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Glycerate-3-phosphate, produced by CO_2 fixation in the Calvin cycle, is critical for the synthesis of the D1 protein of photosystem II

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Abstract

We demonstrated recently that, in intact cells of *Chlamydomonas reinhardtii*, interruption of CO_2 fixation via the Calvin cycle inhibits the synthesis of proteins in photosystem II (PSII), in particular, synthesis of the D1 protein, during the repair of PSII after photodamage. In the present study, we investigated the mechanism responsible for this phenomenon using intact chloroplasts isolated from spinach leaves. When CO_2 fixation was inhibited by exogenous glycolaldehyde, which inhibits the phosphoribulokinase that synthesizes ribulose-1,5-bisphosphate, the synthesis de novo of the D1 protein was inhibited. However, when glycerate-3-phosphate (3-PGA), which is a product of CO_2 fixation in the Calvin cycle, was supplied exogenously, the inhibitory effect of glycolaldehyde was abolished. A reduced supply of CO_2 also suppressed the synthesis of the D1 protein, and this inhibitory effect was also abolished by exogenous 3-PGA. These findings suggest that the supply of 3-PGA, generated by CO_2 fixation, is important for the synthesis of the D1 Protein. It is likely that 3-PGA accepts electrons from NADPH and decreases the level of reactive oxygen species, which inhibit the synthesis of proteins, such as the D1 protein.
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Keywords: Calvin cycle; CO_2 fixation; D1 protein; Glycerate-3-phosphate; Photoinhibition; Photosystem II

1. Introduction

Light-dependent damage to photosystem II (PSII) is unavoidable in plants, algae and cyanobacteria. However, photodamaged PSII does not accumulate in living cells under normal light conditions as a consequence of the rapid and efficient repair of PSII [1]. When the repair of PSII occurs more slowly than the photodamage to PSII, photodamaged PSII accumulates, with resultant photoinhibition of PSII [2–4]. The susceptibility of PSII to photoinhibition is enhanced when light stress is combined with other types of environmental stress [5].

The extent of photodamage to PSII is related primarily to the intensity of light [6–8]. We demonstrated recently that photodamage to PSII occurs in two steps, with step 1 occurring at the oxygen-evolving complex (OEC) and being mediated by UV and strong blue light and step 2 occurring at the reaction center of PSII and being mediated by visible light absorbed by photosynthetic pigments [9]. Recent studies in pea (*Pisum sativum* L.) [10] and a green alga (*Chlamydomonas reinhardtii*) [11] demonstrated that the photoinhibition that occurs upon interruption of the Calvin cycle is not attributable to acceleration of photodamage to PSII.

The repair of photodamaged PSII involves several steps, as follows: proteolytic degradation of the D1 protein; synthesis de novo of the precursor to D1 (known as pre-D1); insertion of the newly synthesized pre-D1 into the thylakoid membrane concomitantly with the assembly of other components of PSII; maturation of the D1 protein via the carboxy-terminal processing of pre-D1; and the assembly of the oxygen-evolving machinery [1,12]. The system for the

Abbreviations: CBB, Coomassie brilliant blue; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; 3-PGA, glycerate-3-phosphate; glycolate-2-P, glycolate-2-phosphate; glycolate-2-P, glycolate-2-phosphate; OEC, oxygen-evolving complex, PSII, photosystem II; pre-D1, the precursor to the D1 protein; ROS, reactive oxygen species; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate

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repair of PSII is inactive in darkness and active in light, and the switch from inactive to active at the step at which pre-D1 is synthesized is regulated by the light-dependent synthesis of ATP [13].

The D1 protein is encoded by the *psbA* genes in the plastid genome. The rate of turnover of this protein is the highest among all the proteins in PSII. The expression of the *psbA* genes is regulated by light at the posttranscriptional level, for the most part, in algae [14–18] and higher plants [19,20]. The rate of translation of *psbA* mRNA may be regulated by the ratio of levels of ATP and ADP [15,21] and the redox potential [16,17]. We showed recently that the translation of *psbA* mRNA is inhibited by reactive oxygen species (ROS), such as H_2O_2 and singlet oxygen ($^1O_2$) [6–8].

Furthermore, we have showed that, in *Chlamydomonas reinhardtii*, interruption of the Calvin cycle by glycolaldehyde, an inhibitor of phosphoribulokinase, or by the missense mutation of the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) inhibits the synthesis of proteins and, in particular, synthesis of the D1 protein. As a result, the repair of photodamaged PSII cannot proceed [11]. These findings lead us to hypothesize that the interruption of CO_2 fixation reduces the production of 3-PGA that accepts electrons from NADPH and thus produces ROS leading to inhibit the synthesis of the D1 protein. However, this hypothesis cannot be evaluated in *Chlamydomonas reinhardtii*, since intact cells do not incorporate exogenously 3-PGA. Therefore, it is essential to use isolated chloroplasts, which incorporate 3-PGA and other intermediates of the Calvin cycle [22].

