pressure liquid chromatography and characterized by physico–chemical methods. The enzyme activity was considerably less if NaF was omitted from the assay. This is presumably due to the presence of a phosphatase.

It is suggested that the kinase participates in the metabolism of the D-galactose, liberated during rapid turnover of IF which occurs as a result of osmotic stimulation and which also is present in the cell under normal growth conditions [6,16].

On increasing the osmotic value of the algal suspension medium with NaCl, there was a marked increase in galactokinase activity. This increase was dependent on [NaCl] (fig.1); the highest activity was found at 0.1 M (230 mosM) beyond which there was no significant change. In a separate experiment the time course of the increase of galactokinase activity was measured after stimulation with 0.1 M NaCl. After a lag of 60 min, the enzyme level increased rapidly reaching a maximum of 160 min and remained constant after this time (fig.2). The enzyme level in control experiments showed a slight decline with time; this is presumably due to a decrease in the concentration of nutrient materials in the medium. When the effect of osmotic stimulus in the presence of cycloheximide was tested, the galactokinase activity was identical to the control experiment (fig.2). Addition of NaCl to the enzyme assay medium did not

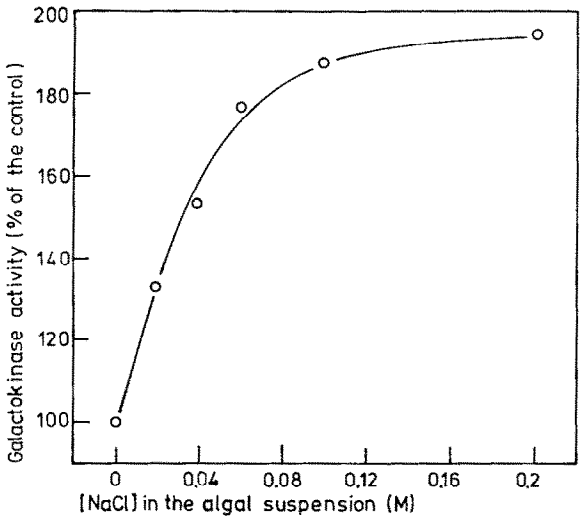

Fig.1. Effect of osmotic stimulation (with NaCl) of *P. malhamensis* on the level of galactokinase. The final algal suspensions in the nutrient medium contained varying [NaCl]; these were acrated for 3 h (see [13]). The cells were then harvested and the enzyme extracted and assayed as in section 2.

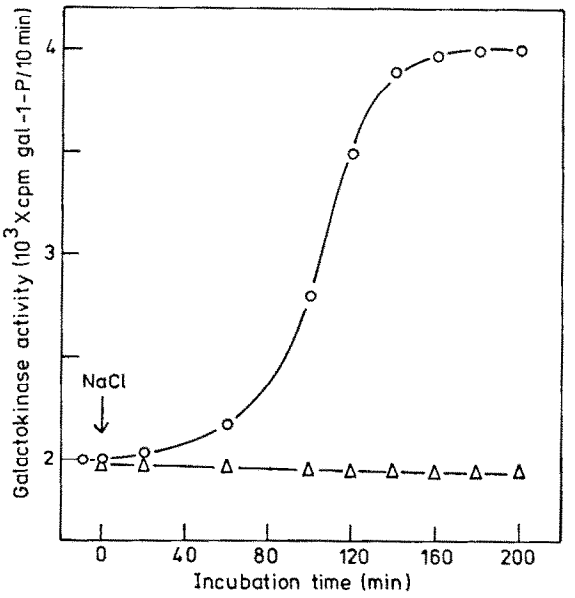

Fig.2. Time course of the development of galactokinase level in *P. malhamensis* in the absence (—○—) and in the presence (—△—) of cycloheximide (50 μg/ml algal suspension). The initial osmotic value of 60 mosM of the algal suspension medium was raised to 230 mosM with NaCl (final conc. 0.1 M). Cycloheximide was added 10 min prior to the addition of NaCl. The cell suspensions were aerated and the enzyme extracted as in section 2. The enzyme levels in the control experiments (devoid of NaCl and cycloheximide) were exactly the same as those in the presence of cycloheximide.

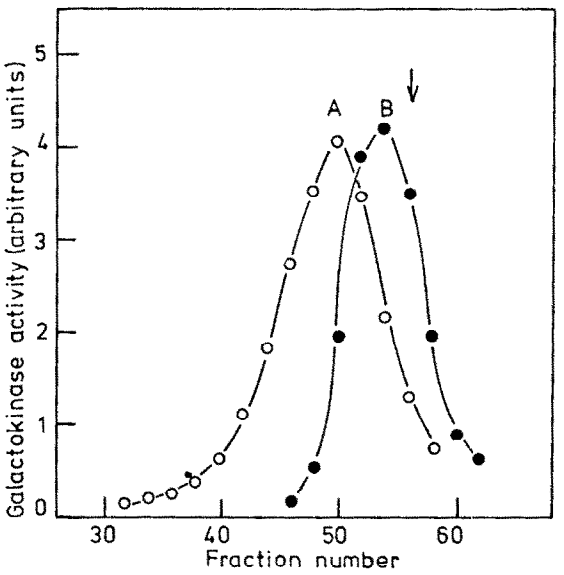

Fig.3. Distribution of galactokinase activity from *P. malhamensis* grown in media containing ^2H_2O (peak A, –○–) and H_2O (peak B, –●–), after equilibrium centrifugation on a cesium formate gradient. The equilibrium position of the α-amylase marker is indicated by the arrow. The direction of decreasing density is from left to right.

result in the activation of galactokinase. These results suggest that the elevated level of the enzyme is due to de novo synthesis. Osmotically stimulated de novo synthesis of the enzyme was also demonstrated by density labelling, followed by isopycnic centrifugation (fig.3).

The hydrolysis of IF in the alga is catalyzed by an α-galactosidase; the increase in the level of this enzyme due to de novo synthesis is known [12] to parallel the changes in galactokinase activity discussed here. It has also been shown that the osmotic stimulus results in a high level of IF in the cell and a 5-fold increase in turnover of this metabolite [6]. In vivo experiments on the degradation of IF have shown that whereas free glycerol rapidly attains a high level after osmotic stimulus, the pool of free galactose increases slowly and to a much smaller extent [16]. The enhanced level of galactokinase accompanying osmotic shock can be equated with low concentration of galactose in the cell under these conditions. The disappearance of galactose may also involve an active transgalactosylase (see [17,18]) however, we have failed to detect this activity in the algae.

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