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ROLE OF GASEOUS TRANSMITTERS NITRIC OXIDE AND HYDROGEN SULFIDE IN CELL REDOX REGULATION

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Nitric Oxide(II) in the Biology of Chlorophyta

E. V. Ermilova*

St. Petersburg State University, St. Petersburg, 199034 Russia

**e-mail: e.ermilova@spbu.ru*

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Abstract—NO is a gaseous signaling redox-active molecule that functions in various eukaryotes. However, its synthesis, turnover, and effects in cells are specific in plants in several aspects. Compared with higher plants, the role of NO in Chlorophyta has not been investigated enough. However, some of the mechanisms for controlling the levels of this signaling molecule have been characterized in model green algae. In *Chlamydomonas reinhardtii*, NO synthesis is carried out by a dual system of nitrate reductase and NO-forming nitrite reductase. Other mechanisms that might produce NO from nitrite are associated with components of the mitochondrial electron-transport chain. In addition, NO formation in some green algae proceeds by an oxidative mechanism similar to that in mammals. The recent discovery of *L*-arginine-dependent NO synthesis in the colorless alga *Polytomella parva* suggests the existence of a protein complex with enzyme activities that are similar to animal nitric oxide synthase. This latter finding paves the way for further research into potential members of the NO synthases family in Chlorophyta. Beyond synthesis, the regulatory processes to maintain intracellular NO levels are also an integral part for its function in cells. Members of the truncated hemoglobins family with dioxygenase activity can convert NO to nitrate, as was shown for *C. reinhardtii*. In addition, the implication of NO reductases in NO scavenging has also been described. Even more intriguing, unlike in animals, the typical NO/cGMP signaling module appears not to be used by green algae. S-nitrosylated glutathione, which is considered the main reservoir for NO, provides NO signals to proteins. In Chlorophyta, protein S-nitrosation is one of the key mechanisms of action of the redox molecule. In this review, we discuss the current state-of-the-art and possible future directions related to the biology of NO in green algae.

Keywords: Chlorophyta, NO, nitrate reductase, NO-synthase, S-nitrosation

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INTRODUCTION

Nitric oxide(II) (NO) belongs to the category of redox-active molecules, which coordinate many physiological and biochemical processes in organisms of different levels of organization [1–3]. Since the late 1990s, after the role of NO in inflammatory reactions and neurotransmission processes in mammals [4–6] was revealed, a comprehensive study of this gaseous free radical in plants began [7, 8]. Using various representatives of higher plants as an example, it has been established that NO is involved in the regulation of such processes of growth and development as seed germination, flowering and fruit ripening, and root development, as well as in adaptation to unfavorable environmental conditions [9–16]. 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 [17]. An analysis of the reductive and oxidative enzymatic and nonenzymatic pathways of endogenous NO synthesis revealed that control by this multifunctional signaling molecule is achieved mainly through posttranslational protein modifications (PTMs) [8, 18–21].

However, most studies in the field of NO functions and mechanisms of control of its intracellular levels (NO biology) in photosynthetic organisms are carried out on higher plants. Compared with Embryophyta functions, NO in algae has been insufficiently studied. This creates a significant gap in understanding of the mechanisms by which this signaling molecule is formed and how it is used in plants in general, especially given the important role of algae in marine, freshwater, and terrestrial ecosystems. The results obtained over the past decade show that green algae, along with higher plants, are a promising experimental model for studying the evolution of NO-dependent regulatory networks. This review discusses the latest data on the mechanisms of control of intracellular NO levels and its role and signaling functions in Chlorophyta.