In the present study, we investigated the effects of exogenously supplied intermediates in the Calvin cycle, such as glycerate-3-phosphate (3-PGA), glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), on the synthesis of the D1 protein in intact chloroplasts from spinach leaves when CO_2 fixation by Rubisco is interrupted by glycolaldehyde or a reduced supply of CO_2. Our results demonstrated that inhibition of protein synthesis via limitation of CO_2 fixation in intact chloroplasts could be overcome by exogenous 3-PGA or glycerate, a precursor to 3-PGA, but not by GAP or DHAP. Our findings indicate that the inhibition of protein synthesis that results from limitation of CO_2 fixation is due to depletion of 3-PGA. This compound accepts electrons from NADPH with resultant decreases in levels of reactive oxygen species (ROS) that inhibit protein synthesis.

2. Materials and methods

2.1. Plant materials and isolation of intact chloroplasts

Spinach was grown at 25 °C with a light/dark cycle of 12 h/12 h. For all experiments, fully developed leaves were harvested 1 h after the start of the light period. Intact chloroplasts were isolated by previously described methods [23,24] with minor modifications. The following procedures were carried out at 0–4 °C. Immediately after harvest, 20 g of spinach leaves were homogenized briefly in 200 ml of a solution of 330 mM sorbitol, 5 mM $MgCl_2$, 2 mM ascorbate, 0.1% BSA, and 10 mM $Na_4P_2O_7$–HCl (pH 6.5). The homogenate was filtered through six layers of gauze and the filtrate was centrifuged at 1700×*g* for 1 min. The resultant pellets were resuspended in 6 ml of Medium A (330 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 50 mM HEPES–KOH, and 0.2% BSA, pH 7.6) and the suspension was loaded on the top of Percoll step-wise gradients (10%, 40% and 70% Percoll in Medium A) and centrifuged for 5 min at 2500×*g*. The chloroplasts suspended in 70% Percoll were collected and diluted with 20 ml volumes of Medium A. After centrifugation of this suspension at 1700×*g* for 3 min, pelleted chloroplasts were resuspended in 0.5 ml of Medium A. Chloroplast intactness, verified by comparing the rate of ferricyanide-dependent uncoupled electron transport in chloroplasts before and after osmotic shock, was more than 90% [25]. Chlorophyll concentrations were determined as described by Arnon [26].

2.2. Measurement of the light-dependent evolution of oxygen

The rate of the light-dependent evolution of oxygen was measured at 25 °C by monitoring light-dependent change in oxygen concentration with a Clark-type oxygen electrode. Actinic white light at a photon flux density of 1000 μmol photons m^{-2} s^{-1} was provided by halogen lamps.

2.3. Labeling of newly synthesized proteins

Intact chloroplasts corresponding to 25 μg of Chl were incubated at 25 °C for 10 min in light at 100 μmol photons m^{-2} s^{-1} in 250 μl of Medium B [330 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.2 mM KH_2PO_4, 10 mM $NaHCO_3$, 25 μM each amino acid (Amino acid mixture minus methionine; Promega, Madison, WI, USA) and 50 mM HEPES–KOH, pH 7.6]. The uptake of methionine was initiated by addition of 250 μCi ml^{-1} [^{35}S]methionine (Amersham Biosciences, Piscataway, New Jersey) and was allowed to proceed at 25 °C for 10 min in light at 100 μmol photons m^{-2} s^{-1}.

