Regulation of glutamine synthetase by metal-catalyzed oxidative modification in the marine oxyphotobacterium Prochlorococcus

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Abstract

The inactivation of glutamine synthetase (GS; EC 6.3.1.2) by metal-catalyzed oxidation (MCO) systems was studied in several Prochlorococcus strains, including the axenic PCC 9511. GS was inactivated in the presence of various oxidative systems, either enzymatic (as NAD(P)H+NAD(P)H-oxidase+Fe3+) or non-enzymatic (as ascorbate+Fe3+ +O2). This process required the presence of oxygen and a metal cation, and is prevented under anaerobic conditions. Catalase and peroxidase, but not superoxide dismutase, effectively protected the enzyme against inactivation, suggesting that hydrogen peroxide mediates this mechanism, although it is not directly responsible for the reaction. Addition of azide (an inhibitor of both catalase and peroxidase) to the MCO systems enhanced the inactivation. Different thiols induced the inactivation of the enzyme, even in the absence of added metals. However, this inactivation could not be reverted by addition of strong oxidants, as hydrogen peroxide or oxidized glutathione. After studying the effect of addition of the physiological substrates and products of GS on the inactivation mechanism, we could detect a protective effect in the case of inorganic phosphate and glutamine. Immunochemical determinations showed that the concentration of GS protein significantly decreased by effect of the MCO systems, indicating that inactivation precedes the degradation of the enzyme. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Metal-catalyzed oxidation; Glutamine synthetase; Cyanobacterium; Regulation; Prochlorococcus

1. Introduction

Prochlorococcus is a marine oxyphotobacterium of global importance, due to its abundance in intertropical oceans and its significant contribution to the total primary production (for a review, see [1]). Besides its ecological interest, Prochlorococcus shows many unusual features among cyanobacteria, in particular regarding several aspects of the photosynthetic apparatus [2–5]. The occurrence in the nature of different ecotypes [6–9], adapted to high and low light (HL and LL, respectively), has been proposed as one of the keys explaining the ability of Prochlorococcus to grow over a large range of irradiances (more than three orders of magnitude) and nutrients (i.e. from the surface, with mostly reduced forms of nitrogen, down to 200 m depth, where nitrates become predominant). Some of these aspects have been extensively studied during the last years, establishing Prochlorococcus as one of the model marine microorganisms, and leading to the sequencing of the genome of three Prochlorococcus strains, representative of the above mentioned ecotypes: those of MED4 (HL) and MIT9313 (LL) have been fully sequenced by the American Department of Energy (DOE) Joint Genome Institute (JGI), while the genome of Prochlorococcus SS120 (LL) is currently being sequenced by the French Genoscope.

The nutrient assimilation in Prochlorococcus has received comparatively little attention, due to the problematic culturing of this microorganism [10] and the availability of only one axenic strain to date [11]. Consequently, only a few papers have dealt with nitrogen assimilation [10–12]. However, nitrogen is considered one of the main limiting nutrients in the oceans [1] and thus a further knowledge of the nitrogen assimilatory pathways could help to explain how Prochlorococcus can proliferate in regions with very low nitrogen levels; furthermore, on the basis of the comparison between the genomes of MED4 and MIT9313, a striking genetic diversity has been described in this pathway from different ecotypes of Prochlorococcus [13,14] suggesting that the differential
availability of nitrogen sources along the water column could be a major force driving the evolution of Prochlorococcus into different ecotypes.

We have previously studied the physiological regulation of glutamine synthetase (GS) in Prochlorococcus [10]. GS is a pivotal enzyme in the metabolism of photosynthetic organisms, and of cyanobacteria in particular [15,16], catalyzing the incorporation of ammonia into glutamine. This enzyme, together with glutamate synthase, represents the connecting point between the carbon and nitrogen metabolisms, and consequently shows a fine regulatory system that has been extensively characterized in enterobacteria [17,18]. GS from Escherichia coli has been shown to be regulated by metal-catalyzed oxidation (MCO), inducing an inactivation of the enzyme that precedes selective degradation by specific proteases [19–22]. MCO systems are involved in many physiological and pathological processes, as protein turnover, oxygen toxicity, aging, pulmonary diseases, arthritis, regulation of metabolic pathways, etc. [23–26]. MCO systems seem to be involved in the control of GS in different organisms, from enterobacteria [19] to animals [27]. We showed the occurrence of this regulatory system for the first time in photosynthetic organisms [28] and it has been later described as well in cyanobacteria [29,30] and higher plants [31].

