

## Oxygen Uptake, Acidification of Medium and Nitrate Uptake Induced by Blue Light in Nitrate-Starved *Chlorella* Cells

Akio Kamiya

Laboratory of Chemistry, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa, 199-0195 Japan

**Blue light-induced oxygen uptake of the colorless mutant of *Chlorella kessleri* (No. 9.80) was 30–40% higher in the presence of exogenous glycine than in its absence. None of the other amino acids tested had this effect. Moreover, mutant cells in which glutamine synthetase was inhibited by methionine sulphoximine, accumulated approximately 65% more ammonium ions under blue irradiation in the presence of exogenous glycine than in its absence. The protein kinase C inhibitors, staurosporine or K252a, reduced the enhancement of oxygen uptake by approximately 40%. The present results indicate that blue light-dependent deamination of endogenous glycine might be a prerequisite for enhanced oxygen uptake in *Chlorella*. This blue light-induced oxygen uptake was not influenced by the inhibitors of protein phosphatase, calyculin A or okadaic acid. On the contrary, calyculin A and okadaic acid had a marked effect on the acidification of the suspension medium and nitrate uptake induced by blue light in *Chlorella* cells. The different responses to the inhibitors of protein kinase and phosphatase suggest the presence of different pathways among the blue light signal transduction operating on oxygen uptake, acidification of the medium and nitrate uptake in *Chlorella*.**

**Key words:** Blue light — *Chlorella* — Glycine — K252a — Oxygen uptake — Protein phosphatase — Staurosporine.

In the past 30 years, several regulatory influences of short-wavelength visible radiation on the basic metabolic reactions of unicellular green algae have been reported. An enhancement of oxygen uptake (Kowallik and Gaffron 1966, Pickett and French 1967, Schmid and Schwarze 1969, Kamiya and Miyachi 1974), increase in the breakdown of endogenous starch reserves (Kowallik and Schätzle 1980, Kamiya 1985), increase in degradation of cellular proteins (Miyachi and Miyachi 1987, Kamiya 1989, 1998), increase in the activities of several enzymes (Kamiya and Miyachi 1975, Kowallik and Ruyters 1976, Ruyters 1987), acidification of the suspension medium, and enhancement of the uptake of exogenous nitrate (Kamiya 1996, 1997) are the

Abbreviations: CA, calyculin A; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMSO, dimethyl sulphoximide; MSX, methionine sulphoximine; PCV, packed cell volume.

most notable examples. However, the pathways of blue light signal transduction leading to the various responses are not yet clear, although there is a report that phosphorylation of two proteins (120 kDa and 34 kDa) bound to the plasma membrane was enhanced by blue light in *Chlorella saccharophila* (Mätschke et al. 1997).

Because ammonium ions increase starch breakdown in *Chlorella* cells under dark conditions (Miyachi and Miyachi 1985), Kamiya (1989) proposed that the enhanced oxygen uptake under blue light might be a light-independent consequence of the accumulation of ammonia, resulting from the light-induced uptake of nitrate from the medium. Inhibitors of protein kinase C, staurosporine and K252a (Tamaoki et al. 1986, Yamada et al. 1987, Matsumoto and Sasaki 1989), significantly reduced the oxygen uptake, starch breakdown, acidification of the suspension medium and nitrate uptake induced by blue light (Kamiya 1997, 1998), which supports the above hypothesis. However, the oxygen uptake was not induced by pulsed blue light, whereas the acidification of the suspension medium and nitrate uptake were enhanced by the same pulsed blue light (Kamiya 1996, 1998), which suggest that there are different pathways in the blue light signal transduction in *Chlorella*.

In the present study, we investigated the effects of exogenous amino acids, which may increase the level of intracellular ammonia, on the blue light-induced oxygen uptake. We also studied the effects of two protein phosphatase inhibitors, calyculin A and okadaic acid on the acidification of medium and nitrate uptake induced by blue light, since these inhibitors affected the blue light-dependent H<sup>+</sup> pump in *Vicia* guard cells (Kinoshita and Shimazaki 1997).

### Materials and Methods

**Culture conditions**—The experiments were conducted with wild-type and two non-photosynthetic pigment mutants of the unicellular green alga, *Chlorella kessleri*, Fott et Novákova, 211-11 h. Wild-type, colorless mutant 9.80 and yellow mutant 211-11 h/20 were obtained from the Culture Collection, Institute for Plant Physiology, University Göttingen, Germany. For growth, wild-type or mutant cells were transferred from agar slants into 500 ml Erlenmeyer flasks containing 200 ml nutrient solution composed of 0.81 g KNO<sub>3</sub>, 0.68 g NaNO<sub>3</sub>, 0.12 g MgSO<sub>4</sub>, 0.41 g NaH<sub>2</sub>PO<sub>4</sub>, 0.14 g Na<sub>2</sub>HPO<sub>4</sub>, 11 mg FeCl<sub>3</sub>, 16.7 mg Ca(NO<sub>3</sub>)<sub>2</sub>, 2.4 mg H<sub>3</sub>BO<sub>3</sub>, 0.7 mg ZnSO<sub>4</sub>, 0.2 mg MnCl<sub>2</sub>, 0.04 mg CuSO<sub>4</sub>, 0.02 mg (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> and 10 g glucose per liter. The algal suspensions were kept under continuous shaking (110 rpm) at 28°C in dark-