NO SYNTHESIS MECHANISMS IN CHLOROPHYTA

The mechanisms that regulate NO levels in photosynthetic organisms are one of the most controversial

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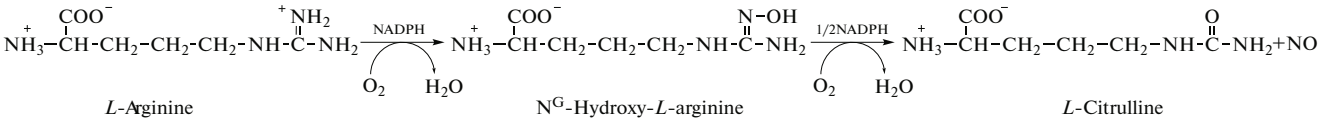

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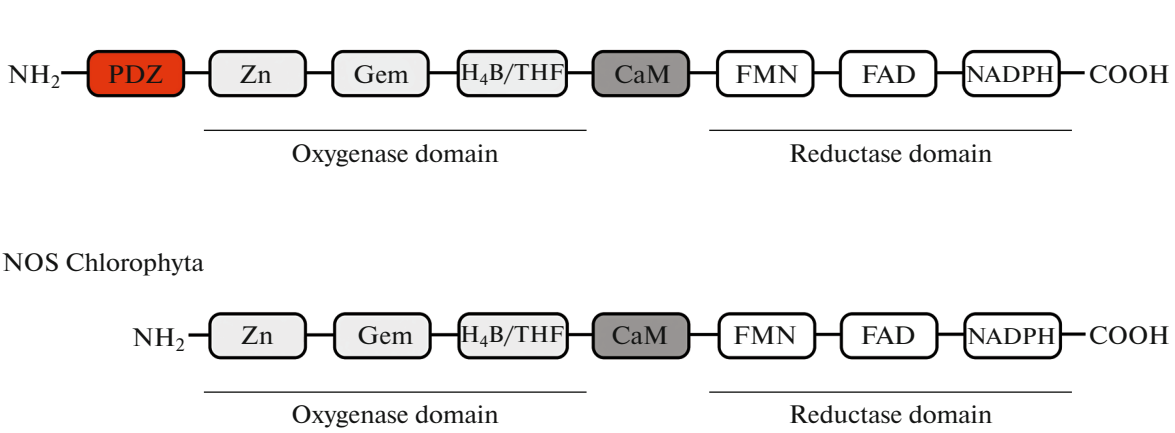

Fig. 1. The oxidative mechanism of NO formation (a) and NOS structure in mammals and green algae (b).

topics in the biology of this redox signaling molecule. Green algae are able to synthesize NO using oxidative or reductive pathways [22–26].

The oxidative pathway of NO formation is based on the formation of nitric oxide and *L*-citrulline from *L*-arginine via two-step oxidation in the presence of O_2 and the reduced form of NADPH by NO synthases ([EC 1.14.13.39] (NOS); Fig. 1a). This mechanism was first identified in mammals that use three NOS isoforms [27]. Mammalian NOS are two-domain proteins that consist of an N-terminal oxygenase domain (NOSoxy) and a C-terminal reductase domain (NOSred) (Fig. 1b). The NOSoxy domain binds arginine, protoporphyrin IX (heme), and tetrahydrobiopterin (BH4). The second domain, NOSred, binds NADPH, FMN, and FAD [28, 29]. Both domains are linked by a calmodulin-binding sequence (CaM). The two conserved cysteine residues of the NOSoxy domain in each monomer form a zinc binding site that facilitates NOS dimerization.

The NOS of green single-celled algae, which belong to the class Mamiellophyceae, was the first to be characterized in plant NOS: *Ostreococcus tauri* (OtNOS), *O. lucimarinus* and *Bathycoccus prasinos* [22, 30, 31]. Interestingly, OtNOS is capable of extremely rapid NO synthesis, which is uncharacteristic of animal or bacterial NO synthases [32].