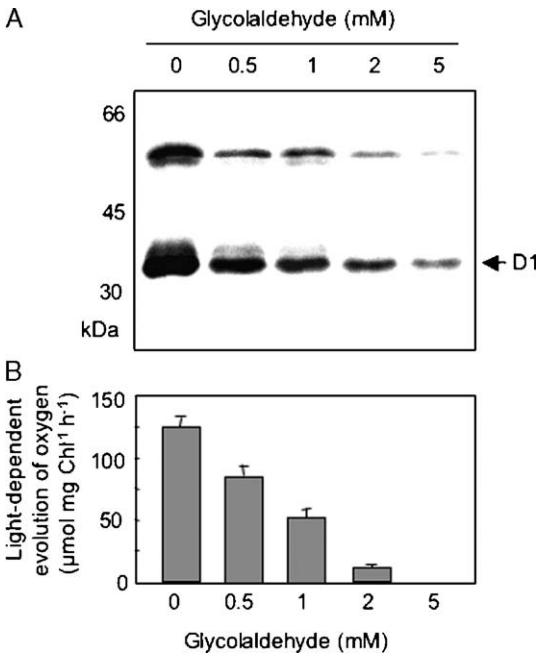

Fig. 1. Glycolaldehyde, an inhibitor of phosphoribulokinase, inhibits the synthesis de novo of plastid genome-encoded proteins. (A) Newly synthesized proteins in intact chloroplasts (100 μg Chl ml^{-1}) were labeled with [^{35}S]methionine (250 μCi ml^{-1}) in the presence of various concentrations of glycolaldehyde in light at 100 μmol photons m^{-2} s^{-1} for 10 min. Proteins from chloroplasts corresponding to 10 μg of Chl were applied to each well. Three independent experiments were performed and essentially the same results were obtained in each case. (B) The rate of light-dependent evolution of oxygen was measured in intact chloroplasts in light at 1000 μmol photons m^{-2} s^{-1} in the presence of various concentrations of glycolaldehyde as indicated. Values shown are means ± S.D. (bars) of results from three independent experiments.

The uptake of methionine was terminated by addition of 200 μg ml^{-1} chloramphenicol.

2.4. Pulse-chase experiments

Intact chloroplasts corresponding to 25 μg of Chl were incubated at 25 °C for 10 min in light at 100 μmol photons m^{-2} s^{-1} in 250 μl of Medium B that contained 100 μCi ml^{-1} [^{35}S]methionine. Then, chloroplasts were collected by centrifugation at 1700×*g* for 1 min and resuspended in 250 μl of Medium B that contained 10 mM unlabeled methionine. The chase was initiated by turning on light at 100 μmol photons m^{-2} s^{-1} at 25 °C. After designated periods of chase, chloroplasts were collected by centrifugation at 2000×*g* for 3 min after the addition of protease inhibitors, namely, 2 μg ml^{-1} of antipain, 2 μg ml^{-1} of leupeptin, and 100 μg ml^{-1} of phenylmethylsulfonyl fluoride, and radiolabeled proteins were analyzed as described below.

2.5. Analysis of radiolabeled proteins

Total proteins from chloroplasts that corresponded to 10 μg of Chl were solubilized in 50 μl of 2% SDS plus 0.1 M dithiothreitol by heating at 60 °C for 3 min and fractionated by electrophoresis on a 15% polyacrylamide gel that contained 6 M urea and 0.08% SDS. To assign individual protein bands, proteins were stained with 0.25% of Coomassie brilliant blue (CBB). The labeled proteins on the dried and fixed gel were exposed to an imaging plate (BAS-III; Fuji-photo Film, Tokyo, Japan) and visualized with a Bio-image Analyzer (BAS 2000; Fuji-photo Film).

2.6. Immunoblotting analysis

Intact chloroplasts corresponding to 25 μg of Chl were incubated at 25 °C in light at 100 μmol photons m^{-2} s^{-1} in 250 μl of Medium B that contained 10 mM unlabeled methionine for 0 or 90 min. Then, the chloroplasts were collected by centrifugation at 2000×*g* for 3 min after addition of the above-mentioned protease inhibitors. Total chloroplast proteins that corresponded to 0.5 μg of Chl were solubilized by heating in 50 μl of 2% SDS plus 0.1 M dithiothreitol at 60 °C for 3 min and fractionated on a 15% polyacrylamide gel that contained 6 M urea and 0.08% SDS. Separated proteins were blotted onto a nitrocellulose membrane and stained with 1% Ponceau S. The D1 proteins on the membrane were reacted with antibodies raised in rabbit against the purified D1 protein from spinach. Each band of immunologically reactive protein was detected with peroxidase-linked second antibodies and enhanced chemiluminescence was examined after reaction with immunoblotting detection reagents (RPN 2106; Amersham Biosciences, Tokyo, Japan). Chemiluminescence was detected by exposure of the membrane to X-ray film.

3. Results

3.1. Glycolaldehyde inhibits protein synthesis in isolated intact chloroplasts

To demonstrate the effects of interruption by glycolaldehyde of CO_2 fixation on protein synthesis in chloroplasts, we monitored the incorporation of [^{35}S]methionine into newly synthesized proteins in intact chloroplasts isolated from spinach leaves (Fig. 1). When such chloroplasts were incubated in light for 10 min in the presence of [^{35}S]methionine, two proteins of about 32 kDa and 55 kDa were strongly radiolabeled. Immunoblotting analysis using antibodies against the D1 protein revealed that the lower band corresponded to the D1 protein whereas the upper band appeared to be another protein