ness. The cell mass increased significantly from 1 to 2 d after inoculation. Growth ceased after 2–3 d due to exhaustion of nitrogen and carbon sources in the medium. For experimental use, 4- to 5-day-old cultures i.e., starved cells, were used, since starved *Chlorella* cells demonstrate marked oxygen uptake in response to blue light (Kamiya and Miyachi 1974). After separation from their nutrient solution by centrifugation for 5 min at  $3,000 \times g$ , the cells were washed with distilled water by resuspending and centrifuging. The packed cell volume (PCV) was determined by centrifugation (10 min,  $3,000 \times g$ ) of 1-ml cell suspensions in micro-haematocrit tubes.

**Irradiation**—Blue and red light were supplied from a 500 W xenon lamp. The radiation was passed either through a Corning glass filter 5–60 (blue light;  $340 < \lambda < 510$  nm; Corning Glass Works, New York, U.S.A.) or a dichroic filter combined with a cold filter (CF-B) (red light;  $580 < \lambda < 730$  nm; Nihon Shinku Kogaku, Tokyo, Japan). The fluence rate was adjusted by neutral density filters (TND; Toshiba Kasei Kogyo, Tokyo, Japan) to  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ , as determined by a radiometer (model 65A; YSI-Kettering, Yellow Springs, Ohio, U.S.A.).

**Measurement of amino acids**—All amino acids used in the experiments were solubilized into  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5) at a final concentration of 2 mM (Kamiya and Kowallik 1991). The amino acid concentration in the medium was determined by the cyanid method (Yemm and Cocking 1955).

**Measurement of oxygen uptake**—After the resuspension of 0.02 ml PCV algal cells in 5 ml of 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5), oxygen uptake was determined polarometrically using a Clark-type oxygen electrode (Rank Brothers Ltd., Botolphsham, Cambridge, U.K.) as described previously (Kamiya and Miyachi 1974).

**pH measurement**—Algal cells resuspended in 10 ml of 1 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5) and placed in an open water-jacketed glass box (diameter, 3.0 cm) connected to a thermostat (28°C). Changes in pH were followed by a pH electrode (Orion Research Co. Ltd., Boston, Ma, U.S.A.) connected to an Orion pH/ion analyzer (EA 940; Orion Research, Co. Ltd.). Output voltage was recorded continuously (NP-0361, Rikadenki, Tokyo, Japan).

**Determination of nitrate and ammonia concentration**—The nitrate concentration in the medium was determined using a nitrate electrode (Orion Research Co. Ltd.) connected to an Orion pH/ion analyzer (Kamiya 1997). In this experiment, we used the yellow mutant of *C. kessleri*, in which a pronounced blue-light effect was observed on nitrate uptake, while in other experiments we used the colorless mutant. Intracellular ammonia concentration was determined in methanol extracts (1 ml algal suspension plus 4 ml methanol according to Miyachi and Miyachi [1987]) by the phenol-hypochlorite method of Solorzano (1969). Briefly, the procedure consisted of successive addition of phenol solution, sodium nitroprusside solution and freshly prepared oxidizing reagent containing sodium citrate and hypochlorite solution to the methanol extracts. The color was allowed to develop for 1 h and absorbance was recorded at 640 nm.

**Chemicals**—Amino acids were purchased from Ajinomoto Co. (Tokyo, Japan). Staurosporine, K252a, calyculin A and okadaic acid were purchased from Sigma Co. (St. Louis, MO, U.S.A.) and solubilized in dimethyl sulphoxide (DMSO). The DMSO concentration in the medium never exceeded 0.2% (v/v). DCMU was solubilized in methanol.

## Results

**Effect of glycine on blue light-induced oxygen uptake**—The addition of glycine (2 mM final conc.) to the starved colorless *Chlorella* mutant cells suspended in 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5) at 28°C did not enhance the minimal respiratory-oxygen uptake in darkness. However, under blue light, oxygen uptake was enhanced by 35% by the addition of glycine (Fig. 1). This increase was saturated at 4 min after the addition of glycine, and remained constant thereafter for at least 14 min (Fig. 1 inset). The enhancement of blue light-induced oxygen uptake appears to be specific to glycine, since none of the other amino acids tested showed similar enhancement (Table 1), although L-serine and L-alanine showed slight enhancement in five measurements. On the other hand, oxygen uptake enhanced by blue light was decreased by 10–25% in the presence of L-lysine, L-histidine, L-arginine, L-leucine, L-aspartic acid or L-glutamine. The reason for these changes is not clear.