In the present work, we addressed the regulation of GS in Prochlorococcus by metal-catalyzed oxidative mechanisms, both enzymatic and non-enzymatic; we studied the effect of anaerobiosis, several possible enhancing and protective agents to characterize the oxidative inactivation by determining GS transferase activity in time-course experiments on samples subjected to the different treatments; finally we measured the effect of MCO systems on the GS protein concentration by immunochemical techniques, in order to understand whether these systems could be involved in the regulation of GS turnover in Prochlorococcus.

2. Materials and methods

2.1. Strains and culturing

Prochlorococcus strains PCC 9511 (high-irradiance-adapted, axenic), MED4 (high-irradiance-adapted) and SS120 (low-irradiance-adapted) were routinely cultured in polycarbonate Nalgene flasks (10 l) using PCR-S11 medium as described by Rippka and coworkers [11]. The sea water used as basis for this medium was kindly provided by Carlos Massó de Ariza and the Instituto Espanol de Oceanografia (Spain). Cells were grown in a culture room set at 24°C under continuous blue irradiances (40 and 4 µE/m²/s for high- and low-irradiance-adapted strains, respectively). Growth was determined by measuring the absorbance of cultures at 674 nm. Cells at the exponential phase of growth (A674 = 0.05) were harvested by centrifugation at 30 100 × g for 5 min in an Avanti J-25 Beckman centrifuge equipped with a JA-14 rotor. After pouring most of the supernatant and carefully pipetting out the remaining medium, the pellet was directly resuspended in cold 50 mM Tris–HCl pH 7.6 (2 ml buffer/l culture), and stored frozen at −20°C until used.

2.2. Preparation of cell-free extracts

Prochlorococcus cells were broken by thawing the cells in the same Tris buffer used for storage. The broken material was centrifuged at 16 100 × g for 10 min and the resulting supernatants were used for determining GS activity and protein in all the experiments described.

2.3. Inactivation assays

For inactivation by the ascorbate system different amounts of cell extracts were incubated at 4°C in a solution containing 50 mM Tris buffer pH 7.6, 0.01 mM FeCl₃ and 1 mM ascorbate. The inactivation reactions were initiated by the addition of iron. All solutions were freshly prepared before each experiment. The ascorbate solution was prepared as follows: 100 µl of 20 mM dithiothreitol (DTT) was added to 9 ml of 10 mM ascorbate, in order to keep ascorbate under reduced conditions; after carefully neutralizing this solution with 1 M NaOH, the volume was completed to 10 ml. For inactivation by the NAD(P)H system, incubations were carried out at different temperatures in a solution containing 50 mM Tris buffer pH 7.6, 0.2 mM FeCl₃ and 5 mM NADH or NADPH. Aliquots of inactivation reaction mixtures were removed at various times and assayed immediately for enzyme activity.

To validate the effect of different reagents, enzymes and radical scavengers on the inactivation of GS, these were added at the concentrations indicated in the text and aliquots were taken up at appropriate times. The order to add reagents was always: ascorbate/NADH, protective/enhancing agent(s), and iron, except for the following cases: substrates and products of the GS reaction were added before all other reagents. Amino acids were preincubated for 5 min with the GS samples prior to addition of the MCO components. Hydrogen peroxide was added after all other components of the assays. Phosphate was prepared by neutralization of phosphoric acid.

Distilled water further purified with a Millipore MilliQ system was used in the preparation of the inactivation reaction mixtures to avoid the effect of metal traces.

2.4. Enzymatic assays

GS transferase activity was determined as previously described [10], during 30 min at 37°C. The composition of the reaction mixture was: 100 mM glutamine, 10 mM sodium hydroxylamine, 50 µM manganese chloride, 10 µM ADP and 50 mM sodium arsenate in 0.2 M MOPS.
pH 7. One unit of activity is the amount of enzyme that transforms 1 μmol of substrate per minute.

Chlorophyll concentration was determined [32] and used to standardize the enzymatic activities. Error bars shown in all figures correspond to standard deviations for four independent experiments.

