



## Regulation of ionic fluxes and protein-release from *Anabaena* HA101 by exogenous abscisic acid

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**Abstract.** The physiological effects of exogenously applied abscisic acid (ABA) on *Anabaena* HA101 grown under normal cultural conditions were similar to the effects induced in plants or algae by the endogenous accumulation of ABA under certain stress conditions. The plasma membrane permeability of *Anabaena* HA101 to ions was greatly reduced by exogenous ABA. *Anabaena* HA101 did not release a significant quantity of protein whilst it was exposed to ABA. When it was subsequently transferred to ABA-free medium, substantial release of protein took place. The released proteins may not be involved in ion transport mechanism when they are still associated with the cell's plasmalemma. It is suggested that the reduction in cell's respiratory activity caused by exogenous ABA treatment is the main factor controlling the influx of phosphate and efflux of protons across the plasmalemma of *Anabaena* HA101. Exogenous ABA, unlike endogenous ABA accumulated in plant tissues, did not play an important role in the regulation of the water potential of *Anabaena* HA101.

**Key words:** Abscisic acid; *Anabaena*; Cyanobacteria; Ionic flux; Plasmalemma; Protein-release.

### Introduction

It has been reported that wilting (Stewart *et al.*, 1986), water stress (Bensen *et al.*, 1988), osmotic stress (Creelman and Zeevaart, 1985) and salt stress (LaRosa *et al.*, 1985) all induce the accumulation of abscisic acid (ABA) in plant tissues. It is well known that endogenous ABA plays an important role in the biochemical and physiological activity of plant tissues and usually causes inhibition of growth and development (Zeevaart and Creelman, 1988). Indeed, the adaptation of plants to the various stresses mentioned above are accelerated by ABA and this might often ensure the plant's survival under the adverse conditions. The adaptive mechanisms in plants to stresses are: (1) Osmoregulation by means of the accumulation of proline or sugars in the plant's tissues (Stewart and Voetberg, 1985) or in algae (Ahmad and Hellebust, 1988); (2)

Change in the membrane permeability or ion uptake ability of plant tissues (Grunwaldt *et al.*, 1978), and (3) Induction of the biosynthesis of certain proteins (Mason *et al.*, 1988) or a specific protein (Singh *et al.*, 1985). In this study, it was found that exogenous ABA could alter the plasma membrane's permeability to ions, reduce oxygen-uptake and induce the release of protein from *Anabaena* cells without changing the water potential of the organism significantly.

### Materials and Methods

#### *Anabaena* Culture

A pure strain of *Anabaena* HA101 (A gift from DR. T. C. Huang, Institute of Botany, Academia Sinica, Taipei, Taiwan) was used. It was grown in liquid batch culture using the same medium and growth condition as described by Huang *et al.* (1988).

### Harvesting *Anabaena* Samples

When the cultures was at mid-log phase, it was centrifuged at 5000 g for 10 min at room temperature. After washing the cells twice with deionized water, they were transferred to the incubation medium that was to be used in the particular experiment.

### Uptake of Phosphate

The *Anabaena* cells were suspended in absorption solution at 25°C containing 0.5 mM calcium sulfate, 1 mM monopotassium phosphate and ABA at one of the following concentrations: 2, 4, 6, or  $8 \times 10^{-4}$  M. The cultures were aerated continuously. After one to four hours of incubation, the uptake of phosphate by the *Anabaena* samples was rapidly terminated by centrifugation in wire mesh baskets at 50 g for 5 min. This centrifugation also ensured that the *Anabaena* samples had a uniform water content (Maas *et al.*, 1979). After the fresh weight measurements, the *Anabaena* cells were dried overnight at 65°C, and analyzed for phosphate by the phosphomolybdate method of Taussky and Shorr (1953).

