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ORIGINAL ARTICLE

NOS-mediated NO production and protein S-nitrosylation in Mamiellophyceae

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Abstract

Nitric oxide (NO) functions as a signaling molecule in many biological processes in green algae and higher plants. Although the mechanisms of NO synthesis in most plants are the subject of ongoing research and debate, a functional NO synthase (NOS) has been characterized only in *Ostreococcus tauri*. To date, the question of whether NO synthesis occurs in other NOS-containing members of the class Mamiellophyceaea, which gave rise to the core Chlorophyta, has not been elucidated. We found that, like *O. tauri*, *O. lucimarinus* and *Bathycoccus prasinos* grow on arginine as the sole nitrogen source, and their NOSs function and produce NO in cells. Moreover, in *O. tauri, O. lucimarinus*, and *B. prasinos*, NO exerts its biological functions through protein S-nitrosylation. Collectively, our data suggest that both NO and S-nitrosylated proteins are important mediators in the process of cell growth in NOS-containing representatives of Mamiellophyceae. Thus, we have updated the data related to protein S-nitrosylation as an evolutionarily conserved mechanism regulating many aspects of cell signaling in plants.

Keywords Arginine · *Ostreococcus Bathycoccus* · Nitric oxide

Introduction

NO is a gaseous signaling redox-active molecule that functions in various eukaryotes (Bredt and Snyder 1992; Wendehenne et al. 2001; 2004). In higher plants, this molecule is involved in the regulation of many processes such as seed germination, flowering, fruit ripening, root development, and adaptation to unfavorable environmental conditions (Bethke et al. 2006; Fancy et al. 2017; González-Gordo et al. 2019; He et al. 2004; Neill et al. 2008; Qiao and Fan 2008; Sun et al. 2014; Yu et al. 2014). In addition, NO plays an important role in the symbiosis of leguminous plants with rhizobia, acting as a metabolic intermediate in the phytoglobin–NO cycle during hypoxia (Berger et al. 2019). In green algae, NO has also been implicated in various cell functions, including control of nitrate assimilation (Sanz-Luque et al. 2015; Zalutskaya et al. 2023), macronutrient stress responses (Filina et al. 2019; Foresi et al. 2022; Lapina et al. 2022; Minaeva et al. 2017), hypoxia and salt stress acclimation (Chen et al. 2016; Zalutskaya et al. 2021), and impact on proline and putrescine biosynthesis (Zalutskaya et al. 2020).

In animals, NO signaling is mainly based on NO binding to heme iron of soluble guanylate cyclases (sGC) to produce cGMP, which then activates the cGMP-dependent protein kinase pathway (Francis et al. 2010; Martinez-Ruiz et al. 2011). In five green algae, 11 proteins homologous to sGC were identified (Astier et al. 2019). However, all sGCs of green algae lacked cysteine residues, which are necessary for the reception of the NO molecule by sGC. This indicates that none of these proteins can respond to the action of NO, like animal sGCs. In addition, the analyzed *Chlamydomonas reinhardtii* sGCs, CYG12, CYG56, and CYG11 did not show dependence on NO (de Montaigu et al. 2010). Moreover, CYG11 has been characterized as a potential CO_2 sensor (Horst et al. 2019). The available data indicate that plants do not use the classic NO/cGMP module (Astier et al. 2021). Apparently, in the process of evolution in animals and plants, a divergence of controlled NO signaling pathways occurred (Astier et al. 2021; Ermilova 2023).

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The major NO-dependent signaling mechanism in higher plants is thought to associate with posttranslational S-nitrosylation of proteins (Astier et al. 2019). In S-nitrosylation, the NO molecule reacts with the thiol group of cysteine in the presence of an electron acceptor, and a covalent S-NO–S-nitrosothiol bond is formed (Smith and Marletta 2012). Among green algae, S-nitrosylation has been analyzed in *C. reinhardtii* and *Chlorella vulgaris* (Henard et al. 2017; Morisse et al. 2014). The ability for S-nitrosylation is also possessed by the closely *C. reinhardtii* related nonphotosynthetic alga *Polytomella parva* (Lapina et al. 2022). Therefore, like higher plants, representatives of core Chlorophyta classes, Chlorophyceae and Trebouxiophyceae, use this type of posttranslational modification. However, to confirm the assumption about the key role of S-nitrosylation in the action of NO on Chlorophyta, it is necessary to analyze this protein modification in representatives of the class Mamiellophyceae, which gave rise to the core Chlorophyta (Leliaert et al. 2011).