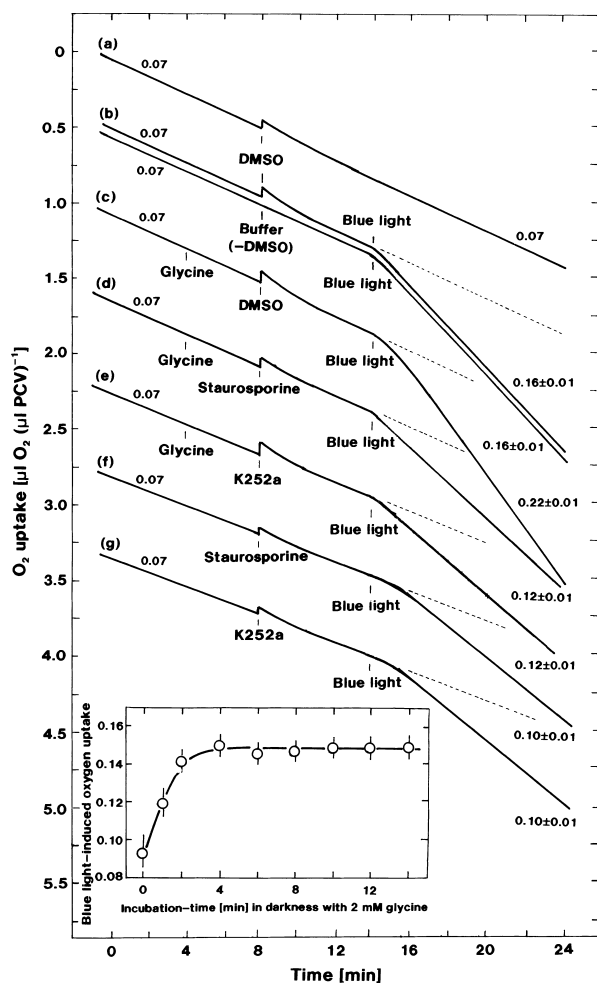
The increase in oxygen uptake under blue light in the presence of glycine was reduced by approximately 40% in the presence of staurosporine or K252a (Fig. 1d, e). The two protein phosphatase inhibitors, calyculin A and okadaic acid, had no measurable effect on the blue light-induced oxygen uptake, even in the presence of 2 mM glycine (Fig. 2).

In the starved cells in which glutamine synthetase was inhibited by 2 mM methionine sulphoximine (MSX), ammonia was accumulated continuously in darkness. This MSX-induced accumulation of ammonia was approximately 20–30% higher in the cells exposed to blue light than in the dark (Fig. 3). Furthermore, in the cell suspensions supplied with exogenous glycine, the accumulation of

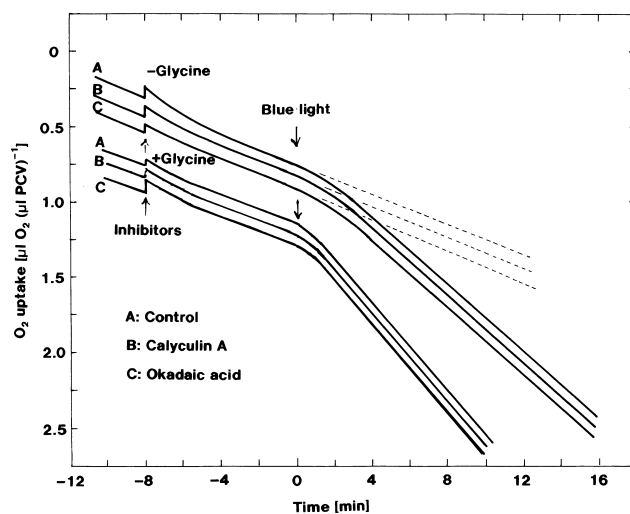
**Table 1** Effects of exogenous amino acids on blue light-induced oxygen uptake in a colorless mutant of *C. kessleri*

Amino acid	Blue light-induced oxygen uptake [% of dark control]
Control	111.6 ± 11.6
L-Lysine	73.9 ± 9.5
L-Serine	135.0 ± 10.1
L-Alanine	137.5 ± 6.0
Glycine	193.4 ± 6.9
L-Glutamine	85.7 ± 4.3
L-Glutamic acid	100.0 ± 5.0
L-Histidine	76.2 ± 5.2

Algal cells (0.02 ml PCV) grown for 5 d were washed and suspended in 5 ml 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5). Amino acids were added as aqueous solution (pH 6.5) at final concentration of 2 mM. Amino acid uptake was adjusted to  $2 \mu\text{mol ml}^{-1}$  PCV by adjusting application period.