### Proton Extrusion

The *Anabaena* samples were each transferred to 10 ml of well aerated incubation medium containing 1 mM calcium chloride, 1 mM monosodium phosphate (pH 6.0), 1  $\mu$ M Fusicoccin and in some cases also ABA (2 or  $4 \times 10^{-4}$  M). The pH of the culture, which was stirred continuously, was initially adjusted to 6. After one hours, the cells were rapidly collected by filtration. The amount of proton released into the incubation medium was determined by back titration of the filtrate to the initial pH with 0.01 N NaOH.

### Protein-Release

The *Anabaena* samples were pre-incubated at 25°C in Stage I incubation medium containing 0.5 mM calcium sulfate, 1 mM monopotassium-phosphate and in some cases ABA at  $4 \times 10^{-4}$  M for one hour. The cells were collected by centrifugation and transferred to the Stage II incubation medium which was either identical to the Stage I medium used or differed only in that it did not contain ABA. The protein released into the pre-incubation or subsequent incubation medium was dialyzed overnight against cold de-ionized water and precipitated with cold 5% TCA. The precipitated protein was collected by centrifugation, and dissolved in

0.1 N NaOH at room temperature. The soluble protein was assayed by Lowry's method as modified by Peterson (1977).

### Oxygen Consumption

The dark respiration rate of *Anabaena* samples was determined using the Clark-electrode as described by Cooper (1977). The incubation medium that contained 1 mM potassium-phosphate buffer pH 7.0, 1% sucrose and either no ABA or  $4 \times 10^{-4}$  M ABA was aerated for three hours at 25°C. Five ml of aerated medium, in which 0.5 g (fresh weight) *Anabaena* was suspended, was placed in the oxygen analyzer's sample chamber and the rate of oxygen utilization by the *Anabaena* in the dark was recorded at 30 min intervals.

### Water Potential

The *Anabaena* samples that were suspended in incubation medium with or without  $4 \times 10^{-4}$  M ABA for one to four days were collected by centrifugation in wire mesh baskets at 50 g for 5 min. A Wescors model Hr 33T microvoltmeter with thermocouple psychrometer chamber was then used to measure the water potential of the *Anabaena* samples.

## Results

In the presence of  $4 \times 10^{-4}$  M ABA *Anabaena* HA 101 took up very little phosphate compared to the ABA-free controls (Fig. 1). By increasing the ABA concentration of the culture, the phosphate-uptake by *Anabaena* declined progressively up to an ABA concentration of  $4 \times 10^{-4}$  M. Higher ABA concentrations did not reduce the phosphate uptake further (Fig. 2). The dark oxygen uptake by *Anabaena* was also inhibited by ABA. However, the data from the time-course experiments show that there was an initial 30 min burst in the oxygen consumption by *Anabaena* whereafter the rate of oxygen uptake decreased markedly with time (Fig. 3). It is known that Fusicoccin promotes proton efflux from plant tissues (Ullrich and Novacky, 1990). However, this enhancement effect of Fusicoccin on proton secretion by *Anabaena* was significantly reduced by the ABA treatment. At a concentration of  $4 \times 10^{-4}$  M, the ABA had a stronger effect than at  $2 \times 10^{-4}$  M (Table 1).

A large amount of protein was released into the incubation medium after the *Anabaena* was transferred from the pre-incubation medium containing ABA at