2.5. Immunochemical techniques

Electroimmunoassay (EIA, or rocket immunoelectrophoresis) was performed as previously described [10] following the method of Axelsen and Bock [33]. Rabbit antibodies (300 μl) anti-GS from Synechocystis PCC 6803 were added to 15 ml of a solution containing 40 mM Tris pH 8.6, 100 mM glycine, 600 mM calcium lactate and 1.5 mM sodium azide, previously melted and kept at 56°C for 15 min; this mixture was used to prepare the electrophoretic bed onto a GelBond (FMC Bioproducts) film sheet. Due to the low GS concentration in standard crude extracts, the extracts for EIA were four-fold concentrated. Samples were subjected to the indicated MCO treatments, and at the indicated times, aliquots were removed and frozen using a quick isopropyl alcohol-based system (ensuring a decrease of temperature of 1°C per minute). Twenty-five μl of each incubation mixture was loaded into each well. The electrophoresis was carried out overnight in a flat bed apparatus (FBE 3000, Pharmacia) at 200 V and 10°C. Plates were washed with 15 mM NaCl for 48 h to remove non-crossreacting proteins, washed with distilled water for 2 h, and then stained with Coomassie blue to detect the immunoprecipitates. Chlorophyll concentration was used to standardize the measured values of the rocket areas.

2.6. Protein determination

Protein of soluble fractions was determined according to the Bio-Rad Protein Assay, according to the instructions of the manufacturer.

2.7. Anaerobic studies

For anaerobic experiments incubations were done in cuvettes fitted with rubber caps and two glass stopcocks that served as inlet and outlet ports for the introduction of argon. The addition of different reagents and the extraction of aliquots for GS enzymatic assays were performed with gas-tight hypodermic syringes. The cuvettes containing the incubation mixtures and those containing the reagents were previously treated with five vacuum/argon cycles.

2.8. Genomic sequences of Prochlorococcus

Preliminary sequence data on the Prochlorococcus MED4 and MIT9313 genomes were obtained from the DOE JGI at http://www.jgi.doe.gov/tempweb/JGI_microbial/html/prochlorococcus/prochlo_pickastrain.html.

2.9. Chemicals

All chemicals used were of standard analytical grade (Sigma, Merck and Pharmacia). Horseradish peroxidase, bovine liver catalase, and superoxide dismutase were purchased from Sigma.

3. Results

We have studied in this work the effect of enzymatic and non-enzymatic MCO systems on the GS inactivation from three Prochlorococcus strains: one axenic (PCC 9511, HL-adapted) and two non-axenic (MED4, HL-adapted, and SS120, LL-adapted). The results from experiments using extracts from any of these strains were very similar, and consequently we will show only experiments performed with Prochlorococcus PCC 9511.

3.1. Inactivation of GS from Prochlorococcus by the iron/ascorbate system

The oxygen radical-generating system composed of Fe^{3+} and ascorbate provoked a clear inactivation of GS after 2 h (Fig. 1), that did not happen in the absence of O_2 (not shown). The inactivation was more marked when Fe^{3+} was replaced by Fe^{2+} in the system with ascorbate. Furthermore, addition of Fe^{2+} alone was sufficient to induce a GS inactivation similar to that of Fe^{3+} and ascorbate. Interestingly, ascorbate alone produced also a significant

![Fig. 1. Inactivation of GS from Prochlorococcus by the iron/ascorbate system. Incubation mixtures contained crude extracts with (●) no addition; (○) 0.1 mM FeCl_3; (△) 0.1 mM FeSO_4; (□) 1 mM ascorbate; (●) 0.1 mM FeCl_3+1 mM ascorbate; (△) 0.1 mM FeSO_4+1 mM ascorbate. The mixtures were incubated at 4°C and samples removed at the indicated times for GS transferase determinations. Data are means of four experiments ± standard deviations. 100% of GS activity corresponded to 5.4 ± 0.4 U/mg chl.](http://www.jgi.doe.gov/tempweb/JGI_microbial/html/prochlorococcus/prochlo_pickastrain.html)
but lower degree of GS inactivation, while Fe$^{3+}$ alone had no effect on GS.

Further characterization of the Fe$^{3+}$/ascorbate MCO system was carried out, studying the effect of different enzymes, amino acids and other reagents (Table 1). None of the tested amino acids was able to avoid inactivation (arginine, histidine, methionine, lysine, serine and proline; not shown). Enzymes inducing the disappearance of oxygen radicals (as peroxidase, catalase or superoxide dismutase) protected against inactivation, although to a lower extent in the case of superoxide dismutase. Addition of azide, a reagent acting as an inhibitor of both peroxidase and catalase [19] and as a scavenger of singlet oxygen, enhanced the inactivation of GS.