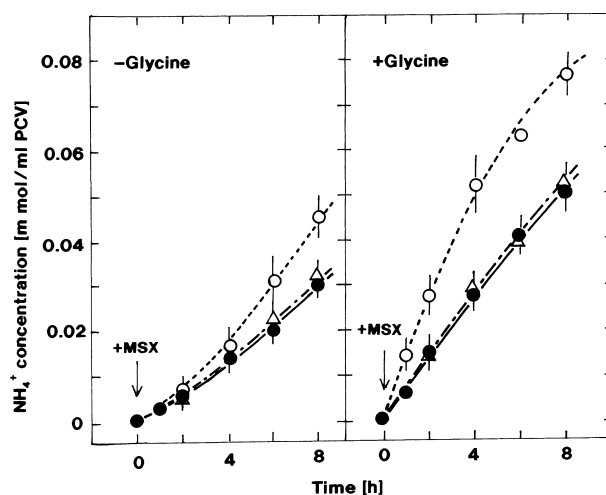


**Fig. 1** Effects of staurosporine and K252a on glycine-enhanced blue light-induced oxygen uptake in a colorless mutant of *C. kessleri*. Algal cells (0.02 ml PCV) grown for 5 d were washed and suspended in 5 ml 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5). (a) Dimethyl sulfoxide (DMSO, final concentration, 0.2% (v/v)) was added to the algal suspension after 8 min. (b) Blue light ( $340 < \lambda < 510$  nm,  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was given after 14 min. (c) Glycine (2 mM, final concentration) was added after 4 min. Staurosporine (d) and K252a (e), solubilized in DMSO, were added 4 min after the addition of glycine. For comparison, effects of staurosporine (f) and K252a (g) without glycine on blue light-induced oxygen uptake are shown (Kamiya 1998). Addition of DMSO, glycine, staurosporine and K252a alone did not influence the rate of oxygen uptake in darkness. Values in figures indicate the rate of oxygen uptake ( $\mu\text{l O}_2 (\mu\text{l PCV})^{-1} \text{min}^{-1}$ ). Inset shows the time course of blue light-induced oxygen uptake during the incubation with glycine. Algal cells were incubated with glycine (final concentration, 2 mM) for indicated periods of time in the dark. Thereafter, they were irradiated with blue light for 10 min. Blue light had little effect on the uptake of glycine during the experimental period. Oxygen uptake was determined with a Clark-type oxygen electrode (Kamiya and Miyachi 1974). The value at time zero indicates blue light-induced oxygen uptake without glycine ( $0.092 \mu\text{l O}_2 (\mu\text{l PCV})^{-1} \text{min}^{-1}$ ) and expressed as the rate obtained after subtracting that of dark  $\text{O}_2$  uptake. Vertical bars indicate standard deviation.



**Fig. 2** Effects of calyculin A and okadaic acid on blue light-induced oxygen uptake in the presence of glycine in a colorless mutant of *C. kessleri*. Algal cells (0.02 ml PCV) were suspended in 5 ml 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5). Calyculin A and okadaic acid were solubilized in DMSO and added at a final concentration of 1.0 and 2.0  $\mu\text{M}$ , respectively. Glycine (final conc. 2 mM) was added 10 min prior to time zero.

ammonia was increased 90% by blue light than under darkness during the 4-h exposure period. This suggests that

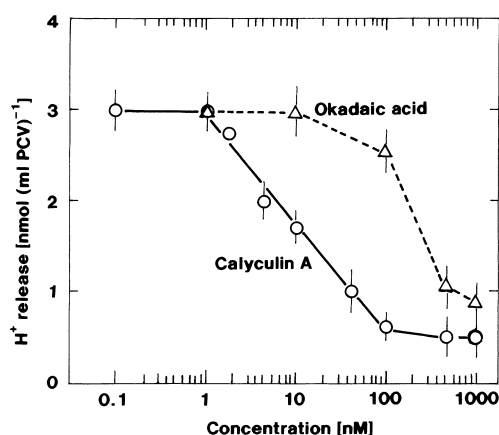


**Fig. 3** Effect of glycine on the release of ammonia in the presence of MSX in nitrate-grown colorless mutant of *C. kessleri*. Algal cells (0.2 ml PCV) were grown in nitrate-medium, washed and resuspended in 10 ml 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 6.5). MSX (final concentration, 2 mM (left)) and MSX plus glycine (final concentration, 2 mM (right)) were added at time zero. Open circles, blue light,  $340 < \lambda < 510$  nm; open triangles, red light,  $580 < \lambda < 730$  nm; closed circles, dark control. Blue- and red-irradiance,  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ . For determination of ammonia concentration, see Materials and Methods.

blue light enhances the activity of glycine oxidases *in vivo*, has been observed *in vitro* by Schmid and Schwarze (1969).

