CHANGES IN cGMP PHOSPHODIESTERASE LEVELS DURING GROWTH AND DIFFERENTIATION IN *BLASTOCLADIELLA EMERSONII*

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1. Introduction

Blastocladiella emersonii, a unicellular phycomycete represents a useful organism for experimental studies on the regulatory role of cyclic nucleotides (see ref [1] for a recent review of the cell cycle). Thus, the levels of cAMP** and cGMP** as well as enzyme activities concerning their metabolism were found to change markedly during the life cycle [2–5]. In all living systems so far studied, cGMP is synthesized via guanylate cyclase and hydrolysed via cyclic nucleotide phosphodiesterases. In principle, any alteration in the amount or activity of either of these two enzymes would affect the intracellular cGMP concentration. Thus, it is important to find out if these two enzyme activities are altered as a function of the cell cycle.

cGMP levels in *B. emersonii* are low during the growth stage rising about 50–100-fold at a defined stage in its life cycle, during sporulation [3]. We have demonstrated that *B. emersonii* contains independent specific enzymes involved in the hydrolysis of cAMP and cGMP [6]. The present communication shows that the specific activity of cGMP phosphodiesterase dramatically changes throughout the life cycle of *B. emersonii*, particularly during germination and sporulation. The results indicate that the observed variation in cGMP levels when cells sporulate [3] reflects the changes in enzymatic activities, mainly in cGMP phosphodiesterase.

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**Abbreviations: cAMP, cyclic 3′,5′-adenosine monophosphate; cGMP, cyclic 3′,5′-guanosine monophosphate.

2. Materials and methods

2.1. *Organism and growth conditions*

Stocks of *B. emersonii* were kept at 26°C on Difco PYG agar plates. Collection of zoospores, growth conditions and techniques used to induce sporulation were essentially the same as described elsewhere [2]. For germination experiments, zoospores obtained according to Soll et al. [7] were inoculated into DM_4 growth medium [2] and the cell types scored by the 'spinner flask assay' of Soll et al. [7].

2.2. *Preparation of extracts*

Cells were harvested by centrifugation (3 min, 1000 × *g*) at 4°C, resuspended in a buffer containing 50 mM Tris–HCl, pH 8.0, 5 mM $MgCl_2$ 1 mM 2-mercaptoethanol, 0.15 mg/ml of phenylmethane sulphonyl fluoride and 10% glycerol (v/v) (Buffer A) and lysed in the French press cell at 1000 psi for the zoospores and 3000 psi for the vegetative cells. These crude preparations were assayed directly.

2.3. *Assay of the cGMP phosphodiesterase*

cGMP phosphodiesterase activities were determined as described [6]. A typical incubation mixture contained in 0.1 ml of buffer A: 40 000–80 000 cpm [^{3}H]cGMP (New England Nuclear); 100 μM unlabeled cGMP and 20–60 μg of protein extract. After 10 min at 30°C the reactions were terminated by plunging the incubation tubes for 2 min into boiling water. 50 μg of snake venom were added to each tube and incubation proceeded for another 15 min at 30°C. At the end of this period 1.0 ml of a 1:3 slurry of Bio-Rad AG1 X-2 resin was added to the mixtures. After centrifugation, radioactivity of 1.0 ml of the

supernatant was determined in a liquid scintillator spectrometer. For every enzyme activity determination, a zero time value was determined and subtracted from the experimental results. The results reported were always the mean of duplicate experiments.

Protein was determined by the method of Lowry et al. [8] using bovine serum albumin as a standard.

3. Results

B. emersonii has a cGMP specific phosphodiesterase [6]. Under standard assay conditions, the specific activity of zoospore crude extracts lies between 16–20 nmol/mg protein/10 min.

Enzyme activities in homogenates from cells at different stages of growth have been measured and are listed in table 1. A marked decline in specific activity during germination, as compared to zoospore and germling activities, has been found. The enzyme activity remains constant at low levels throughout exponential growth, beginning to rise when the sporangia start papillae formation.