Using large-scale analysis of genomes and transcriptomes, NOS homologues were identified in some algae [33, 34]. However, only in 11 representatives of Chlorophyta, these homologues contained both domains typical of mammalian NOS, which were connected to each other by a region identical to the CaM-binding sequence of OtNOS [33]. It should be emphasized that OtNOS retains 70% of its activity in the absence of CaM [22]. The available data suggest that NOS Chlorophyta most likely do not interact with CaM. In addition, unlike mammalian NOS, proteins of this family in algae have lost the conserved amino-acid residues of the N-terminal hook, but contain an atypical Zn-binding region [34] (Fig. 1b). Another distinctive feature of the structural organization of NOS in green algae is that they bind an H4B analogue, presumably tetrahydrofolate (H4F), which, for example, in OtNOS, acts as an electron donor [22, 35]. Understanding whether these proteins are true NOS will allow the analysis of their biochemical properties.

It is noteworthy that, in addition to NOS of the "archetypal" or "standard" type characteristic of mammals, proteins that are structurally different from them have also been found [33, 36]. Since, in some cases, inhibitory analysis revealed NOS-like activities in representatives that do not contain a canonical NOS, it was suggested that multimeric complexes

consisting of individual components, NOSoxy and NOSred, may be involved in the formation of NO from arginine [21, 23].

However, the fact that only a few representatives of Chlorophyta contain NOS orthologues raises the question of the significance of this enzyme for algae in general. In addition, in green algae, the possibility of NO formation through the oxidative pathway with the use of polyamines, as in some higher plants, was not revealed [37].

An analysis of the available data indicates that the oxidative pathway of NO formation has not been preserved in the majority of Chlorophyta in the course of evolution. At the same time, most green algae are able to effectively assimilate and restore nitrate, which is further reduced to nitrite. The reduction pathway of NO formation is based on its formation from nitrite.

The reduction of nitrate to nitrite is carried out by the enzyme nitrate reductase (NR). Plant enzymes are found in the cytosol and consist of two subunits, each of which contains three prosthetic groups: FAD, heme b_{557}, and the molybdenum cofactor (Moco), which is a molybdopterin complex with molybdenum [38]. Enzyme domains are redox centers in which electrons are sequentially transferred from NADPH to FAD, heme, and Moco [25]. It is noteworthy that the enzyme has two activities: diaphorase and terminal. In diaphorase or dehydrogenase activity, electrons from NADPH are used to reduce electron acceptors such as ferrocyanide or cytochrome *c*. The terminal activity of NR determines the reduction of nitrate using electrons received from donors such as FMN, methylviologen, or bromophenol blue.

Experimental data obtained on higher plants suggested that Mo-containing NR is the main enzyme responsible for the generation of NO from nitrite in plants [8, 39, 40]. At the same time, under standard conditions, the activity that ensures the reduction of nitrite is about 1% of the total NR activity [41]. This suggests a minimal contribution of the enzyme to NO synthesis. It is possible that other Moco-dependent enzymes may be responsible for the formation of NO in plants [42].

At present, the nitrite-dependent formation of NO has been experimentally proven only in several species of green algae [25, 43–46]. The molecular mechanisms of redox molecule generation have been studied in most detail in the model alga *Chlamydomonas reinhardtii*. An analysis of the nitrite-dependent generation of the signal molecule in this green alga allowed us to reconsider the role of NR in this process. Thus, it was shown that during the formation of NO from nitrite, NR interacts with another Mo-containing protein, NOFNiR (NO-forming nitrite reductase) [44] (Fig. 2). NOFNiR synthesizes NO independently of the Mo center of NR, but uses electrons supplied by the diaphorase activity of NR. It is not yet clear whether a similar NR-NOFNiR dual system is also used by other green algae and higher plants.

Learning adaptation of *C. reinhardtii* to hypoxia showed that the nitrite-dependent formation of NO occurs in the absence of functional NR [47], which means that it is carried out using another mechanism independent of NR. In higher plants, components of the mitochondrial electron transport chain (mtETC) are involved in NO synthesis [48, 49]. The role of mtETC in the formation of NO has been studied in *Chlorella sorokiniana* and *C. reinhardtii* [43, 50] (Fig. 2). However, the mechanisms of nitrite-dependent NO synthesis require further study involving a larger number of objects.

