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Glycinebetaine alleviates the inhibitory effect of moderate heat stress on the repair of photosystem II during photoinhibition

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Abstract

Transformation with the bacterial gene *codA* for choline oxidase allows *Synechococcus* sp. PCC 7942 cells to accumulate glycinebetaine when choline is supplemented exogenously. First, we observed two types of protective effect of glycinebetaine against heat-induced inactivation of photosystem II (PSII) in darkness; the *codA* transgene shifted the temperature range of inactivation of the oxygen-evolving complex from 40–52 °C (with half inactivation at 46 °C) to 46–60 °C (with half inactivation at 54 °C) and that of the photochemical reaction center from 44–55 °C (with half inactivation at 51 °C) to 52–63 °C (with half inactivation at 58 °C). However, in light, PSII was more sensitive to heat stress; when moderate heat stress, such as 40 °C, was combined with light stress, PSII was rapidly inactivated, although these stresses, when applied separately, did not inactivate either the oxygen-evolving complex or the photochemical reaction center. Further our studies demonstrated that the moderate heat stress inhibited the repair of PSII during photoinhibition at the site of synthesis *de novo* of the D1 protein but did not accelerate the photodamage directly. The *codA* transgene and, thus, the accumulation of glycinebetaine alleviated such an inhibitory effect of moderate heat stress on the repair of PSII by accelerating the synthesis of the D1 protein. We propose a hypothetical scheme for the cyanobacterial photosynthesis that moderate heat stress inhibits the translation machinery and glycinebetaine protects it against the heat-induced inactivation.

Keywords: *codA* gene; Glycinebetaine; Heat stress; Photoinhibition; Photosystem II; Protein synthesis; Repair

1. Introduction

Heat stress is one of the main environmental factors that limits the growth and productivity of plants [1,2]. It seems likely that photosynthesis is most sensitive to such heat stress among various physiological processes [3]. Inhibition of photosynthesis under heat stress is common to plants in tropical and subtropical regions and the temperature zones where plants are exposed periodically to high temperatures [4]. Among various machineries of photosynthesis, the photosystem II complex (PSII) is particularly sensitive to heat, and even a short period of exposure to high temperatures irreversibly inactivates the oxygen-evolving complex of PSII [5,6]. The photochemical reaction center of PSII is also inactivated by heat, but is less sensitive to heat than the oxygen-evolving complex [5,7].

We have shown that photoinhibition of PSII is stimulated by abiotic stress, such as low-temperature [8], high-salt [9–11], and oxidative stress [12,13]. The CO_2 limitation stress also enhanced the extent of photoinhibition in PSII [14,15]. Since the extent of photoinhibition is a result of balance between the rate of light-induced inactivation (photodamage) and the rate of repair, we investigated the effect of abiotic stress on the rates of photodamage and repair separately. The results demonstrate that abiotic stress, such as cold [8,16,17], salt [11] and oxidative

Abbreviations: BQ, 1,4-benzoquinone; Chl, chlorophyll; PAM, Synechococcus sp. PCC 7942 transformed with the empty vector (control); PAMCOD, *Synechococcus* sp. PCC 7942 transformed with a vector that contains the *codA* gene

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[12,13], inhibits the repair of PSII, but does not affect the photodamage to PSII [18,19]. The step that is affected by abiotic stress is the translation of *psbA* mRNA [11–13].

Glycinebetaine (*N*,*N*,*N*-trimethylglycine; hereafter betaine) is accumulated in a variety of plants and microorganisms under abiotic stress, such as low temperature, salinity and drought [20–23]. We previously transformed the cyanobacterium *Synechococcus* sp. PCC 7942 (hereafter, *Synechococcus*) with the *codA* gene for choline oxidase from *Arthrobacter globiformis*, which synthesizes betaine from choline [24,25]. Transformed cells, designated as PAMCOD [25] synthesized betaine *in vivo* from exogenously supplied choline and accumulated betaine at levels of 60 to 80 mM in the cytoplasm. We further explored that the accumulation of betaine protected PSII against photoinhibition under low-temperature stress and salt stress [24,25]. A recent study demonstrated that betaine counteracted the inhibitory effect of salt stress on the translation of *psbA* mRNA [26].

A similar system was developed in higher plants by genetic engineering with the *codA* gene for biosynthesis of betaine in *Arabidopsis thaliana* and we observed that the resultant transgenic plants accumulated betaine in leaves at levels of about 1 mM [27]. We have shown that the transformation enhanced the tolerance to moderate heat stress during germination of seeds and growth of plants [28,29]. Transformation with cDNA for betaine aldehyde dehydrogenase also allowed *Nicotiana tabacum* plants to synthesize betaine and enhanced the tolerance to moderate heat stress [30,31].

In the present study, we demonstrated, using PAMCOD cells of *Synechococcus,* that moderate heat stress inhibited the synthesis of the D1 protein of PSII and that the accumulation *in vivo* of betaine counteracted the inhibitory effects of moderate heat stress in the synthesis of the D1 protein. In addition, the betaine accumulation protected the oxygen-evolving complex and the photochemical reaction center when *Synechococcus* cells were incubated at high temperatures in darkness.