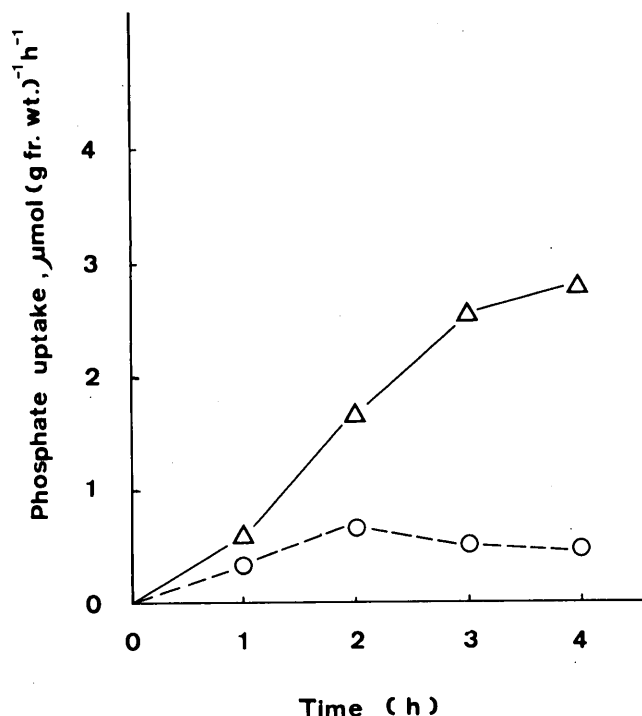


Fig. 1. The effect of  $4 \times 10^{-4}$  M ABA on phosphate uptake by *Anabaena* HA101 at 25°C.  $\Delta$ - $\Delta$ - $\Delta$ : Control;  $\circ$ - $\circ$ : Treated with ABA.

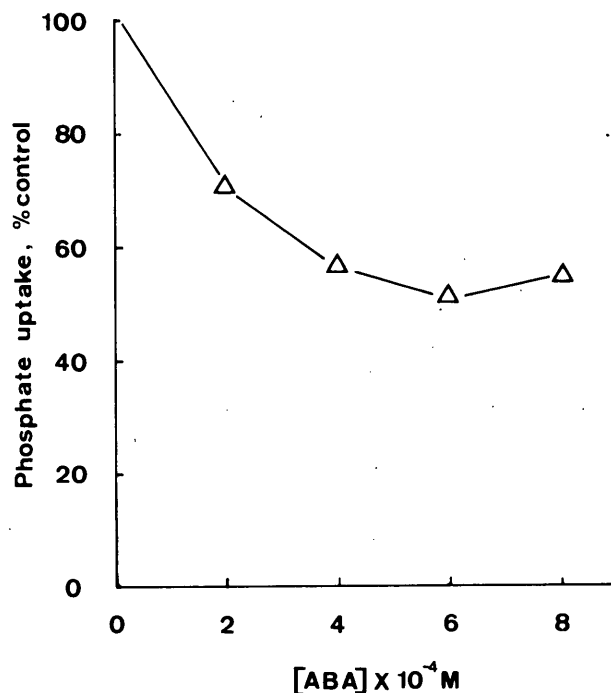


Fig. 2. The effect of ABA's concentration on its inhibition of phosphate uptake by *Anabaena* HA101.

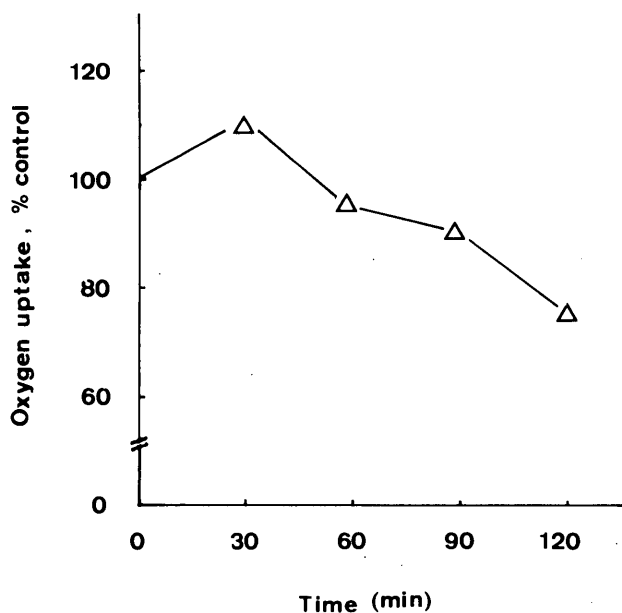


Fig. 3. Time-course of oxygen-uptake by *Anabaena* HA101 during treatment with  $4 \times 10^{-4}$  M ABA at 25°C. The rate of oxygen-uptake was  $54.4 \mu\text{mol/g fresh weight}$  before the ABA was added to the incubation medium.