The class Mamiellophyceae is a monophyletic group in the phylum Chlorophyta (Marin and Melkonian 2010). The most studied representatives of this class belong to three genera, i.e., *Bathycoccus*, *Micromonas*, and *Ostreococcus* (Piganeau et al. 2011). The members of these genera have small genomes (from 13 to 23 Mb), small size (0.7 to 2 µm), and simple cellular organization (single chloroplast and mitochondrion) (Derelle et al. 2006; Moreau et al. 2012; Palenik et al. 2007; Peers and Niyogi 2008; Worden et al. 2009). Notably, functional NO synthase (NOS) was characterized in *Ostreococcus tauri* (Foresi et al. 2010). Purified recombinant *O. tauri* NOS catalyzes NO formation and L-citrulline from L-arginine (Weisslocker-Schaetzel et al. 2017). In addition, NOS-like sequences were identified in the genomes of *Bathycoccus prasinos* and *O. lucimarinus* (Kumar et al. 2015). However, NO production in cell suspension of these algae has not been analyzed. The latter fact has limited our knowledge of the biological significance of NOS in Mamiellophyceae. In this study, we investigated arginine (Arg)-dependent growth and NO accumulation in *B. prasinos* and *O. lucimarinus.* In addition, protein S-nitrosylation has been described for the first time in NOS-containing representatives of Mamiellophyceae.

Materials and methods

Algal strains and cultivation conditions

Ostreococcus tauri RCC4221, *O. lucimarinus* RCC754, and *B. prasinos* RCC4222 were obtained from the Roscoff Culture Collection (http://www.roscoff-culture-collection.org/). Algal strains were grown in a modified ASP12 culture medium (McFadden and Melkonian 1996; http://www.ccac.uni-koeln.de/) and cultivated in 250-mL flasks in a chamber (KBWF 240, Binder GmbH, Tuttlinger, Germany) at 20 °C under continuous illumination by white light (effluence rate of 45 photons µmol m^{-2} s^{-1}) with continuous agitation (90 rpm). To determine the growth rates, different media were used depending on nitrogen source: standard ASP12 with $NaNO_3$, or this medium with nitrate replaced by arginine at various concentrations. At each harvesting time, the number of cells was counted microscopically (×90 objective) using a counting chamber. Four hundred cells from each sample were scored for three biological replicates.

Measurement of NO

To measure the NO levels, the cells grown in ASP12 were collected at the exponential phase of growth (6-day and 4-day cultures of *Ostreococcus* species and *B. prasinos*, respectively) by centrifugation (9000 × g, 10 min), washed with N-free medium, incubated in 5 mM arginine for the time indicated, and then exposed to 1 µM 4-amino-5-methylamino-2′7′-difluorofluorescein diacetate NO-specific dye (DAF-FM DA, Sigma-Aldrich, St. Louis, MO, USA). After this treatment, the cells were washed, resuspended in arginine-containing medium, and incubated for an additional 30 min to allow the complete de-esterification of the intracellular diacetates; then, the intracellular generation of NO was evaluated using a microplate reader CLARIOstar (BMG, Ortenberg, Germany). The excitation and emission wavelengths were set at 483 ± 14 and 530 ± 30 nm, respectively. The fluorescence levels were expressed as arbitrary units (fluorescence levels per 10^7 cells). Cell autofluorescence was subtracted from the total fluorescence obtained. Three technical replicates per condition were included on each plate, and each experiment was performed three times independently.

Confocal microscopy

For the NO detection by confocal microscopy, *O. lucimarinus* RCC754 and *B. prasinos* RCC4222 cells were treated as described above. Images were acquired with a Leica TCS SP5 confocal microscope (Leica-Microsystems, GmbH, Wettzlar, Germany) equipped with 100 × oil immersion objective. Excitation was performed with a 488-nm argon laser. The signals arising from the DAF-FM DA were collected on the channel between 500 and 544 nm and 645 and 728 nm to separate signals arising from the DAF-FM DA and endogenous chlorophyll, respectively.

S-nitrosylated protein labeling and Western blotting

Ostreococcus tauri, *O. lucimarinus*, and *B. prasinos* (5 10^7 cells mL^{-1}) grown in standard medium were collected at the