Addition of 2 mM hydrogen peroxide to the reaction mixture containing 0.01 mM Fe$^{3+}$ and 1 mM ascorbate promoted a slight acceleration of the GS inactivation at the beginning of the experiment (not shown), enhancing the inactivation after 2 h compared with the standard Fe$^{3+}$ and ascorbate system (Table 1); when Fe$^{3+}$ concentration was raised to 0.1 mM under the same conditions, the inactivation of GS was reinforced (not shown). Addition of hydrogen peroxide alone did not have any effect on GS inactivation. Mannitol and sodium formate, reagents acting as scavengers of hydroxyl radicals, had no significant effect on the inactivation of GS. Addition of 4 mM Mn$^{2+}$ to the iron/ascorbate system completely avoided GS inactivation. On the contrary, addition of β-mercaptoethanol further inactivated the enzyme.

We tested the effect of the physiological substrates and products of GS on the metal-catalyzed inactivation of the enzyme: glutamate, ammonium, ATP, glutamine, ADP and Pi (Table 2). Pi and glutamine addition, either alone or in combination, protected clearly GS against inactivation. On the contrary, ATP addition induced a stronger inactivation of the enzyme. We observed however that the ATP-promoted inactivation is unrelated with the MCO system and occurs even in the absence of both iron and ascorbate (not shown).

### Table 2

<table>
<thead>
<tr>
<th>Addition</th>
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<tr>
<td>None</td>
<td>100 ± 7.4</td>
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<tr>
<td>MCO (Fe$^{3+}$/ascorbate)</td>
<td>40 ± 0.2</td>
</tr>
<tr>
<td>MCO+2 mM H$_2$O$_2$</td>
<td>33 ± 0.1</td>
</tr>
<tr>
<td>2 mM H$_2$O$_2$</td>
<td>97 ± 2.0</td>
</tr>
<tr>
<td>MCO+2 mM azide</td>
<td>33 ± 0.1</td>
</tr>
<tr>
<td>MCO+0.6 μM peroxidase</td>
<td>100 ± 3.9</td>
</tr>
<tr>
<td>MCO+1 μM catalase</td>
<td>94 ± 2.0</td>
</tr>
<tr>
<td>MCO+50 μM superoxide dismutase</td>
<td>62 ± 7.0</td>
</tr>
<tr>
<td>MCO+4 mM Mn$^{2+}$</td>
<td>99 ± 1.2</td>
</tr>
<tr>
<td>MCO+5 mM β-mercaptoethanol</td>
<td>8 ± 4.2</td>
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</table>

Samples were incubated in the presence of the indicated products, and aliquots removed after 60 min to assay transferase activity. The MCO system was composed of 0.01 mM FeCl$_3$ and 1 mM ascorbate. Values are means of four independent determinations ± standard deviations. 100% of GS activity corresponded to 5.4 ± 0.4 U/mg chl.
Interestingly, a significant degree of inactivation was also detectable after addition of NADPH or NADH alone, but not when Fe$^{3+}$ only was added to samples. The enzymatic character of this process is consistent with its dependence on the temperature: GS inactivation was strongly enhanced when temperature was increased from 25 to 30°C (not shown).

Histidine, proline, arginine, lysine and serine had no effect on the iron/NADH system (not shown), while the sulfhydryl-containing amino acid cysteine strongly enhanced the inactivation of GS (Table 3), leading to a complete lack of enzymatic activity after 60 min. A similar effect was observed when L-mercaptoethanol, another thiolic reagent, was added to samples. Addition of manganese and also peroxidase or catalase completely avoided inactivation (as did with the iron/ascorbate system), but in this system the possible protective effect of superoxide dismutase was almost non-detectable.

Table 4 shows the effects of the substrates and products of the GS reaction on the inactivation due to the iron/NADH system. Addition of P$_i$ protected GS against inactivation in a more marked manner than previously observed with the iron/ascorbate system (Table 1); on the other hand, ADP had no effect and ATP induced an inactivation independent of the MCO system, as described above. However, in this system addition of glutamine (not shown) did not prevent inactivation, in contrast to the results found with the iron/ascorbate system.

3.3. Effect of thiolic compounds

The enhanced inactivation provoked by cysteine or β-mercaptoethanol addition was further analyzed (Fig. 3), including other thiolic compounds as DTT or DTE, commonly used in different enzymatic studies to keep active sites of the enzymes in their reduced form. All these agents were able to produce a marked GS inactivation (particularly in the cases of β-mercaptoethanol and DTT) in the absence of a MCO system. Furthermore, addition of Fe$^{3+}$ to the samples accelerated the inactivation promoted by cysteine alone, leading to a complete loss of GS activity after only 30 min (Fig. 3). This effect was completely prevented by addition of the chelator EDTA (not shown). The inactivation provoked by thiolic compounds was not reverted by addition of oxidants as hydrogen peroxide or oxidized glutathione (not shown).