Table 1. Effect of ABA on the Fusicoccin (FC)-enhanced proton secretion by *Anabaena* HA101

Treatment	H <sup>+</sup> secretion, nmol (g fr.wt.) <sup>-1</sup> h <sup>-1</sup>
Control	23.0
FC (1.0 μM)	50.1
FC (1.0 μM) + ABA (2 × 10 <sup>-4</sup> M)	20.3
FC (1.0 μM) + ABA (4 × 10 <sup>-4</sup> M)	16.9

Table 2. Effect of exposing *Anabaena* HA101 during the initial (Stage I) and/or subsequent one hour incubation stage (Stage II) to  $4 \times 10^{-4}$  M ABA on the amount of protein released into the medium

Treatment		Protein, μg (g fr.wt.) <sup>-1</sup> h <sup>-1</sup>	
Stage I	Stage II	Stage I	Stage II
-ABA	-ABA	2.5	2.7
+ABA	-ABA	2.2	19.1
+ABA	+ABA	2.3	2.5

$4 \times 10^{-4}$  M (Stage I) to the subsequent ABA-free medium (Stage II) (Table 2, Figs. 4 and 5). Only a small amount of protein was released by the *Anabaena* when both the pre-incubation medium at Stage I and the subsequent medium at Stage II contained ABA or when neither contained ABA (Table 2). The data obtained

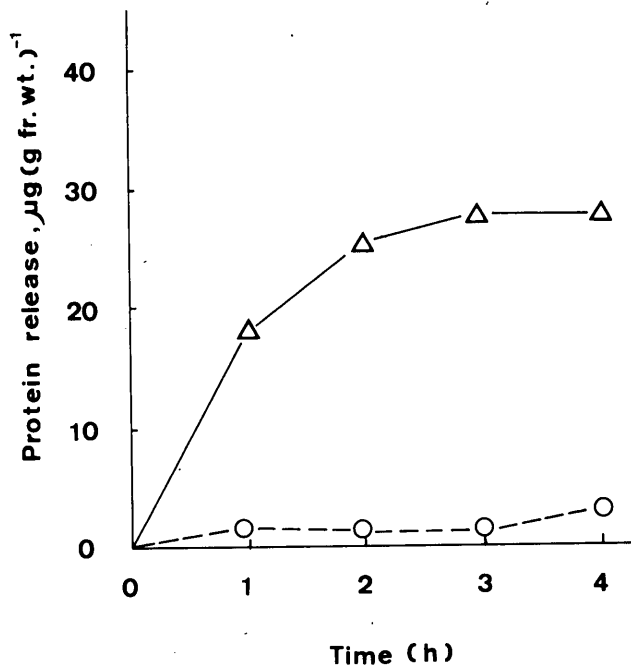


Fig. 4. The time-course of protein release from *Anabaena* HA101.  $\Delta$ - $\Delta$ - $\Delta$ : Pre-treated with ABA at  $4 \times 10^{-4}$  M for one hour then transferred to ABA-free medium;  $\circ$ - $\circ$ - $\circ$ : Pre-treatment and subsequent medium both contained ABA at  $4 \times 10^{-4}$  M.