exponential phase of growth by centrifugation (9000 × g, 10 min), washed with N-free medium, and incubated in 5 mM arginine for the time indicated. For protein extraction, algal cells were resuspended in HENS buffer: 100 mM HEPES, pH 8.0, 1 mM EDTA, 0.1 mM neocuproine, and 1% SDS (Thermo Fisher Scientific, No. 90106, Rocksford, IL, USA) and vortexed with zirconia-silica beads (0.1 and 0.5 mm) using homogenizer Minilys (BertinTechnologies, France). After centrifugation (10,000 × g, 10 min), the protein concentration was determined by staining with a Pierse BCA Protein Assay Kit (Thermo Fisher Scientific, No. 23227, Rocksford, IL, USA). Protein (100 µg) in 100 µL of HENS buffer were used per sample. To block free cysteine thiols, 2 µL of 1 M sulfhydryl-reactive compound, MMTS (20 mM final concentration), was added to 100 µL of each sample and incubated for 30 min at room temperature. Proteins were precipitated by adding six volumes of pre-chilled (− 20 °C) acetone, and the samples were frozen at − 20 °C to remove unreacted MMTS. The precipitated proteins were pelleted by centrifugation (10,000 × g, 10 min) and resuspended in 100 µL of HENS buffer. To each 50 µL of sample, 1 µL of the non-biological reagent iodoTMT (Thermo Fisher Scientific, No. 90105, Rocksford, IL, USA) was added, and then, 2µL of 1 M sodium ascorbate was added to the mixture. These steps were performed in the dark. For negative control reactions, 2 µL of ultrapure water instead of sodium ascorbate was added to the samples. The reaction proceeded for 2 h at room temperature. Then, 10 µL of 5 × reducing Laemmli sample buffer (Laemmli 1970) was added to 40 µL of the labeled samples. After separation by SDS-PAGE on a 12% polyacrylamide gel, proteins were transferred to nitrocellulose membranes (Carl Roth, Karlsruhe, Germany) by semi-dry blotting (Trans-blot SD, Bio-Rad, Bio-Rad Laboratories, Geylang, Singapore) and stained with Ponceau S. The blots were blocked in 5% non-fat dry milk in Tris-buffered saline solution with 0.1% Tween 20 for 1 h, prior to an incubation of 1 h in the presence of primary anti-TMT antibody (1:1000). As a secondary antibody, the horseradish peroxidase conjugated anti-mouse IgG was used at a dilution of 1:20,000. The membranes were incubated with Super Signal West Pico Chemiluminescent Substrate for 5 min, and then, the films were scanned using a Bio-Rad ChemiDocTMMP Imaging System. Ponceaus S-stained membranes were used as a loading control.

Statistical analysis

The values for the quantitative experiments described above were obtained from at least three independent experiments with no fewer than three technical replicates. Data represent the mean ± SE. When necessary, statistical analyses were followed by a Student's *t* test (p value < 0.01).

Results

S-Nitrosylation in *O. tauri*

In *O. tauri*, NOS is known to generate nitric oxide, and NO production is increased upon the addition of Arg (Foresi et al. 2010; Weisslocker-Schaetzel et al. 2017). In our experiments, the amount of NO increased 1 h after transfer into 5 mM Arg and reached a maximum (approximately 1.9-fold) after 24 h of incubation (Fig. 1a). Upon further cultivation in Arg-containing medium, the NO level again decreased to values approximately 1.4-fold higher than the control. Notably, the transfer of cells into a fresh nitrate-containing medium led to a slight increase in NO levels by about 1.5-fold after 24 h.

To assess whether the arginine-derived NO is biologically active, we determined potential S-nitrosylation in cells incubated in the presence of nitrate or 5 mM Arg. The Pierce S-nitrosylation Western blot kit assay was used to determine protein S-nitrosylation in *O. tauri* (Fig. 1b). The probes were treated with the non-biological reagent iodoTMT to label S-nitrosylated proteins instead of HPDP-biotin, resulting in less background during Western blot detection. Probes were then identified using anti-TMT antibodies. Incubation with 5 mM arginine for 24 h produced intense band about 22 kDa. Interestingly, the same band was present in a sample of cells incubated with nitrate. An additional control without MMTS treatment showed that protein nitrosylation in these cells was specific. The results implied that the concentration of NO formed from Arg in both types of cells was sufficient to lead to S-nitrosylation of the proteins. The 56-kDa band detected in all samples is presumably nonspecifically labeled due to the presence of unblocked free cysteine thiols in this protein(s).