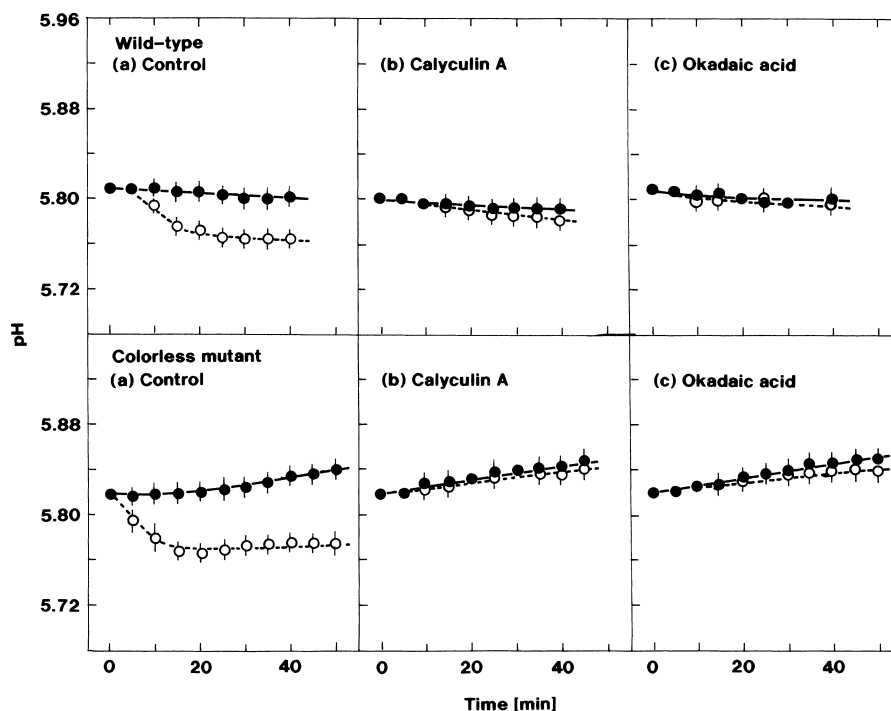
In contrast to the absence of a significant effect of calyculin A and okadaic acid on the glycine-induced enhancement of the blue-light-induced oxygen uptake, these inhibitors were found to have a marked effect on the action of blue light on both acidification of suspension medium and nitrate uptake in *Chlorella* cells.

**Effect of calyculin A and okadaic acid on the blue light-induced acidification of algal suspension**—In darkness, the pH of the suspension medium (1 mM NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>-buffer) of heterotrophically-grown, starved colorless mutant and that of the 10<sup>-5</sup> M DCMU-treated wild-type cells of *Chlorella* did not change significantly from the original pH of approximately 5.8 (wild-type, ΔpH=0–0.01; mutant, ΔpH=0–0.02) over the course of 40–50 min (Fig. 4a). Exposure to blue light, however, resulted in acidification of the medium. Immediately after the onset of light, the pH began to drop, falling by approximately 0.05 within 15–20 min, and remaining constant thereafter (Fig. 4a). Irradiation of blue light for 3 min was enough to induce the same degree of acidification; the pH continued to drop for 10–15 min in the following dark, and then stabilized as under continuous irradiation (data not shown). Acidification of the medium was inhibited by the addition

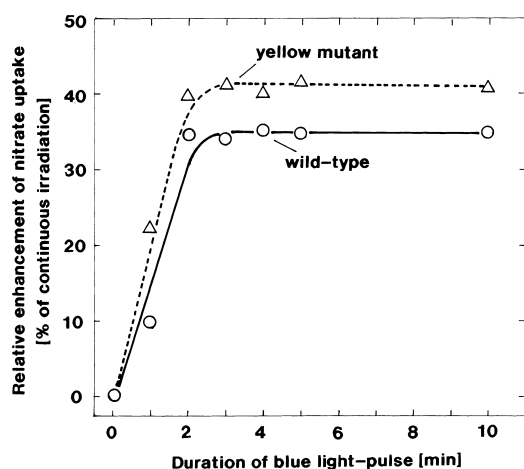


**Fig. 5** Effects of calyculin A and okadaic acid at various concentrations on the blue light-induced acidification of suspension media of colorless mutant cells of *C. kessleri*. Algal cells were incubated with various concentrations of calyculin A or okadaic acid for 20 min under blue light or in the dark. All samples contained DMSO (final concentration, 0.2% (v/v)). Vertical bars indicate standard deviation. Other conditions are described in Fig. 4.

of the phosphatase inhibitors, calyculin A (1 μM) and okadaic acid (1 μM), in both the wild-type and mutant cells



**Fig. 4** Inhibition by calyculin A and okadaic acid of blue light-induced acidification of suspension medium of wild-type and colorless mutant cells of *C. kessleri*. Cell concentration of wild-type and colorless mutant was 0.2 and 0.3 ml PCV per 10 ml 1 mM NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 5.8), respectively. Calyculin A and okadaic acid (final concentration, 1 μM) were added 5 min prior to time zero. Control contained 0.2% (v/v) DMSO. Photosynthesis of wild-type was inhibited by 10<sup>-5</sup> M DCMU. Other conditions are described in Fig. 3.