The decrease in enzyme activity during germination has been more closely followed in experiments on which distinct cell types characteristic of this transition were scored [7]. As can be seen in fig.1, the enzyme activity does not drop sharply during zoospore germination (as found to occur with cAMP phosphodiesterase [2]), but falls gradually, to reach its lowest levels only after transformation of round cells to germlings takes place. Further support for this observation was obtained in experiments in which

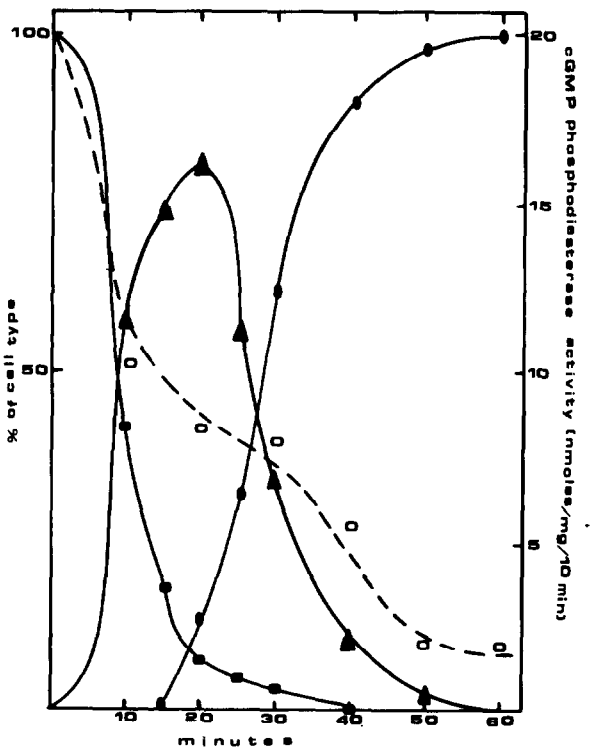

Fig.1. cGMP phosphodiesterase activity during zoospore germination. Zoospores were obtained according to Soll et al. [7]. Zero time corresponds to the time of innoculation of zoospores into DM_4 growth medium. At different times cells were harvested by centrifugation and the enzymatic activity determined. Percent of cell types at each point was determined by examination under phase contrast microscope [7]. (■——■) zoospores; (▲——▲) round cells; (●——●) germlings; (□——□) enzyme specific activity.

Table 1
cGMP phosphodiesterase activity in extracts of cells at different phases of growth

Source of enzyme	cGMP phosphodiesterase (nmol/mg protein/10 min)
Zoospores	16–20
Germlings	3.0
2 h cells	1.5
6 h cells	0.8
Papillated cells	4–5

Zoospores (4×10^5/ml) were innoculated into DM_4 growth medium and at different times cells were harvested by centrifugation and enzymatic activity was determined.

germination was induced in the presence of cycloheximide. This drug blocks the conversion of round cells to germlings [9]. Data in fig.2 show that, under these conditions, the enzyme activity only dropped from 18 to 9 nmol/mg/10 min and remained at this level. It would, thus, appear that conversion to germlings is necessary to accomplish total decrease in enzyme specific activity during germination process.

Activity throughout sporulation was followed in experiments where growth was interrupted during the exponential growth phase by removal of nutrients and sporulation elicited by incubation in 0.1 mM $CaSO_4$. The results obtained are shown in fig.3. At the time of growth medium replacement (6 h of growth) cGMP