O. lucimarinus

Arginine-dependent growth and NO formation in *O. lucimarinus*

Because *O. tauri* grows on Arg (Foresi et al. 2022), we examined this amino acid as a potential N source for *O. lucimarinus*. N-free medium was supplied with external Arg (1 mM or 5 mM), and the growth of the alga in Arg-containing media was determined (Fig. 2a). Supplementation of 1 mM Arg resulted in slightly slower growth and lower final yields compared to nitrate-containing medium. Interestingly, the addition of 5 mM Arg to the medium did not improve growth characteristics compared to cells grown in 1 mM Arg (Fig. 2a). Moreover, in 5 mM Arg-containing medium, cells consistently exhibited a longer lag phase and slower growth than in cultures supplemented with 1 mM

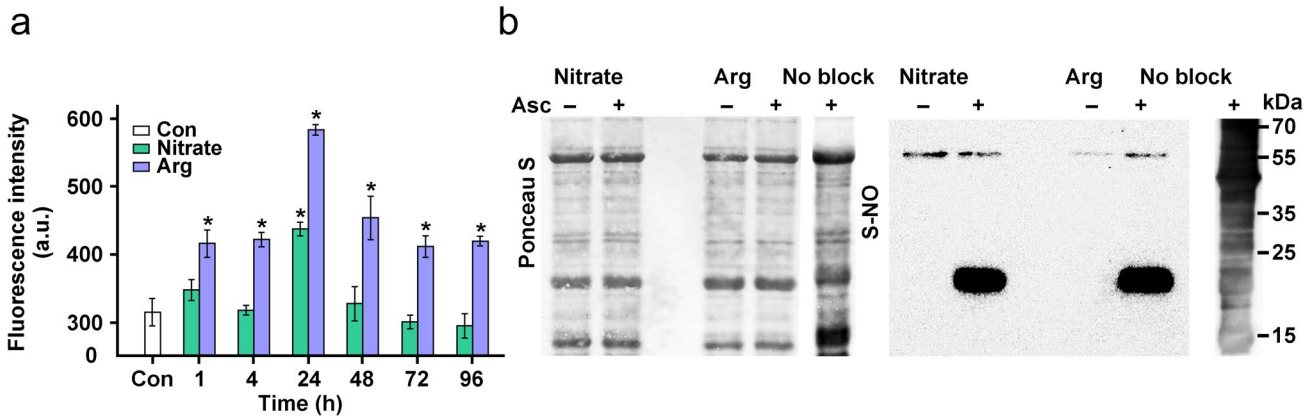

Fig. 1 Effects of Arg on NO production and protein S-nitrosylation in *O. tauri*. **a** Cells were grown in nitrate-containing medium (Con) and then transferred to fresh control medium or 5 mM Arg-containing medium for the indicated times. Fluorescence intensity due to NO formation was determined using 1 µM DAF-FM and expressed as arbitrary units per 10^7 cells. Cell autofluorescence was subtracted from the total fluorescence obtained. Means ± SEs of three biological replicates. The asterisk denotes significant differences between control (Con) and test variants according to the Student's *t* test (*p* value < 0.01). **b** The samples used were in nitrate-containing-medium or 5 mM Arg-containing medium for 24 h. The degree of protein S-nitrosylation was measured by Western blot with an anti-TMT antibody. Labeling with a non-biological iodoTMT™ reagent was performed with or without 5 mM ascorbate (Asc). Ponceau S staining was used to confirm the equal loading of the different samples. The right-hand lane represents the unblocked sample; the other samples were blocked with MMTS

Arg. Taken together, these data indicate that L-arginine can be used as a nitrogen source. As in *O. tauri* (Foresi et al. 2022), the growth of *O. lucimarinus* was inhibited by the NOS inhibitor L-NAME (Online Resource 1a).

Similar to *O. tauri*, a gene encoding NOS (e_gwEuk.16.4.1,Phycocosm) was found in *O. lucimarinus* (Santolini et al. 2017). Potential signal peptide of OlNOS was not identified using the TargetP-2.0 server (https://services.healthtech.dtu.dk/services/TargetP-2.0/), suggesting its cytoplasmic localization. Therefore, we wondered whether the addition of Arg increases NO generation. We found that the fluorescence signal was highest in cells incubated in 5 mM Arg, with a maximum level after 2 h of incubation (5.4-fold increase), and then, NO levels decreased after 24 h of growth. The same trend, only with lower fluorescence intensity levels, was observed after addition of 1 mM Arg. Cells incubated in nitrate-containing media also showed accumulation of NO with the highest induction of 2.6-fold after 2 h (Fig. 2b). A rise in NO levels in cells did not correlate with the gene expression (Online Resource 2a), hinting the involvement of post-transcriptional control.

We confirmed these results using confocal microscopy (Fig. 2c). After incubation with the NO-specific dye DAF-FM DA, the 5-mM Arg-treated cells displayed green NO fluorescence. In nitrate-containing medium, *O. lucimarinus* also showed a signal, but weaker than in cells incubated in 5 mM Arg. These analyses indicate that external Arg had an impact on NO production but some NO was also generated from intracellular Arg.