MECHANISMS OF NO ENZYMATIC TURNOVER IN CHLOROPHYTA

To eliminate the damaging effect of NO, organisms must control its intracellular levels. Thus, *C. reinhardtii* contains truncated hemoglobin 1 (THB1), which has NO-dioxygenase activity and can interact with NR [51]. THB1 accepts electrons from NR and converts NO to nitrate in the presence of oxygen (Fig. 2), i.e., functions as an alternative electron acceptor. Interestingly, this mechanism has been described in two algae, *Chattonella subsalsa* and *Heterosigma akashiwo* (Raphidophyceae), in which chimeric genes encode both THB and NR [52]. Another truncated *C. reinhardtii* hemoglobin, THB2, which also has NO-dioxygenase activity, is required (in addition to THB1) to control intracellular NO levels under conditions of phosphorus deficiency [53, 54]. It is assumed that truncated hemoglobins are involved in the modulation of NO levels and, due to this, can control NO-dependent signaling pathways [45, 51, 53, 54].

In addition, NO, as shown recently, can serve as a substrate for NO reductase *C. reinhardtii*, which catalyzes the formation nitric oxide(I) (N_2O) in mitochondria and chloroplasts [55–57]. In the light, the process of formation is catalyzed by FLV proteins, and in the dark it is catalyzed by cytochrome P450 - CYP55 [57]. It is noteworthy that CYP55 is synthesized and functions mainly at a low oxygen content in the environment [58]. Because the various Chlorophyta use both synthesis mechanisms N_2O [57], this way of controlling NO levels in green algae may be the most conserved.

NO levels in cells can also be regulated by the oxidation of NO derivatives (RNS) with the antioxidant tripeptide glutathione (GSH, Glu-Cys-Glu), which leads to the formation of S-nitrosoglutathione (GSNO), which is considered as the main intracellular NO reservoir [59]. Because GSNO acts as a NO buffer, its levels are important for NO homeostasis. In this regard, S-nitrosoglutathione reductase (GSNOR), which catalyzes the irreversible conversion of GSNO into oxidized glutathione, is substan-