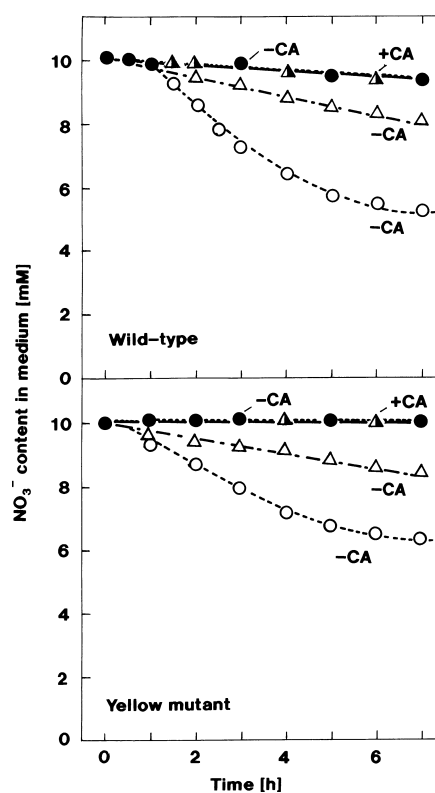
**Fig. 6** Enhancement of nitrate uptake by blue light-pulses of various durations as compared with that by continuous irradiation in wild-type and yellow mutant cells of *C. kessleri*. Algal cells (0.4 ml PCV) were suspended in 10 ml 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer + 10 mM  $\text{NaNO}_3$  (pH 6.5). Algal cells exposed to blue light-pulse ( $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for various pulse durations and incubated in the dark for 4 h thereafter. In wild-type cells, photosynthesis was inhibited by  $10^{-5}$  M DCMU. Enhancement is given as the percentage of the enhancement relative to that under continuous irradiation.

under both continuous (Fig. 4b, c) or pulsed irradiation (data not shown). Calyculin A was approximately 20 times more effective than okadaic acid. Acidification was inhibited by 50% either by 10 nM calyculin A, or by 200 nM okadaic acid (Fig. 5).

**Effect of calyculin A on blue light-induced nitrate uptake**—A brief irradiation with blue light was enough to induce the increase in nitrate uptake as in the case of the acidification of the medium. Blue light for 2 min induced a 35–40% increase in nitrate uptake (Fig. 6). This enhancement was maintained, even in darkness throughout the subsequent experimental period (Fig. 7). Calyculin A inhibited the blue light-induced enhancement of nitrate uptake, but had no effect on nitrate uptake in the dark.

## Discussion

Our recent study showed that the effects of blue light on oxygen uptake and breakdown of starch are suppressed by staurosporine and K252a in *Chlorella* cells (Kamiya 1998). Blue light also enhances the release of ammonia, which activates  $\alpha$ -amylase and phosphorylase isolated from wild-type of *C. kessleri*, and exogenous ammonia enhances the breakdown of starch and respiration (Miyachi and Miyachi 1985, 1987). Enhancement of nitrate reductase activity by blue light is also well established (Stoy 1955, Aparicio et al. 1976, Roldan and Butler 1980). However, oxygen uptake and release of ammonia are induced by blue



**Fig. 7** Effect of calyculin A on blue light-pulse-induced nitrate uptake in wild-type and yellow mutant cells of *C. kessleri*. Algal cells (0.2 ml PCV) were suspended in 10 ml 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer plus 10 mM  $\text{NaNO}_3$  (pH 6.5). Open circles, continuous blue irradiation; closed circles, dark control; triangles, pre-irradiated for 5 min with blue light in the presence ( $\blacktriangle$ ) or absence ( $\triangle$ ) of calyculin A (final concentration,  $1 \mu\text{M}$ ). Blue fluence rate,  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Photosynthesis of wild-type cells was inhibited by  $10^{-5}$  M DCMU. All samples contained DMSO (final concentration, 0.2% (v/v)). Shown are typical results of five independent experiments.

light in both nitrate- and ammonia-grown *Chlorella* cells (Kamiya 1998), suggesting that ammonia is released under blue light through a pathway other than nitrate reduction, because ammonia-grown *Chlorella* cells do not possess the nitrate reductase and its activity (Zeiler and Solomonson 1989).