S-Nitrosylation in *O. lucimarinus*

We next asked whether proteins undergo S-nitrosylation in *O. lucimarinus*. Western blotting, as in the case of *O. tauri*, showed one prominent band around 22 kDa in probes from cells incubated in both nitrate and Arg (Fig. 2d). Another low-intensity specific band of ~30 kDa was detected in Arg-treated cells. The appearance of this band is probably due to a higher expression level of some protein(s) in cells incubated with Arg. Thus, similar to *O. tauri*, *O. lucimarinus* utilizes protein S-nitrosylation in a nitrogen-replete environment.

B. prasinos

Arginine-dependent growth and NO formation in *B. prasinos*

Bathycoccus prasinos demonstrated similar growth parameters on both nitrate and Arg as N-sources (Fig. 3a). When cells were grown in 0.1 mM Arg-containing medium, they entered stationary phase earlier than cells in medium with 5 mM Arg. In general, the addition of Arg to N-free medium promoted sustained growth rate of *B. prasinos*. The growth of cells was blocked by L-NAME (Online Resource 1b).

Bathycoccus prasinos genome contains a gene XM_007510876.1 encoding NOS (Santolini et al. 2017). According TargetP-2.0 analysis, *B. prasinos* NOS also does not contain potential transit peptide. Next, the question