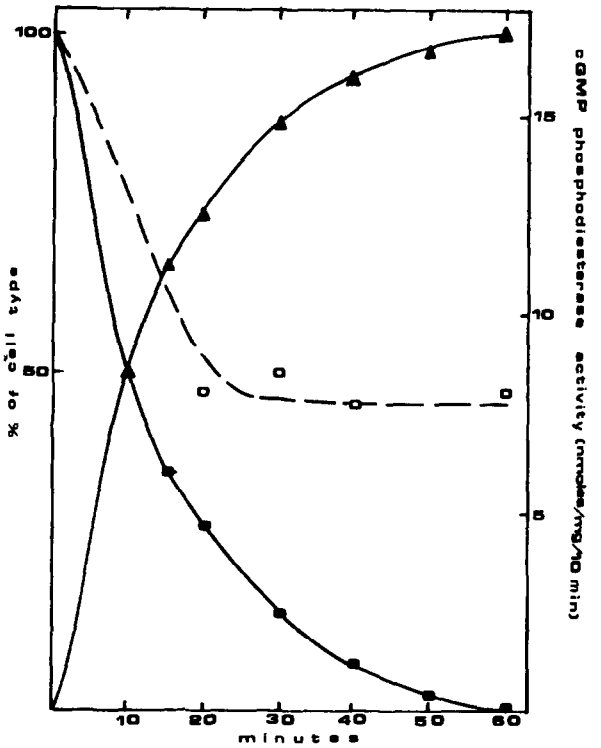

Fig.2. cGMP phosphodiesterase activity during germination in the presence of cycloheximide. Same procedure as in fig.1. Cycloheximide (2 μg/ml) was added at zero time. (■——■) zoospores; (▲——▲) round cells; (□——□) enzyme specific activity.

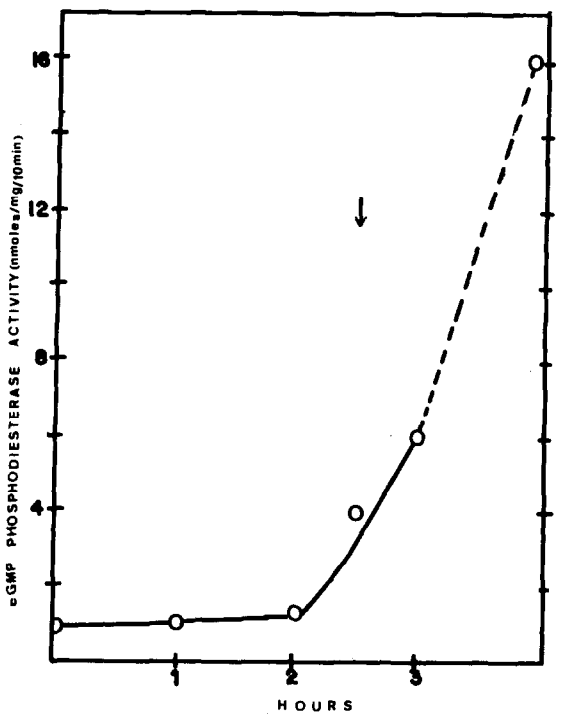

Fig.3. cGMP phosphodiesterase activity during sporulation. At zero time, cells previously grown for 6 h in DM_4 medium were harvested and resuspended in a 0.1 mM $CaSO_4$ solution. At different times samples were removed by centrifugation and cGMP phosphodiesterase activity determined. Arrow represents the time when the first cells to release zoospores were observed. At 4 h over 90% of the cells had sporulated; the released zoospores were selectively collected by filtration on a 30 μM mesh nylon net and their enzymatic activity was measured. (○——○) specific activity of the enzyme; the broken part of this curve does not obligatorily reflect enzyme synthesis but rather its compartmentation in the progeny zoospores.

phosphodiesterase activity is low, in accordance with the levels found for growing cells (see table 1). The enzyme activity remained unchanged for about 2 h after induction of sporulation. However, after this period, phosphodiesterase activity rises sharply and reaches, at the time of spore release, levels 16-fold higher than those found in 6 h cells.

4. Discussion

This report documents a cyclic variation in cGMP phosphodiesterase activity during the life cyclic of *B. emersonii*. The enzyme activity is high in zoospores, gradually drops during germination, remaining low at the growth phase and rises again during the later stage of sporulation.

The rise in cGMP phosphodiesterase activity during sporulation correlated well with the onset of intracellular cGMP accumulation, which occurs between 60 and 150 min prior to cytokinesis and zoospore release, abruptly falling before the end of sporulation [3]. We observed that phosphodiesterase activity remains unaltered for about 120 min, and rises sharply after this time, reaching maximum activity in the newly formed zoospores. Thus, it can be proposed that the observed changes in phosphodiesterase activities may be responsible for the regulation of cGMP levels both during this stage of differentiation and in the zoospores, since guanylate cyclase activity increases at

least 50-fold during sporulation and is even higher in the zoospores [4].

The regulatory mechanisms of cGMP levels during germination is far from being understood. At this stage of the life cycle zoospores lose more than 80% of their cGMP [3] as it occurs with the cGMP phosphodiesterase. Therefore, the determination of guanylate cyclase activities during *B. emersonii* germination might shed some light on the question of cGMP level regulation in this phase of the life cycle.

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