3.4. Effect of the iron/ascorbate MCO system on the GS protein concentration in crude extracts of Prochlorococcus

We utilized EIA to determine relative GS protein concentration in crude extracts subjected to the effect of the iron/ascorbate system. Fig. 4 shows the corresponding values measured in Prochlorococcus PCC 9511 samples at 0, 24 and 48 h after addition to the extracts of the different reagents. In all cases, samples treated with the MCO system showed a decrease in the GS antigen concentration.

Table 4

<table>
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<th>Addition</th>
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<tr>
<td>None</td>
<td>100 ± 3.6</td>
</tr>
<tr>
<td>MCO (Fe$^{3+}$/NADH)</td>
<td>41 ± 7.6</td>
</tr>
<tr>
<td>MCO+2.5 mM cysteine</td>
<td>46 ± 0.0</td>
</tr>
<tr>
<td>MCO+0.6 μM peroxidase</td>
<td>98 ± 1.4</td>
</tr>
<tr>
<td>MCO+50 μM superoxide dismutase</td>
<td>48 ± 2.3</td>
</tr>
<tr>
<td>MCO+5 mM β-mercaptoethanol</td>
<td>2 ± 1.4</td>
</tr>
<tr>
<td>MCO+4 mM Mn$^{2+}$</td>
<td>101 ± 3.7</td>
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</table>

Samples were incubated in the presence of the indicated products, and aliquots removed after 60 min to assay transferase activity. The MCO system was composed of 0.2 mM FeCl$_3$ and 5 mM NADH. Values are the means of four independent determinations ± standard deviations. 100% of GS activity corresponded to 5.5 ± 0.2 U/mg chl.
while control samples showed no significant changes. Addition of Mn$^{2+}$ did not prevent the decrease in GS protein concentration induced by the MCO system.

4. Discussion

MCO is one of the covalent modifications used in cells to tag proteins to be later degraded [19,34–36]. In this work we addressed the MCO of GS from Prochlorococcus, studying the iron/ascorbate system. Incubation mixtures were prepared with the following treatments: control (no addition, light gray), addition of 0.1 mM FeCl$_3$/1 mM ascorbate (medium gray) and addition of 0.1 mM FeCl$_3$/1 mM ascorbate+4 mM MnCl$_2$ (dark gray). At the indicated times, samples were removed, frozen and later subjected to immunoelectrophoresis, as described in Section 2.

It is widely accepted that oxidation due to the iron/ascorbate system occurs as follows: ascorbate reduces Fe$^{3+}$ to Fe$^{2+}$, that is the cation interacting with the metal binding sites of GS; besides, auto-oxidation of ascorbate produces hydrogen peroxide that reacts with Fe$^{2+}$ bound to GS, inducing the appearance of oxygen reactive species that oxidize amino acid residues near the active site [19]. Formate and mannitol, two oxygen radical scavengers, are unable to avoid inactivation, suggesting that these radicals are produced in a caged environment, thus preventing the access of scavengers [20,35]. Our results are consistent with this model, indicating that a similar mechanism occurs in Prochlorococcus. Addition of ascorbate or NAD(P)H alone allowed a slight inactivation of GS (Figs. 1 and 2), suggesting that trace concentrations of iron in extracts (coming either from the cells or from impurities of chemicals) are enough for the inactivation to proceed.

Histidine, arginine and cysteine have been shown in some organisms to protect against GS inactivation by MCO systems [28,38]. A possible explanation for this effect derives from these amino acids being residues involved in the inactivation of GS; hence, added amino acids would compete with the residues from the enzyme, preventing inactivation [38–40]. Histidine and cysteine can also act as chelators and scavengers [20]. However, in our case no protection by any amino acid could be detected. Furthermore, we observed that addition of cysteine alone inactivated GS, and in combination with iron this inactivation induced the complete loss of enzymatic activity after 30 min (Table 3 and Fig. 4). Consequently, we studied the effect of other thiolic compounds on GS from Prochlorococcus (Fig. 3). All studied reagents were able to promote different degrees of GS inactivation, that could not be reverted by addition of oxidants. This could suggest the occurrence of another MCO inactivating mechanism, different from the iron/ascorbate or iron/NAD(P)H, and composed of iron and the thiol, as described [21]. This hypothesis is reinforced by the fact that EDTA addition protected GS against the cysteine-promoted inactivation. The importance of these systems is confirmed by the existence of enzymes that protect against thiol/iron- but not against ascorbate/iron-induced inactivation [41]. On the other hand, cysteine and other thiols can generate a thyl radical, by reaction between residues from the thiol and hydroxyl radicals [42,43]; the thyl radical can then damage amino acid residues as histidine in GS, inducing inactivation [40].