2. Materials and methods

2.1. Organisms and culture conditions

In brief, *Synechococcus* sp. PCC 7942 strains PAM (a control strain that had been transformed with the plasmid vector alone) and PAMCOD (a strain that had been transformed with the plasmid vector that included the codA gene for choline oxidase (X84895)) were obtained as described previously [24]. Cells were grown photoautotrophically as described previously in glass tubes (25 mm i.d × 200 mm) at 30 °C under constant illumination from incandescent lamps at 70 μmol photons $m^{-2}s^{-1}$ in BG-11 medium [32] supplemented with 20 mM HEPES–NaOH (pH 7.5) in the presence of 1.0 mM choline chloride. Cultures were aerated with sterile air that contained 1% CO2 [33].

2.2. Exposure of cells to heat and light stress

Cells from 3-day-old cultures were harvested by centrifugation at 8000×*g* for 10 min at 30 °C and resuspended in fresh BG-11 medium at a Chl concentration of 5 μg ml^{-1} [10,34]. The resultant suspensions of cells were incubated at 30 °C for 1 h in 100-ml glass tubes in growth chambers under conditions identical to the original culture conditions. When photoinhibition was induced, cells were exposed to light from incandescent lamps at 500 or 1,000 μmol photons m^{-2} s^{-1}. In some experiments, protein synthesis was blocked by inclusion in the medium of 250 μg ml^{-1} lincomycin (Sigma Chemical Co., St. Louis, MO), which was added to the culture medium 10 min before the start of incubation. Heat treatment was carried out at different temperatures in 100-ml glass tubes in growth chambers under normal growth conditions.

2.3. Measurement of photosynthetic activity

We measured the activity of PSII in intact cells by monitoring the oxygen-evolving activity at 30 °C with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, U.K.) in the presence of 1.0 mM 1,4-benzoquinone (BQ) as the electron acceptor and an inhibitor of respiration [33,35]. The sample, in a 3-ml cuvette, was illuminated by light that had been passed through a red optical filter (R-60; Toshiba, Tokyo, Japan) and an infrared-absorbing filter (HA-50; Hoya Glass, Tokyo, Japan). The intensity of light at the surface of the cuvette was 2000 μmol photons m^{-2} s^{-1}.

2.4. Kinetics of changes in the fluorescence of Chl a

The light-induced quenching of the fluorescence of Chl *a* due to reduction of pheophytin [36–38] in intact cells was monitored with a fluorometer (PAM-101; Heinz Walz, Effeltrich, Germany) in the pulse-amplitude modulation mode. The light-induced quenching of Chl fluorescence was measured at 30 °C in the presence of 1 mg ml^{-1} sodium dithionite after the continuous exposure of the sample to actinic light ($\lambda > 520$ nm) from an incandescent lamp (KL-1500 Electronic; Schott Glasswerke, Wiesbaden, Germany) at 2700 μmol photons m^{-2} s^{-1}. The concentration of Chl was determined as described by Arnon et al. [39]. Other experimental details are given in the legends to figures.

2.5. Isolation of thylakoid membranes and immunoblotting analysis of the D1 protein

Thylakoid membranes were isolated from PAM and PAMCOD cells, as described previously [10,34]. Thylakoid membranes were solubilized by incubation for 5 min at 65 °C in 60 mM Tris buffer (pH adjusted to 6.8 with HCl) that contained 2% (w/v) sodium dodecyl sulfate (SDS), 5% (v/v) 2-mercaptoethanol and 10% (v/v) glycerol, and then proteins were separated by polyacrylamide gel electrophoresis [12.5% (w/v) polyacrylamide] in the presence of 0.08% (w/v) SDS and 6 M urea, as described previously [11]. Molecular markers (Kaleidoscope pre-stained standards; Bio-Rad Laboratories, Hercules, CA) were used for estimations of the molecular masses of immunoreactive proteins. After electrophoresis, the separated proteins were blotted onto a nitrocellulose membrane (Schleicher & Schuell Inc., Keene, NH) in a semi-dry transfer apparatus (Atto, Tokyo, Japan). Then, the D1 protein was detected immunologically with an ECL Western blotting kit according to the protocol supplied with the kit (Amersham International, Buckinghamshire, UK).

The total D1 protein was detected with antibodies raised in rabbits against a synthetic oligopeptide that corresponded to the DE loop of the D1 protein (amino acids 234–242, counted from the amino terminus of the D1 protein from spinach). These antibodies recognize the products (D1) of the *psbAI*, *psbAII* and *psbAIII* genes because the amino acid sequence of the AB loop is exactly the same in the products of these genes. As second antibodies, we used horseradish peroxidase-linked antibodies raised in donkeys against rabbit immunoglobulin G (Amersham International).

The antibodies raised in rabbit against D1 were kindly provided by Prof. Kimiyuki Satoh (Department of Biology, Okayama University, Okayama, Japan). A digital camera system (LAS-1000; Fuji Photo Film Co., Tokyo, Japan) was used to monitor signals from blotted membranes and to quantify the D1 protein.

2.6. Northern blotting analysis

Total RNA was isolated from cells and Northern-blotting analysis was performed as described previously [11,40]. Equal amounts of RNA (4 μg) from each sample were loaded in individual wells of the gel and rRNA was visualized by staining with ethidium bromide. Hybridizations were performed at 55 °C according to standard procedures. Two oligonucleotides of 66 bp, one corresponding to the untranslated sequence of the *psbAI* gene for D1:1 and the other to the similar untranslated sequences of *psbAII* and *psbAIII* genes for D1:2, were used as transcript-specific probes for *psbAI* and *psbAII–psbAIII*