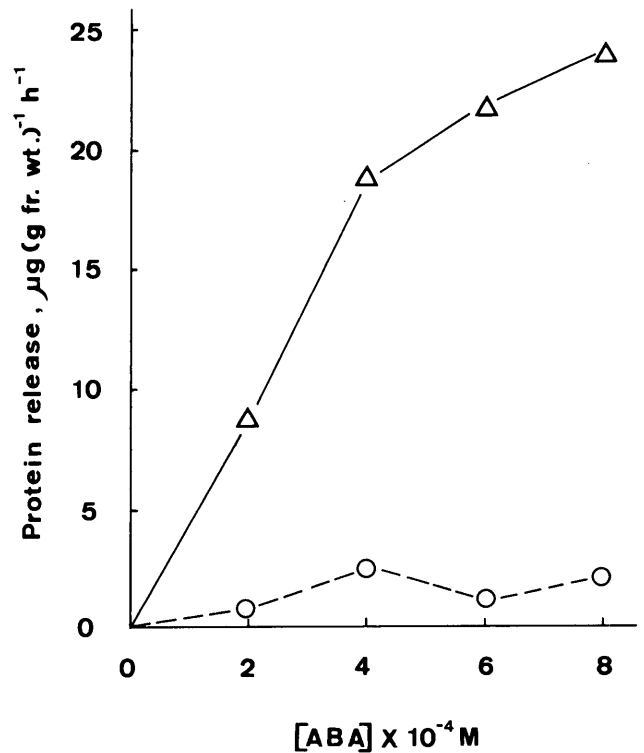


Fig. 5. The effect of the ABA concentration during a one hour pre-treatment on the subsequent protein release by *Anabaena* HA101 in ABA-free medium.  $\circ$ - $\circ$ - $\circ$ : Control;  $\Delta$ - $\Delta$ - $\Delta$ : Pre-treated with ABA.

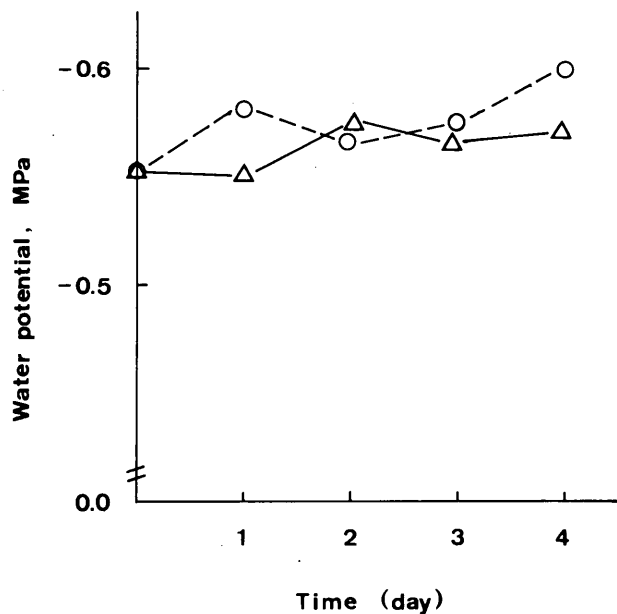


Fig. 6. The effect of  $4 \times 10^{-4}$  M ABA on the water potential of *Anabaena* HA101.  $\Delta$ - $\Delta$ - $\Delta$ : Control;  $\circ$ - $\circ$ - $\circ$ : Treated with ABA.

from the time-course experiment (Fig. 4) show that most of protein was released from the *Anabaena* during the first two hours of incubation in the ABA-free second medium. Thereafter, smaller amounts of protein were released. For the release of protein from the *Anabaena*,  $2 \times 10^{-4}$  M and  $4 \times 10^{-4}$  M ABA were most effective in the Stage I medium because ABA concentration higher than  $4 \times 10^{-4}$  M did not increase the subsequent protein release in the Stage II ABA-free medium very much.

The ABA treatment did not affect the water potential of the *Anabaena* cells significantly although there was a tendency for the treated cells to have a slightly lower water potential than the untreated cells (Fig. 6).

## Discussion

The ABA treatment reduced the permeability of the plasmalemma of *Anabaena* HA101 to ions. As the data in Figs. 1 and 2 and Table 1 show, the influx of phosphate and efflux of protons decreased when the

*Anabaena* was grown in a medium containing ABA. Several studies have shown that osmotic stress alters the membrane permeability of plant cells (Munns and Passioura, 1984; Shalhevet *et al.*, 1976) whilst salt stress inhibits phosphate uptake by barley roots (Maas *et al.*, 1979) and those stresses induced plants to accumulate ABA (Creelman and Zeevaart, 1985; LaRosa *et al.*, 1985).