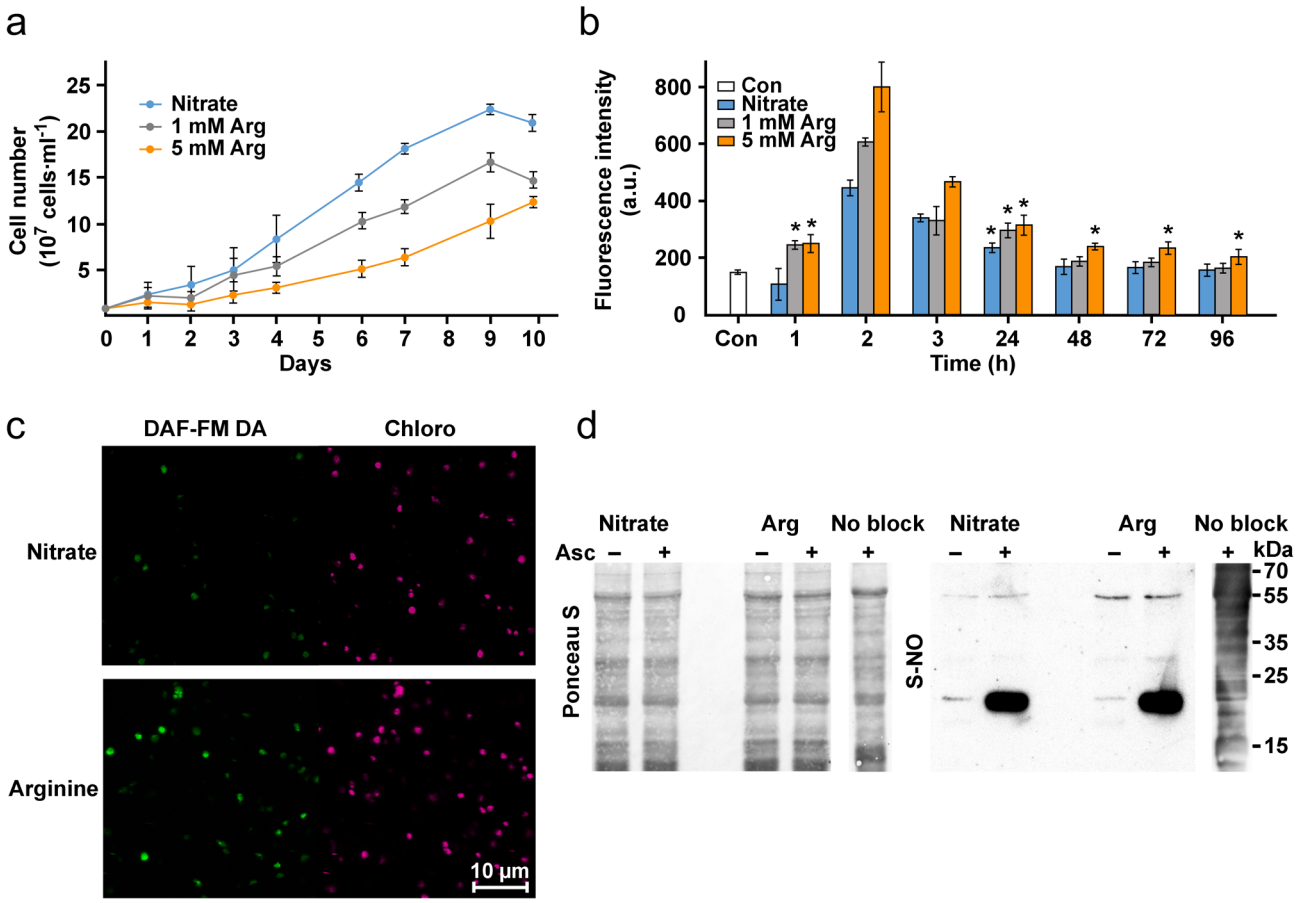

Fig. 2 Effects of Arg on growth, NO production and protein S-nitrosylation in *O. lucimarinus*. **a** Cells were grown in nitrate-containing medium and then transferred to fresh control medium or Arg-containing media for the indicated times. **b** Fluorescence intensity due to NO formation was determined using 1 µM DAF-FM and expressed as arbitrary units per 10^7 cells. Cell autofluorescence was subtracted from the total fluorescence obtained. Means ± SEs of three biological replicates. The asterisk denotes significant differences between control (Con) and test variants according to the Student's *t* test (*p* value < 0.01). **c** NO visualization by confocal microscopy. Images of cells in nitrate-containing medium or incubated in 5 mM Arg-containing medium for 2 h under. The left-hand panels show DAF-FM fluorescence (green color), while the right-hand panels show chlorophyll autofluorescence (violet color). Scale bar: 10 µm. **d** The samples used were in nitrate-containing -medium (Con) or 5 mM Arg-containing medium for 2 h. The degree of protein S-nitrosylation was measured by Western blot with an anti-TMT antibody. Labeling with a non-biological iodoTMT™ reagent was performed with or without 5 mM ascorbate (Asc). Ponceau S staining was used to confirm the equal loading of the different samples. The right-hand lane represents the unblocked sample; the other samples were blocked with MMTS

arose about the cells' ability to generate NO. Cells incubated in nitrate-containing medium showed negligible NO accumulation (Fig. 3b). 0.1 mM Arg did not increase the fluorescence level. However, the addition of 5 mM arginine resulted in an enhancement in NO levels with a maximum after 1 h of incubation. These data are consistent with the images obtained by confocal microscopy (Fig. 3c). Similar to *O. tauri* (Foresi et al. 2022) and *O. lucimarinus* (Online Resource 2a), the gene encoding NOS in B. *prasinos* showed no significant differences in expression under the conditions tested (Online Resource 2b). Overall, our results showed that *B. prasinos* NOS, which appears to be located in the cytoplasm, is functional.

S-Nitrosylation in *B. prasinos*

The Pierce S-nitrosylation Western blot method identified S-nitrosylated proteins in *B. prasinos* (Fig. 3d). We found that in this alga, endogenous S-nitrosylation of proteins is also a concomitant factor in cell growth and is independent of external Arg. Notably, as in both *Ostreococcus* species, the most intense band around 22 kDa was also detected in *B. prasinos*.