In the present study, the addition of glycine enhanced the blue light-induced oxygen uptake by approximately 30–40%, but this enhancement was suppressed by staurosporine or K252a. Moreover, blue light stimulated the release of ammonia from exogenous glycine in the presence of MSX (see Fig. 3), which is assumed to be catalyzed by glycine oxidase (Schmid and Schwarze 1969). We previously observed that the addition of MSX to the cultures in the dark caused the release of ammonia, and increased the rate of starch breakdown (Kamiya 1989). Thus, we assumed that glycine oxidase is phosphorylated by the action

of blue light in *Chlorella* cells. Schmid and Schwarze (1969) previously found that glycine oxidase activity in the mixture of the enzyme and substrate was enhanced by irradiation with blue light. Therefore, it will be necessary to determine whether glycine oxidase is really phosphorylated by the action of blue light. In this connection, it is interesting that blue light induced the phosphorylation of distinct proteins (120 kDa and 34 kDa) bound to the plasma membrane in *Chlorella saccharophila* (Mätschke et al. 1997).

Protein phosphatases, which catalyze protein dephosphorylation, are crucial regulators of many cellular processes in animals and plants (Cohen 1989, Smith and Walker 1996). According to their substrate specificity, protein phosphatases are classified into serine/threonine- and tyrosine-type phosphatases. The former can be further subdivided into types 1, 2A, 2B, and 2C. Type-1 protein phosphatase is ubiquitous to all eukaryotes (Cohen 1989). Recent in vitro studies have indicated that type-1 and type-2A phosphatases are markedly inhibited by calyculin A and okadaic acid at nanomolar concentrations, whereas the type-2B, type-2C, and tyrosine-type phosphatases are not sensitive to these inhibitors at the same concentrations (Ishihara et al. 1989). Furthermore, type-1 phosphatase is more sensitive to calyculin A than to okadaic acid, whereas type-2A is more sensitive to okadaic acid than to calyculin A.

The present study showed that blue light-induced acidification of the suspension medium of wild-type and colorless mutant cells of *C. kessleri*, was inhibited by approximately 60% by calyculin A and okadaic acid and was much more sensitive to calyculin A than to okadaic acid. The  $IC_{50}$  of calyculin A was 10 nM whereas that of okadaic acid was 200 nM, suggesting that type-1 protein phosphatase is most likely involved in the blue light-induced acidification. This blue light-induced acidification is largely inhibited by vanadate ( $V_2O_5$ ,  $Na_3VO_4$ ), without affecting the enhancement of oxygen uptake by blue light (Kamiya 1996). This suggests that the pH change induced by blue light is associated with  $H^+$ -pump activity rather than the release of  $CO_2$  by respiration. On the other hand, the uptake of nitrate induced by 5 min of blue light in wild-type and yellow mutant cells of *C. kessleri* was completely eliminated by the presence of calyculin A. On the contrary, blue light-enhanced oxygen uptake is not influenced by calyculin A or okadaic acid and, the pulsed blue light does not enhance the oxygen uptake, which are different from the acidification of the suspension medium and nitrate uptake induced by blue light (Kamiya 1996, 1998). Therefore, there seem to be different pathways among the blue-light signal transductions operating on oxygen uptake, acidification of the medium and nitrate uptake in *Chlorella* cells. Further research is needed to elucidate these differences.

I would like to thank Prof. Dr. W. Kowallik, Bielefeld University, for his valuable suggestions. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