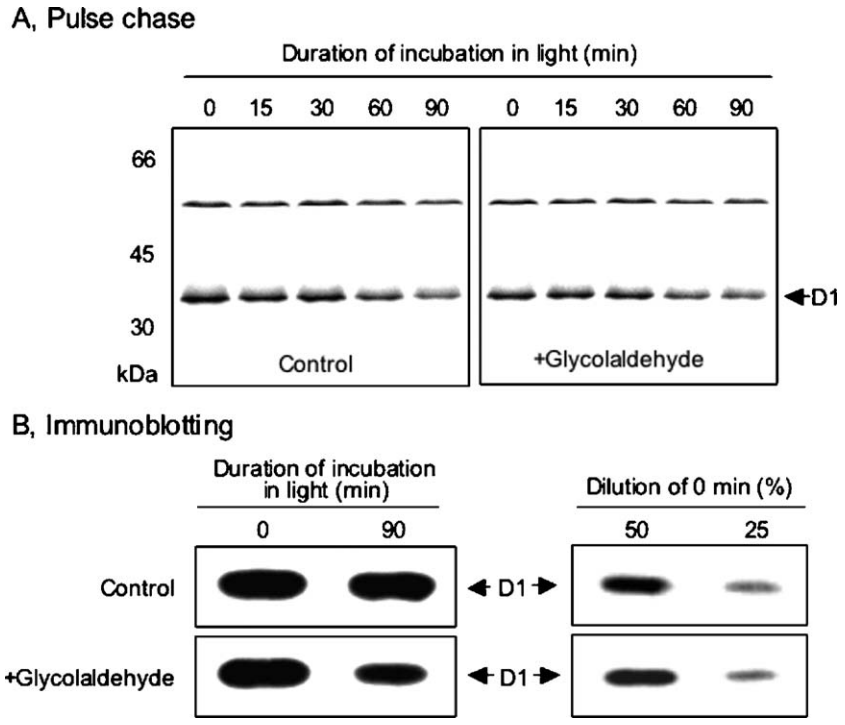

Fig. 2. Glycolaldehyde does not affect degradation of the D1 protein. (A) Pulse-chase analysis of the level of the D1 protein. Intact chloroplasts (100 μg Chl ml^{-1}) were pulse-labeled with [^{35}S]methionine (250 μCi ml^{-1}) as described in Materials and methods and the radiolabel was chased with 10 mM unlabeled methionine in light at 100 μmol photons m^{-2} s^{-1} in the presence of 5 mM glycolaldehyde or in its absence (control). Proteins from chloroplasts corresponding to 10 μg of Chl were applied to each well. Three independent experiments were performed and essentially the same results were obtained in each case. (B) Immunoblotting analysis with antibodies against the D1 protein. Chloroplasts (100 μg Chl ml^{-1}) were incubated in light at 100 μmol photons m^{-2} s^{-1} for 90 min in the presence of 5 mM glycolaldehyde or in its absence (control). Proteins from chloroplasts corresponding to 10 μg of Chl were applied to each well. Three independent experiments were performed and essentially the same results were obtained in each case.

(data not shown). However, glycolaldehyde, an inhibitor of phosphoribulokinase, which is an enzyme in the Calvin cycle [27], reduced the incorporation of radioactive methionine into the D1 protein. Glycolaldehyde at various concentrations had a dose-dependent effect on the extent of radiolabeling of proteins (Fig. 1A) and on the light-dependent evolution of oxygen (Fig. 1B). The effects of glycolaldehyde on the synthesis of chloroplast proteins in intact chloroplasts were similar to those observed in *Chlamydomonas reinhardtii* [11]. Therefore, it seems likely that the repressed synthesis of proteins by inhibition of phosphoribulokinase is common to chloroplasts in vivo and in vitro.

To examine whether the inhibitory effects of glycolaldehyde on the incorporation of methionine into chloroplast proteins were due to inhibition of the synthesis of proteins or to the rapid degradation of newly synthesized proteins, we monitored the degradation of chloroplast proteins in light in the presence of glycolaldehyde and in its absence by pulse-chase radiolabeling with [^{35}S]methionine (Fig. 2A). After proteins had been labeled with [^{35}S]methionine, chloroplasts were incubated in darkness for 5 min in the presence of glycolaldehyde or in its absence, and then they were exposed to light. In the absence of glycolaldehyde, the level of radiolabeled D1 protein decreased gradually to reach 40% of the initial level after incubation for 90 min (Fig. 2A). Glycolaldehyde had no significant effect on the degradation of the D1 protein. These results indicate that the effect of glycolaldehyde on the incorporation of [^{35}S] methionine into chloroplast proteins was attributable to the inhibition of protein synthesis de novo rather than to the rapid degradation of proteins. Immunoblotting analysis with antibodies against the D1 protein showed that the level of the D1 protein decreased to 50% of the initial level during incubation of chloroplasts in light for 90 min in the presence of glycolaldehyde (Fig. 2B). However, there was no decrease in the level of D1 protein in the absence of glycolaldehyde. These results suggest that glycolaldehyde inhibited the synthesis de novo of D1 protein, with a resultant decrease in the level of D1 protein.