Since oxidative inactivation and degradation of GS is considered a physiological response in E. coli [19], we tested the effect of enzymatic MCO systems on the Prochlorococcus GS activity. Similar results were observed with regard to the effect of amino acids, catalase, peroxidase and scavengers, etc. on the iron/ascorbate system

Fig. 4. Immunochemical determination of GS protein concentration in crude extracts of Prochlorococcus PCC 9511 in the presence of the iron/ascorbate MCO system. Incubation mixtures were prepared with the following treatments: control (no addition, light gray), addition of 0.1 mM FeCl$_3$/1 mM ascorbate (medium gray) and addition of 0.1 mM FeCl$_3$/1 mM ascorbate+4 mM MnCl$_2$ (dark gray). At the indicated times, samples were removed, frozen and later subjected to immunoelectrophoresis, as described in Section 2.
(Tables 1 and 3, Fig. 3); hence we conclude that the proposed model of oxygen radical production induced by the iron/NAD(P)H oxidases, inducing GS inactivation [20], is also functional in Prochlorococcus. However, the effect of substrates of the GS on the MCO-promoted inactivation was somehow different: P, either alone or in combination, completely protects GS against inactivation with the iron/NADH system, while in the case of iron/ascorbate produced only partial protection (Tables 2 and 4). In addition, glutamine is protective against the iron/ascorbate, but not against the enzymatic MCO system.

Studies performed on the GS from E. coli showed that the enzyme modiﬁed by oxidation was more rapidly degraded than the native form; these results led to the proposal of oxidative modiﬁcation as a marking step to tag GS, prior to its processing by proteases [24]. Determination of GS antigen levels in Prochlorococcus showed that the MCO systems also act on the intracellular concentration of GS, since samples subjected to the effect of iron/ascorbate showed a decrease in GS protein concentration that did not happen in control, non-treated samples (Fig. 4). Addition of Mn$^{2+}$ to MCO-treated samples did not avoid such decrease, in contrast to the results observed on GS activity (Table 1). Some antioxidants have been shown to protect against inactivation of enzymes, but not against its degradation [44]. However, it has previously been shown in other organisms that Mn$^{2+}$ can prevent the MCO-promoted decrease in GS protein concentration [28]. The increased susceptibility of Prochlorococcus GS to the action of proteases due to the effect of MCO systems could be explained on the basis of conformational changes of the enzyme, inducing the exposure to proteolytic action of GS domains shielded in its native form, as previously proposed [45]. The presence of some of the proteases involved in proteolytic mechanisms (as ClpP and ClpX; [46]) in the genome of Prochlorococcus MED4 is consistent with this hypothesis. Besides, in the sequence of glmA from Prochlorococcus MED4 and MIT9313, two histidine residues are located (positions 272 and 274, respectively) corresponding to the histidines 269 and 271 from E. coli, which have been demonstrated to be involved in the inactivation mechanism [40,47,48]. The participation of NADH-oxidase, an enzyme widely distributed in nature [49], and the involvement of products of the GS reaction in the protection from this inactivation strongly suggest that the described MCO-induced inactivation and degradation of GS oxidatively modiﬁed in Prochlorococcus possesses a physiological role. The presence of similar systems in other cyanobacteria [29,30], green algae [28] and higher plants [31] is noteworthy. Furthermore, it has been proposed in wheat that this inactivating mechanism could mediate a response from GS to oxidative stress produced by speciﬁc conditions in the photosynthetic metabolism [31]. Two possible physiological roles can be considered for the MCO-induced inactivation and degradation of GS: first, as a regulatory mechanism of the enzyme at the level of protein concentration; and second, as a secondary mechanism of protection against oxidative stress, so that damaged proteins are selectively degraded in order to produce new, fully operative enzymes to keep the functioning of metabolism in cells [50].

Acknowledgements

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