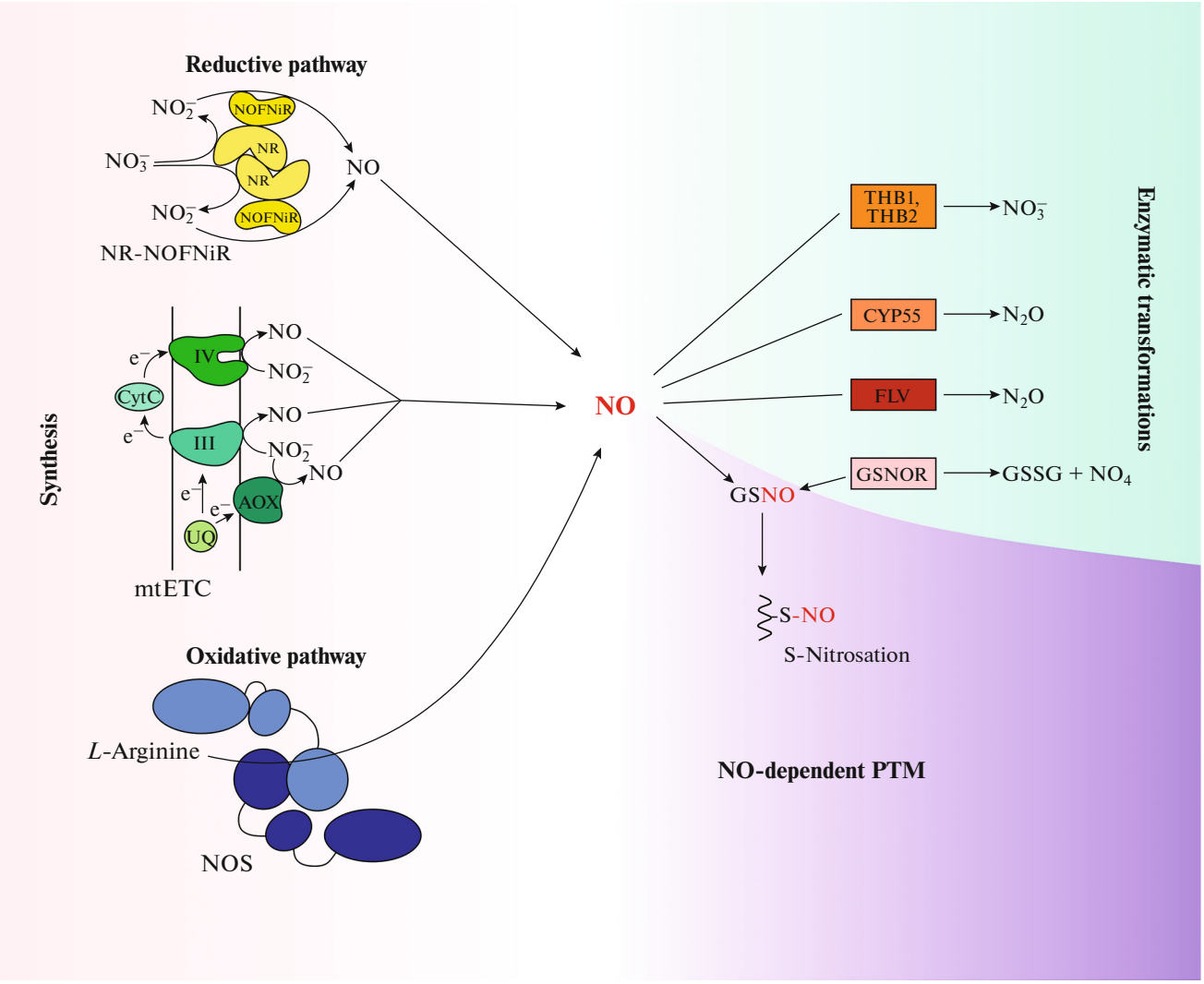

Fig. 2. Mechanisms for controlling intracellular NO levels in Chlorophyta.

tially involved in maintaining the balance of reactive nitrogen species and, ultimately, in controlling the redox state of the cytoplasm [60]. Interestingly, in the *C. reinhardtii* nuclear genome two genes encoding GSNOR isoforms have been identified. The CrGSNOR1 enzyme was characterized and its limited sensitivity to redox-dependent post-translational modifications was established [61]. It is assumed that the levels of nitrosothiols in algal cells are controlled by other enzymes or regulated mainly at the level of NO synthesis. This difference from higher plants may be associated with different needs for the regulation of NO metabolism in Chlorophyta and Streptophyta.

NO ACTION MECHANISMS IN CHLOROPHYTA

In mammals, an NO-dependent signaling pathway has been described in which synthase-produced NO activates soluble guanylate cyclases (sGCs), which catalyze the synthesis of the second messenger cGMP from guanosine 5'-triphosphate. Signal transmission occurs through the subsequent action of cGMP on effector proteins [62].

In five species of green algae (Chlorophyta, order Chlamydomonadales), 11 proteins homologous to sGC were identified [24]. However, all proteins of green algae lack cysteine residues (Cys78 and Cys214), 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 *C. reinhardtii* sGCs, CYG12, CYG56, and CYG11 did not show dependence on NO [63]. Moreover, CYG11 has been characterized as a potential CO_2 sensor [64]. The available data indicate that plants do not use the classic NO/cGMP module [65]. Apparently, in the process of

evolution in animals and plants, a divergence of controlled NO signaling pathways occurred.