3.2. Exogenous 3-PGA abolishes the inhibition of protein synthesis by glycolaldehyde

Glycolaldehyde inhibits phosphoribulokinase and, as a result, it decreases the level of ribulose-1,5-bisphosphate (RuBP), which is the substrate of Rubisco [27]. Thus, we might predict that the availability of 3-PGA would be limited in the presence of glycolaldehyde. To examine whether inhibition of protein synthesis de novo by glycolaldehyde is due to the limited availability of 3-PGA, we investigated the effects of exogenous 3-PGA on the synthesis of proteins in the presence of glycolaldehyde in isolated intact chloroplasts (Fig. 3A). Exogenously supplied 3-PGA is transported into chloroplasts via the action of the phosphate transporter in the chloroplast envelope [22,28]. We confirmed the uptake of 3-PGA into chloroplasts by monitoring the light-dependent evolution of oxygen in the presence of 3-PGA and glycolaldehyde (Fig. 3B).

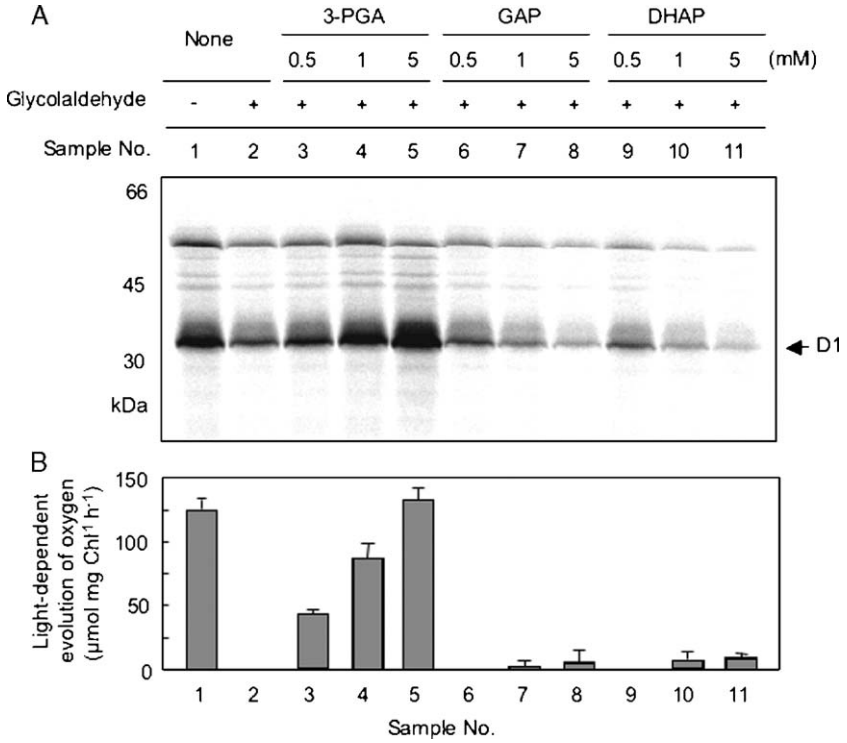

Fig. 3. Exogenous 3-PGA enhances the synthesis de novo of plastid-encoded proteins in the presence of glycolaldehyde, whereas exogenous GAP and DHAP inhibit it. (A) Analysis of proteins after pulse labeling with [^{35}S]methionine. Intact chloroplasts (100 μg Chl ml^{-1}) were incubated in light at 100 μmol photons m^{-2} s^{-1} for 10 min with [^{35}S]methionine (250 μCi ml^{-1}) in the presence of 5 mM glycolaldehyde and various concentrations of 3-PGA, GAP or DHAP. Proteins from chloroplasts corresponding to 10 μg of Chl were applied to each well. Three independent experiments were performed and essentially the same result was obtained in each case. (B) The rate of the light-dependent evolution of oxygen under the same conditions as in (A). Values are means±S.D. of results from three independent experiments.

The results in Fig. 3A indicate that the synthesis of proteins was restored by an exogenous supply of 3-PGA. Moreover, 3-PGA appeared to have a dose-dependent effect on the extent of radiolabeling of proteins (Fig. 3A) and the light-dependent evolution of oxygen (Fig. 3B). By contrast, other intermediates in the Calvin cycle, such as GAP and DHAP, both of which are also transported into chloroplasts by the phosphate transporter [22,28], did not restore the light-dependent evolution of oxygen (Fig. 3B) and failed to abolish the inhibitory effects of glycolaldehyde on the synthesis of D1 protein (Fig. 3A). These intermediates tended, instead, to inhibit the residual synthesis of proteins that occurred in the presence of glycolaldehyde.