## References

- Aparicio, P.J., Roldan, J.M. and Calero, F. (1976) Blue light photoreactivation of nitrate reductase from green algae and higher plants. *Biochem. Biophys. Res. Commun.* 70: 1071-1077.
- Cohen, P. (1989) The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* 58: 453-508.
- Ishihara, H., Martin, B.L., Brautigam, D.L., Karaki, H., Ozaki, H., Kato, Y., Fusetani, N., Watabe, S., Hashimoto, K., Uemura, D. and Hartshorne, D.J. (1989) Calyculin A and okadaic acid: inhibitors of protein phosphatase activity. *Biochem. Biophys. Res. Commun.* 159: 871-877.
- Kamiya, A. (1985) Light-induced changes in the molecular size of starch in colorless *Chlorella* cells. *Plant Cell Physiol.* 26: 759-763.
- Kamiya, A. (1989) Effects of blue light and ammonia on nitrogen metabolism in a colorless mutant of *Chlorella*. *Plant Cell Physiol.* 30: 513-521.
- Kamiya, A. (1996) Blue light-induced acidification of the medium of a nitrate starved *Chlorella* mutant. *Physiol. Plant.* 98: 36-42.
- Kamiya, A. (1997) Blue light-induced acidification of the medium and the uptake of nitrate in a nitrate-starved *Chlorella*. *Photochem. Photobiol.* 66: 288-293.
- Kamiya, A. (1998) Signal transduction in blue light-induced oxygen uptake in *Chlorella* cells. *Physiol. Plant.* 104: 50-55.
- Kamiya, A. and Kowallik, W. (1991) Influences of blue light on uptake and consumption of amino acids in a colorless mutant of *Chlorella*. *J. Plant Physiol.* 138: 279-284.
- Kamiya, A. and Miyachi, S. (1974) Effects of blue light on respiration and carbon dioxide fixation in colorless *Chlorella* mutant cells. *Plant Cell Physiol.* 15: 927-937.
- Kamiya, A. and Miyachi, S. (1975) Blue light-induced formation of phosphoenolpyruvate carboxylase in colorless *Chlorella* mutant cells. *Plant Cell Physiol.* 16: 729-736.
- Kinoshita, T. and Shimazaki, K. (1997) Involvement of calyculin A- and okadaic acid-sensitive protein phosphatase in the blue light response of stomatal guard cells. *Plant Cell Physiol.* 38: 1281-1285.
- Kowallik, W. and Gaffron, H. (1966) Respiration induced by blue light. *Planta* 69: 92-95.
- Kowallik, W. and Ruyters, G. (1976) Über Aktivitätssteigerungen der Pyruvatkinase durch Blaulicht oder Glucose bei einer chlorophyllfreien *Chlorella*-Mutante. *Planta* 128: 11-14.
- Kowallik, W. and Schätzle, S. (1980) Enhancement of carbohydrate degradation by blue light. In *The Blue Light Syndrome*. Edited by Senger, H. pp. 344-360. Springer-Verlag, Berlin.
- Mätschke, M., Riedel, J. and Tischner, R. (1997) Evidence for signal transduction in the stimulation of nitrate uptake by blue light in *Chlorella saccharophila*. *Photochem. Photobiol.* 66: 128-132.
- Matsumoto, H. and Sasaki, Y. (1989) Staurosporine, a protein kinase C inhibitor interferes with proliferation of arterial smooth muscle cells. *Biochem. Biophys. Res. Commun.* 158: 105-109.
- Miyachi, S. and Miyachi, S. (1985) Ammonia induces starch degradation in *Chlorella* cells. *Plant Cell Physiol.* 26: 245-252.
- Miyachi, S. and Miyachi, S. (1987) Some biochemical changes related to starch breakdown induced by blue light illumination and by addition of ammonia to *Chlorella* cells. *Plant Cell Physiol.* 28: 309-314.
- Pickett, J.M. and French, C.S. (1967) The action spectrum for blue-light-stimulated oxygen uptake in *Chlorella*. *Proc. Natl. Acad. Sci. USA* 57: 1587-1593.
- Roldan, J.M. and Butler, W.L. (1980) Photoactivation of nitrate reductase from *Neurospora crassa*. *Photochem. Photobiol.* 32: 375-381.
- Ruyters, G. (1987) Control of enzyme capacity and enzyme activity. In *Blue Light Responses: Phenomena and Occurrence in Plants and Microorganisms*. Edited by Senger, H. Vol. II, pp. 71-88. CRC Press Inc., Boca Raton, FL.
- Schmid, G.H. and Schwarze, P. (1969) Blue light enhanced respiration in

- a colorless *Chlorella* mutant. *Hoppe-Seyler's Z. Physiol. Chem.* 350: 1513-1520.
- Smith, R.D. and Walker, J.C. (1996) Plant protein phosphatases. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 101-125.
- Solorzano, L. (1969) Determination of ammonia in natural waters by the phenol-hypochlorite method. *Limnol. Oceanogr.* 14: 799-801.
- Stoy, V. (1955) Action of different light qualities on simultaneous photosynthesis and nitrate assimilation in wheat leaves. *Physiol. Plant.* 8: 963-986.
- Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F. (1986) Staurosporine, a potent inhibitor of phospholipid/ $\text{Ca}^{++}$  dependent protein kinase. *Biochem. Biophys. Res. Commun.* 135: 397-402.
- Yamada, K., Iwahashi, K. and Kase, H. (1987) K252a, a new inhibitor of protein kinase C, concomitantly inhibits 40K protein phosphorylation and serotonin secretion in phorbol ester-stimulated platelets. *Biochem. Biophys. Res. Commun.* 144: 35-40.
- Yemm, E.W. and Cocking, E.C. (1955) The determination of amino acids with ninhydrin. *Analyst* 80: 209-213.
- Zeiler, K. and Solomonson, L.P. (1989) Regulation of *Chlorella* nitrate reductase: control of enzyme activity and immunoreactive protein levels by ammonia. *Arch. Biochem. Biophys.* 269: 46-54.

(Received September 24, 1999; Accepted February 8, 2000)