It is believed that the main NO dependent signaling mechanism in higher plants is associated with post-translational S nitrosation of proteins [24]. With modifications of this type, the NO molecule reacts with the thiol group of cysteine in the presence of an electron acceptor and the covalent bond S–NO–S-nitrosothiol is formed [66].

Among green algae, S-nitrosation has been analyzed mainly in *C. reinhardtii*. In this model alga about 500 S-nitrosated proteins have been identified, the functions of which are associated with metabolic processes, protein synthesis, folding, and degradation, replication, transcription, and other cell functions [67]. In addition, several *C. reinhardtii* proteins have been characterized with this PTM [68, 69]. Nitric oxide is produced in *C. reinhardtii* cells during nitrate assimilation [70, 71], macronutrient starvation [45, 53, 54, 72, 73], and under hypoxic conditions [47] and is important for the synthesis of proline and putrescine [74]; however, S-nitrosation of proteins has been characterized only under saline stress [75]. The ability for S-nitrosation is also possessed by the closely *C. reinhardtii* related nonphotosynthetic alga *P. parva* [23]. Thus, in order to experimentally confirm the assumption about the key role of S-nitrosation in the action of NO on green algae, it is necessary to analyze this modification of proteins in other representatives of Chlorophyta and under different conditions.

CONCLUSIONS AND OUTLOOK

Recent studies have led to the discovery of NO synthesis by representatives of Chlorophyta. However, despite the widespread use of green algae in scientific research and biotechnology, the mechanisms of NO formation/utilization and its role in signal transduction pathways in these organisms are not yet well understood. One of the main problems in studying the role of NO has been (and still is) deciphering the mechanisms that determine changes in its intracellular levels. Thus, in higher plants, the key role in NO synthesis is attributed to NR. A new *C. reinhardtii* system for the formation of a redox molecule has been discovered, consisting of two enzymes: NO-forming nitrite reductase and NR. At the same time, it remains unclear whether a similar system functions in other algae or, like higher plants, they use NR for NO synthesis.

The discovery of the oxidative pathway of NO synthesis and the characterization of NOS in some Chlorophyta also do not yet allow us to understand how widely this group of organisms uses arginine as a substrate for NO generation and whether complexes consisting of several proteins can functionally replace NOS. The latter assumption is supported by the recent discovery of arginine-dependent NO synthesis in a nonphotosynthetic alga, *P. parva,* which lost NR in the process of evolution.

No less intriguing is the enzymatic conversion of NO. A number of studies have established the participation of truncated hemoglobins with dioxygenase activity in this process. In addition, it turned out that NO reductases, which catalyze the conversion of NO into N_2O can play an important role in reducing NO levels in green algae cells. To clarify all the unresolved issues, it is necessary to study a larger number of objects.

One more intensively developing direction, related to the analysis of the mechanisms of action of this signaling molecule, should be noted separately. Nitric oxide metabolism is regulated by the redox state of cells. Together with other redox molecules, NO is involved in the control of cellular redox processes. In this regard, in Chlorophyta, first of all, a detailed study of the interaction of NO with reactive oxygen species is necessary. In addition, recent data make it doubtful that Chlorophyta use the typical animal NO-cGMP module in their regulatory networks. In the absence of specific receptors in algae, the perception and action of NO is apparently carried out mainly through PTMs. To confirm the correctness of the assumption about the key role of PTMs in the type of S-nitrosation, it is necessary to analyze S-nitrosomes in various representatives of Chlorophyta under physiological conditions under which a redox molecule is generated in cells. In our opinion, further progress in the field of NO biology in Chlorophyta will make it possible to judge the evolution of key components (enzymes and regulatory proteins) of nitric oxide biosynthesis and utilization, as well as those regulatory networks controlled by NO in plants.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The author declares that she has no conflicts of interest.

This article does not contain any studies involving animals or human participants performed by the author.

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