3.3. Limitation of the supply of CO_2 inhibits protein synthesis

To examine the effects of CO_2 fixation on the synthesis of proteins in isolated chloroplasts in further detail, we investigated the effects of the reduced availability of CO_2 on the synthesis of proteins (Fig. 4A). To limit the availability of CO_2, we first incubated chloroplasts in light for 10 min in the absence of CO_2. The net photosynthetic activity fell to 30% of the initial activity but the activity was restored by subsequent addition of CO_2 (Fig. 4B), suggesting that the initial incubation had resulted in limitation of the CO_2 supply. After the initial incubation in the absence of CO_2, the radiolabeling of newly synthesized proteins was initiated by addition of [^{35}S] methionine with the addition of CO_2 or in its absence. During radiolabeling in the light for 10 min, the D1 protein was synthesized in all cases. However, the level of D1 was significantly reduced in the absence of CO_2. These results suggest that interruption of CO_2 fixation by limitation of the supply of CO_2 inhibits the synthesis of proteins in isolated chloroplasts.

3.4. Exogenous 3-PGA and glycerate abolish the inhibition of protein synthesis caused by limitation of CO_2

When the supply of CO_2 is limited, the carboxylation of RuBP by Rubisco is suppressed and the level of 3-PGA falls. To demonstrate that inhibition of protein synthesis by limitation of CO_2 is also due to the reduced availability of 3-PGA, we investigated the effects of exogenous 3-PGA on the synthesis of proteins in the absence of CO_2. Fig. 4A shows that the inhibition of protein synthesis caused by limitation of CO_2 was abolished by exogenously supplied 3-PGA. Furthermore, inhibition of the light-dependent evolution of oxygen by limitation of CO_2 was also abolished by an exogenous supply of 3-PGA (Fig. 4B).

The inhibition of protein synthesis was also abolished by an exogenous supply of glycerate, a precursor to 3-PGA, which is transported into chloroplasts by the glycolate/glycerate carrier in the chloroplast envelope [29] and is then converted, within the chloroplasts, to 3-PGA by glycerate kinase [30]. We found, moreover, that inhibition of the light-dependent evolution of oxygen by limitation of CO_2 was also abolished by glycerate (Fig. 4B).

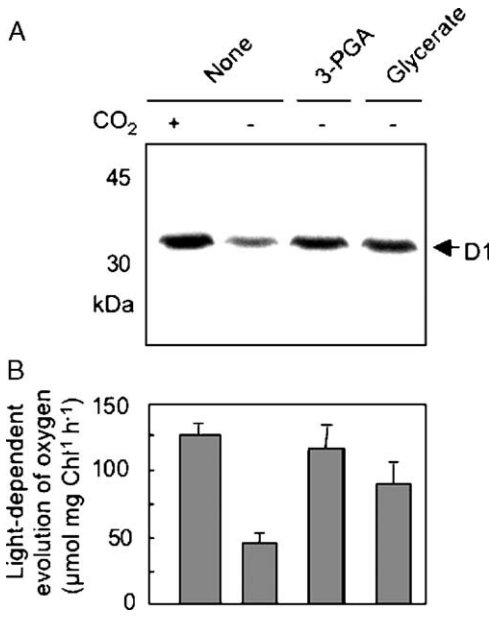

Fig. 4. Exogenous 3-PGA and glycerate enhance the synthesis de novo of plastid-encoded proteins under CO_2-limiting conditions. (A) Analysis of proteins after pulse labeling with [^{35}S]methionine. Intact chloroplasts (100 μg Chl ml^{-1}) were incubated in light at 100 μmol photons m^{-2} s^{-1} for 10 min in the absence of $NaHCO_3$ before radiolabeling and then they were incubated for another 10 min under the same light conditions with [^{35}S]methionine (250 μCi ml^{-1}) in the presence of 10 mM $NaHCO_3$, 5 mM 3-PGA or 5 mM glycerate or in their absence. Proteins from chloroplasts corresponding to 10 μg of Chl were applied to each well. Three independent experiments were performed and essentially the same results were obtained in each case. (B) The rate of the light-dependent evolution of oxygen under the same conditions as in (A). Values are means ± S.D. (bars) of results from three independent experiments.