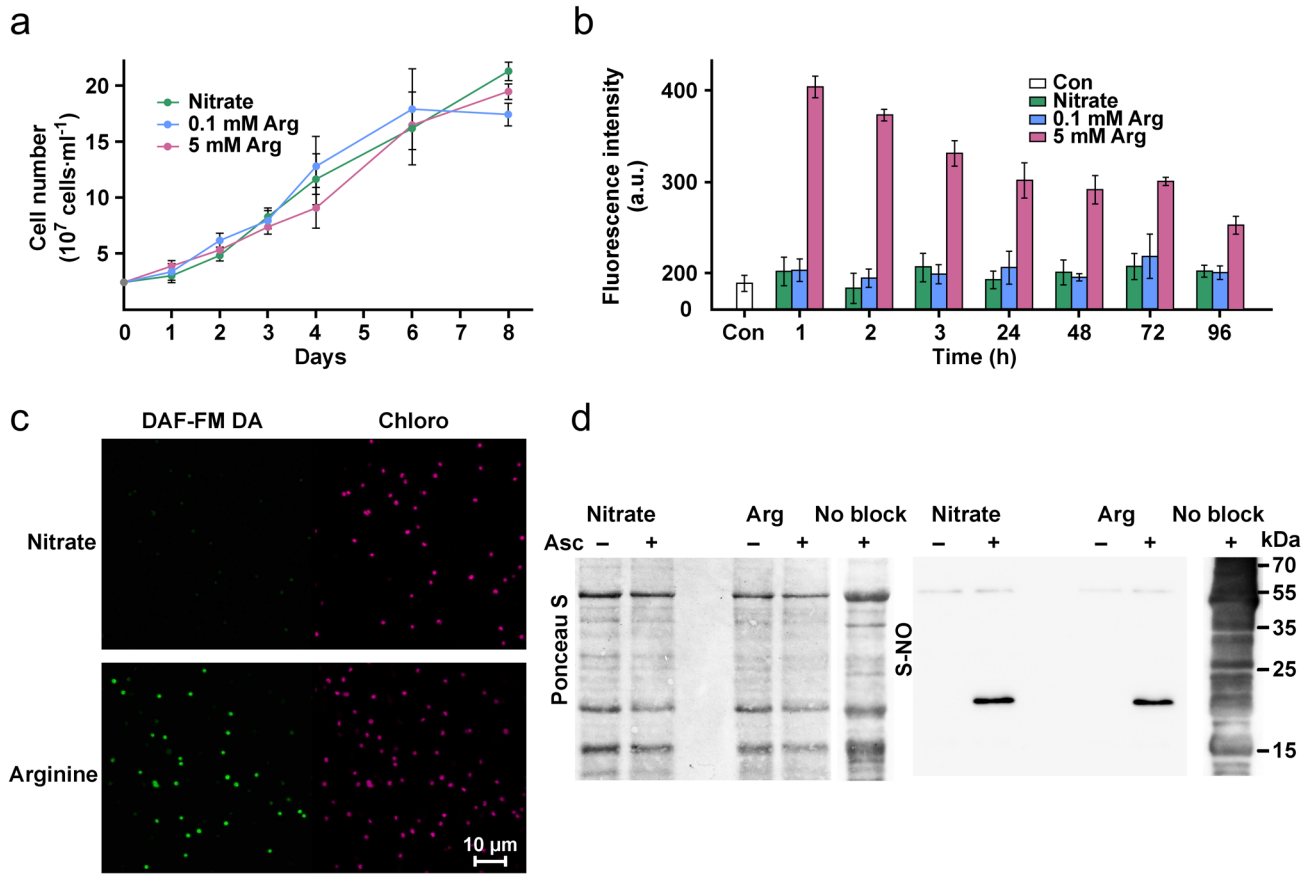

Fig. 3 Effects of Arg on growth, NO production, and protein S-nitrosylation in *B. prasinos*. **a** Cells were grown in nitrate-containing medium and then transferred to fresh control medium or Arg-containing media for the indicated times. **b** Fluorescence intensity due to NO formation was determined using 1 µM DAF-FM and expressed as arbitrary units per 10^7 cells. Cell autofluorescence was subtracted from the total fluorescence obtained. Means ± SEs of three biological replicates. **c** NO visualization by confocal microscopy. Images of cells in nitrate-containing medium or incubated in 5 mM Arg-containing medium for 2 h. The left-hand panels show DAF-FM fluorescence (green color), while the right-hand panels show chlorophyll autofluorescence (violet color). Scale bar: 10 µm. **d** The samples used were in nitrate-containing -medium (Con) or 5 mM Arg-containing medium for 2 h. The degree of protein S-nitrosylation was measured by Western blot with an anti-TMT antibody. Labeling with a non-biological iodoTMT™ reagent was performed with or without 5 mM ascorbate (Asc). Ponceau S staining was used to confirm the equal loading of the different samples. The right-hand lane represents the unblocked sample; the other samples were blocked with MMTS

Discussion

Arg, which has the highest nitrogen-to-carbon ratio among 21 proteinogenic amino acids, can be utilized as an external nitrogen source by various green algae (Laliberté and Hellebust 1990; Zuo et al. 2012; Calatrava et al. 2019). In this study, we found that, like *O. tauri* (Foresi et al. 2022), *O. lucimarinus* and *B. prasinos* grow on Arg as the sole N source (Figs. 2a, 3a). Interestingly, Arg concentrations sufficient to maximize the growth of these algae are between 0.1 and 1 mM, which is even lower than the optimal concentrations for other Chlorophyta. These results confirm previous observations on the broad distribution of transport systems for basic amino acids in microalgae (Kirk and Kirk 1978; Komor et al. 1988).

Despite the fact that the genes encoding NOS are present in the genomes of *O. tauri*, *O. lucimarinus*, and *B. prasinos* (Kumar et al. 2015), the enzyme has only been characterized in *O. tauri* (Foresi et al. 2010). As in *O. tauri*, the NOS genes in *O. lucimarinus* and *B. prasinos* showed no significant differences in transcription levels between cells grown in nitrate and arginine (Online Resource 2). This suggests that expression is controlled at the post-transcriptional level.