ABA also reduced the growth of *Anabaena*. The *Anabaena* population as measured by the turbidity of the culture or by the fresh weight of the *Anabaena* (data not shown) was much smaller after 4 days of ABA treatment than in the controls. This growth inhibition of *Anabaena* may be related to the inhibition of proton extrusion by ABA (Table 1).

The data in Figs. 4 and 5 show that the ABA-treated *Anabaena* releases substantial amounts of protein when they are transferred to an ABA-free incubation medium. Very little protein was released when ABA was present in the second incubation medium (Table 2). Several previous studies demonstrated that osmotic shock induces bacterial cells (Kaback, 1970) and plant cells (Maas *et al.*, 1979; Rubinstein, 1982) to release a small amount of protein. This protein released from the *Anabaena* after the ABA-treatment was terminated may be the stress protein that was synthesized in the *Anabaena* plasmalemma in response to the ABA treatment. Although the physiological functions of this protein should be investigated further, it might not play an important role in the transport mechanism because phosphate influx and proton efflux were inhibited at Stage I before the protein was released from the cells (Table 2). Therefore, it is suggested that the reduction of these ionic fluxes across the plasmalemma may be due to the adverse effect of ABA on the respiratory activity of *Anabaena* (Fig. 3). It is known that phosphate-uptake and proton-secretion are active transport processes that require ATP derived from oxidative phosphorylation (Salisbury and Ross, 1985).

Although ABA is called a stress hormone which enables plants to survive under various stress-conditions, the ABA-treatment did not significantly change the water potential of *Anabaena* (Fig. 6). It is assumed that the proline or sugar which accumulate in plants in response to the accumulation of endogenous ABA did not similarly increase in *Anabaena* HA101 as a result of its exposure to the exogenous ABA treatment.

It is known that ABA accumulates in the tissues or

cells of plants or algae that are grown under water-, osmotic, or salt-stress conditions (Bensen *et al.*, 1988; Creelman and Zeevaart, 1985; LaRosa *et al.*, 1985; Stewart *et al.*, 1986; Zeevaart and Creelman, 1988). There is evidence that endogenous ABA changes the root's hydraulic conductivity (Mackhart *et al.*, 1979) and prevents stomatal opening by blocking potassium ion influx into guard cells (Schauf and Wilson, 1987). In 1988, Pesci also reported that ABA regulated KCl and NaCl influxes into barley leaves. Over-all, it may be concluded from these earlier studies that ABA regulates the plasmalemma's ion transport ability and the release of protein from tissues under stress-conditions. Because exogenous ABA, in the case of *Anabaena* HA101 reduced the plasmalemma permeability, such as the influx of phosphate, efflux of proton, and it also affected the release of protein from the cells. It may be concluded that exogenous ABA in stressed plants have similar physiological functions, except that the exogenous ABA-treatment did not regulate the water potential of *Anabaena* HA101.

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## 離層酸控制藍綠藻離子輸送及蛋白質釋放機制

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離層酸 (ABA) 處理藍綠藻 *Anabaena* HA101 引起的生理反應與其於逆境環境下所產生離層酸者極為相似。離層酸處理使 *Anabaena* HA101 的細胞膜對離子的透過性減少，以致使其生長受到抑制。於離層酸處理時 *Anabaena* HA101 並不會釋放蛋白質，但於處理後，於不含離層酸培養液時該藍綠藻則可把蛋白質放出來。此蛋白質與細胞膜的離子輸送並沒有相關。離層酸處理使 *Anabaena* HA101 呼吸作用減少才是離子透過性減少的主要因素。惟離層酸處理對 *Anabaena* HA101 水勢 (water potential) 之影響並不大。