3.5. Exogenous 3-PGA does not abolish the inhibition of protein synthesis by H_2O_2

We demonstrated previously in cyanobacteria that H_2O_2 inhibits the synthesis of proteins and, as a result, the repair of photodamaged PSII [6]. Therefore, we examined whether H_2O_2 could inhibit the protein synthesis de novo in isolated intact chloroplasts and whether exogenous 3-PGA could abolish such an inhibitory effect of H_2O_2 on protein synthesis. As shown in Fig. 5, both exogenous H_2O_2 and methyl viologen, which accepts electrons from PSI and accelerates the production of O_2^- and H_2O_2 in light [31], inhibited protein synthesis de novo in isolated chloroplasts. However, the inhibitory effects of H_2O_2 and methyl viologen were not abolished by exogenous 3-PGA. These findings indicate that 3-PGA does not support protein synthesis directly.

4. Discussion

4.1. Interruption of CO_2 fixation via the Calvin cycle inhibits protein synthesis de novo

Light energy that is absorbed by photosynthetic pigments is used for the production of NADPH and ATP via photochemical reactions, and these compounds are used

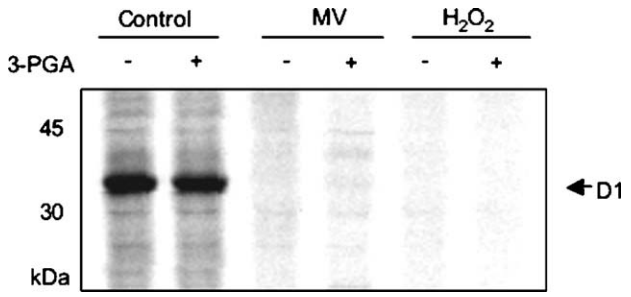

Fig. 5. Exogenous 3-PGA has no effect on the synthesis de novo of plastid-encoded proteins when such synthesis has been inhibited by the presence of H_2O_2 or methyl viologen. Intact chloroplasts (100 μg Chl ml^{-1}) were incubated in light at 100 μmol photons m^{-2} s^{-1} for 10 min with [^{35}S]methionine (250 μCi ml^{-1}) in the presence, as indicated, of 5 μM methyl viologen (MV) or 5 mM H_2O_2 with or without 5 mM 3-PGA. Proteins from chloroplasts corresponding to 10 μg of Chl were applied to each well. Three independent experiments were performed and essentially the same results were obtained in each case.

primarily for the fixation of CO_2 in the Calvin cycle. Rubisco, which is a prerequisite for CO_2 fixation, catalyzes the carboxylation of RuBP to yield 3-PGA. 3-PGA is then converted to triosephosphates, such as GAP and DHAP, at expense of NADPH and ATP, in reactions catalyzed by 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase. Some of the triosephosphates is then recycled to regenerate RuBP. Glycolaldehyde inhibits the phosphoribulokinase that is involved in the recycling of RuBP, ultimately interrupting the fixation of CO_2 [27]. Our results demonstrate that interruption of CO_2 fixation by glycolaldehyde inhibits the synthesis of PSII proteins in chloroplasts (Fig. 1A). This result is consistent with our previous finding, in *Chlamydomonas reinhardtii*, that interruption of CO_2 fixation by glycolaldehyde or by a missense mutation in the gene for Rubisco suppresses the synthesis of PSII proteins [11].

4.2. A possible explanation for the inhibition of protein synthesis by interruption of CO_2 fixation

Fig. 6 shows a simplified representation of the Calvin cycle that can help us to interpret the mechanism responsible for the inhibition of protein synthesis by interruption of CO_2 fixation insofar as it relates to our present results. Interruption of CO_2 fixation decreases the utilization of NADPH with a resultant decrease in the level of $NADP^+$. Since $NADP^+$ is a major acceptor of electrons in PSI, depletion of $NADP^+$ accelerates the reduction of O_2 to generate superoxide (O_2^-), which is immediately converted to H_2O_2 by superoxide dismutase [32]. Several studies have demonstrated, moreover, that interruption of CO_2 fixation enhances the production of H_2O_2 [32–35]. In the photosynthetic apparatus, H_2O_2 is scavenged enzymatically and nonenzymatically [36]. However, when the rate of production of H_2O_2 is raised significantly, the resultant high level of H_2O_2 inhibits the translational elongation of proteins and, in particular, that of the D1 protein, during the repair of PSII in the cyanobacterium *Synechocystis* sp. PCC 6803 [6]. In the present study, incubation of chloroplasts with H_2O_2 or with methyl viologen nonspecifically inhibited the synthesis of chloroplast proteins (Fig. 5), suggesting that the H_2O_2-dependent inhibition of protein synthesis occurs both in chloroplasts and in cyanobacterial cells. The production of H_2O_2 under illumination might, therefore, be involved in the inhibition of the synthesis of chloroplast proteins de novo when the supply of 3-PGA is limited (see Fig. 6, thick arrows). This hypothesis corresponds to the observation that the effects of glycolaldehyde on the