In our experiments, all these species had high and similar levels of NOS-dependent NO production, which could be augmented by the exogenous Arg supplementation (Figs. 1a, b; 2b, c; 3b, c). Interestingly, in *O. lucimarinus* and *B. prasinos*, NO peaked earlier than in *O. tauri*, indicating species specificity of NOS activity. Notably, the NO level decreases with further cultivation in a medium containing Arg. A possible scenario is that the rate of nitric oxide use begins

to exceed the rate of its formation. However, we also cannot exclude changes in the activity of the NO-synthase. In *O. tauri*, following NOS-dependent degradation of Arg, non-enzymatic oxidation of NO leads to the formation of N-oxides (mainly NO_2^-), which are re-incorporated into the primary N metabolism (Foresi et al. 2022). Although the genomes of *O. lucimarinus* and *B. prasinos* also lack genes encoding Arg catabolism enzymes such as L-amine oxidase, arginine deiminase, and arginase, they do contain a homologue of ornithine/arginine decarboxylase. Thus, it remains to be determined whether OlNOS and BpNOS are involved in Arg metabolism and whether nitrite is formed by spontaneous oxidation of NO in these species.

As mentioned above, NOS are present in three species of Mamiellophyceae and absent from two *Micromonas* species. This is not surprising, since algal NOSs are unevenly distributed across the phylogeny of photosynthetic organisms (Jeandroz et al. 2016; Santolini et al. 2017; Santolini 2019). These enzymes have been found not only in green algae (streptophytes and chlorophytes) but also in red and brown algae, diatoms, and dinoflagellates. Moreover, in addition to the "standard" NOS identified in mammals, several different groups of NOS have been described, corresponding to different architectural types of proteins (Santolini 2019; Chatelain et al. 2021). To conclude about the distribution and types of NOSs among Mamiellophyceae, the genomes of more members of this class need to be sequenced.

As NO-mediated PTMs, S-nitrosylation plays a key role in regulating cellular signaling in green algae and higher plants (Astier et al. 2019; Chaudron et al 2025; Wei et al 2025). We found that S-nitrosylation is a route for the transduction of NO bioactivity in NOS-containing Mamiellophyceae as well (Figs. 1b, 2d, 3d). Notably, bands around 22 kDa, corresponding to endogenous SNO-proteins, were similar in *O. tauri*, *O. lucimarinus*, and *B. prasinos*. The results suggest that similar proteins undergo S-nitrosylation in these cells. In plants, the identified proteins that are controlled by S-nitrosylation are mainly related to metabolism, photosynthesis, growth and development, and stress responses, indicating a role for this posttranslational modification in important cellular and metabolic pathways (Morisse et al. 2014; Feng et al. 2019; Liu et al. 2024; Wang et al. 2024; Wei et al. 2025). Further application of multi-omics approaches will be critical to deciphering protein S-nitrosylation networks and their regulatory roles in Mamiellophyceae.

Unexpectedly, the bands visualized in cells grown on Arg were comparable to those in cells grown on nitrate (Figs. 1b, 2d, 3d). Thus, cellular SNO-protein levels do not correlate with NO amounts (Figs. 1–3). One possible explanation is that NOS-mediated NO levels in all cells were sufficient for S-nitrosylation. Another fundamental question of how protein SNOs are formed de novo from NO. In contrast to other PTMs, S-nitrosylation is generally considered to be non-enzymatic (Lancaster 2017). However, in some bacteria, S-nitrosylation of proteins by NO is essentially enzymatic and is strongly dependent on a multiplex enzymatic complex but not on NO levels (Seth et al. 2018). But the possibility of enzymatic S-nitrosylation in plants is still controversial. Recent data indicate that NO signaling in the cyanobacterium *Synechococcus elongatus* PCC7942 is mediated by protein S-nitrosylation (Zalutskaya et al. 2025). Taken together, the results suggest that S-nitrosylation is an evolutionarily conserved post-translational modification in oxygenic organisms that is independent of the NO-generating mechanism.

Overall, our results imply that NOS functions in *O. lucimarinus* and *B. prasinos* and endogenous S-nitrosylation of proteins is a cell signaling mechanism in NOS-containing Mamiellophyceae. Future studies should focus on identifying the target proteins of S-nitrosylation, their precise sites of modification, and the functional consequences of these modifications on algal growth. We are only starting to have a sense of this, and clearly, there is a lot more to learn.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00709-025-02101-w.

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Author contributions EE conceived and designed the research. TL, VS, and VV conducted experiments. EE and TL analyzed data. EE wrote the manuscript.

Data availability The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Declarations

Conflict of interest The authors report there are no competing interests to declare.

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