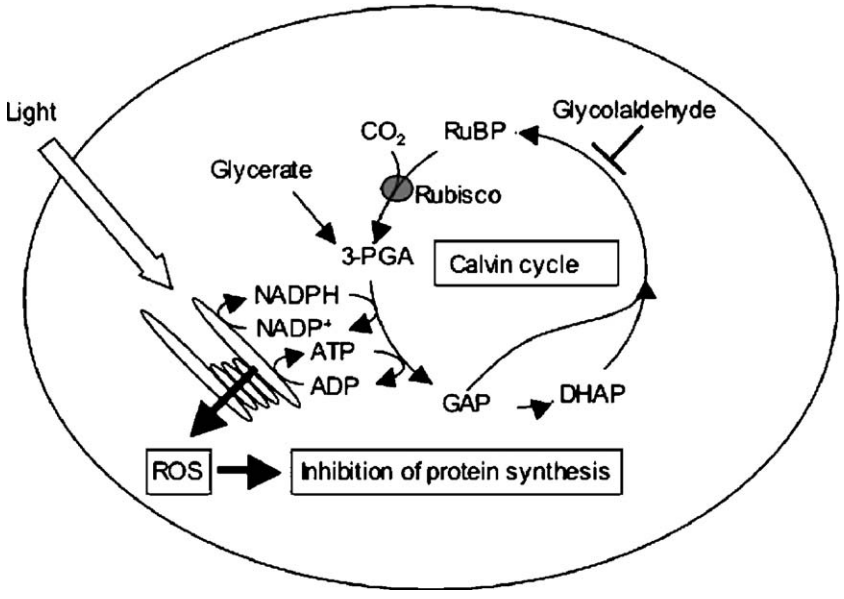

Fig. 6. A hypothetical scheme for the inhibition of protein synthesis upon interruption of the Calvin cycle in isolated chloroplasts. Abbreviations: DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; 3-PGA, glycerate-3-phosphate; ROS, reactive oxygen species; RuBP, ribulose-1,5-bisphosphate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

photoinhibition of PSII depend on the presence of molecular oxygen, which is the precursor to O_2^- that is converted to H_2O_2 [37].

Exogenous GAP and DHAP not only failed to restore protein synthesis and, in fact, inhibited it at high concentrations (Fig. 3A). The conversion of GAP and DHAP to 3-PGA is reverse reactions in the Calvin cycle. Therefore, exogenous GAP and DHAP tend to reduce $NADP^+$ to NADPH and might be expected to accelerate the production of H_2O_2 via O_2^- [36].

Fig. 4A suggests that limitation of CO_2 inhibited protein synthesis de novo, but not completely. The oxygenation of RuBP by Rubisco produces 3-PGA and glycolate-2-phosphate (glycolate-2-P) under CO_2-limiting conditions. This reaction produces 3-PGA much more slowly than the carboxylation reaction and, thus, it cannot contribute efficiently to the generation of 3-PGA [38]. However, glycolate-2-P is metabolized through the photorespiration pathway that involves reactions in chloroplasts, peroxisomes, and mitochondria to generate 3-PGA in plant cells [38]. Supply of 3-PGA by oxygenation of RuBP and photorespiration pathway might, at least partially, have avoided inhibition of protein synthesis by limitation of CO_2. The partial inhibition of protein synthesis by limitation of CO_2 was abolished by exogenous supply of 3-PGA and glycerate that is an intermediate of the photorespiration pathway (Fig. 4A).

4.3. Interruption of CO_2 fixation by environmental stress might inhibit the protein synthesis-dependent repair of PSII after photodamage

The fixation of CO_2 is sensitive to environmental stress. For example, high temperatures inactivate Rubisco activase, which is essential for the activity of Rubisco [39,40]. Dehydration and salt stress close stomata and limit the supply of CO_2 for the carboxylation of RuBP by Rubisco [5,41]. Under such conditions, the production of 3-PGA is limited and the level 3-PGA might fall. It has been shown that the level of 3-PGA falls dramatically under CO_2-limiting [42] and drought [42–44] conditions. Because depletion of 3-PGA inhibits the synthesis of proteins de novo in chloroplasts (Fig. 3A), interruption of CO_2 fixation by environmental stress might be expected to inhibit the protein synthesis-dependent repair of PSII after photodamage. High-temperature stress [45,46], low-temperature stress [4,46] and salt stress [47] have been shown to inhibit the repair of PSII. Although these kinds of stress might inhibit the repair of PSII directly, the inhibitory effects of these various forms of stress might be attributable, in part, to depletion of 3-PGA.

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