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Molecules of Interest

Nitric oxide in plants
To NO or not to NO

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Abstract

The current knowledge on the occurrence and activity of NO in plants is reviewed. The multiplicity of nitrogen monoxide species and implications for differentiated reactivity are indicated. Possible sources of NO are evaluated, and the evidence for the presence of nitric oxide synthase in plants is summarised. The regulatory role of $NO^{\cdot}$ in plant development and in plant interactions with microorganisms, involving an interplay with other molecules, like ethylene or reactive oxygen species is demonstrated. Finally, some other suggestions on potential functions of $NO^{\cdot}$ in plants are indicated. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

For more than 50 years, the only gaseous signalling molecule in the living world known to science was the plant hormone, ethylene. The 1998 Nobel Prize for Medicine heralded the establishment of another, even a smaller one, player of this kind in mammalian cells — nitric oxide. This relatively stable free radical has been initially identified as an endothelium-derived relaxation factor, and later implicated in signal transduction pathways controlling neurotransmission, cell proliferation, platelet inhibition, programmed cell death, and host responses to infection (Wink and Mitchell, 1998). The presence of nitrogen monoxide in plants has been known for some time (Leshem, 1996), and its involvement in regulation of plant growth (Gouvea et al., 1997) and phytoalexin accumulation (Noritake et al., 1996) has been demonstrated. It was however the appearance of two ground-breaking papers (Delledonne et al., 1998; Durner et al., 1998) that demonstrated clearly its regulatory role in plant biology, in this case during interactions with pathogenic microorganisms. Recent excellent reviews of this topic (Bolwell, 1999; Durner and Klessig, 1999), allow me to consider only selected important points of "chemical biology of nitric oxide" (Wink and Mitchell, 1998) in plants.

2. Multiplicity of nitrogen monoxide chemistry

Current views ascribe most of physiological effects of NO action to its radical species — nitric oxide. Based on analogies with animal systems, the same assumption is becoming prevalent in studies of plants, although direct detection and determination of NO forms present in planta are very scarce (e.g. Mathieu et al., 1998). It should be noted however that the NO chemistry is much more complicated and involves an interplay between three species differing in physical properties and chemical reactivity: nitrosonium cation (NO^{+}), nitric oxide radical ($NO^{\cdot}$), and nitroxyl anion (NO^{-}). Their chemistry is not recognised to that extent as the chemistry and implications of that chemistry for

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biological activities of respective dioxygen counterparts: O_2, superoxide radical anion ($^{\cdot}O_2^-$), and hydrogen peroxide (H_2O_2) (Stamler et al., 1992).

There are also important consequences for methodological design of experiments aimed at elucidation of the role of NO in plants, and a cautionary note seems appropriate in this place. Some conclusions are drawn on the basis of experimental results obtained with NO-releasing compounds, and the implicit assumption is that the NO species released is nitric oxide (e.g. Delledonne et al., 1998). Although compounds with clearly defined ability to release $NO^{\cdot}$ are now available, some other commonly used complexes, like nitroprusside are in fact iron–nitrosyl substances with strong NO^+ character (cited after Stamler et al., 1992).

3. Origin of nitrogen monoxide in plants

There are many possible sources of nitrogen monoxide (Fig. 1). Although in animals NO is generated almost exclusively by nitric oxide synthase (NOS, EC 1.14.13.39), in bacteria, fungi, and plants the presence of NO, like the presence of ROS, is intimately implicated in their metabolism, and in fact is one of the elements of nitrogen cycling on Earth. Nitrification/denitrification cycles provide NO as a by-product of N_2O oxidation into the atmosphere. Plants not only react to the atmospheric or soil NO, but are also able to emit substantial amounts of NO (see Durner and Klessig, 1999). Thus, NO could be generated by non-enzymatic mechanisms, e.g. via chemical reduction of NO_2^- at acidic pH or by carotenoids in the presence of light (Cooney et al., 1994). The major origin of NO production in plants however is probably through the action of NAD(P)H-dependent nitrate or nitrite reductases (Yamasaki et al., 1999).

In mammals, NOS catalyses a five-electron oxidation of a terminal guanidino nitrogen of arginine, and insertion of a pair of oxygen atoms (from two activated O_2 molecules) yields citrulline and NO. The reaction is supported by NADPH and flavins, and thiol and tetrahydrobiopterin are additional cofactors. Mechanistically, the enzyme is similar to cytochrome P450. The existence of NOS in plants was inferred from immunoreactivity of protein extracts (Sen and Cheema, 1995), and indications of NOS-type activity in plant tissues (Cueto et al., 1996; Ninnemann and Maier, 1996). Subsequently, NOS induction during plant–pathogen interactions (Delledonne et al., 1998; Durner et al., 1998) and growth phase-dependent NOS localisation (Ribeiro et al., 1999) have been demonstrated. However, the identity of plant enzyme with mammalian-type NOS is far from proved. Both the protein, and the gene(s) coding for the enzyme have not been identified in plants yet. Moreover, as Durner and Klessig (1999) rightly pointed out, there are no data whether tetrahydrobiopterin is synthesised by plants. Conflicting results have also been obtained with regard to Ca^{2+}-dependence. While NOS activity in lupin roots was Ca^{2+}-dependent, similarly to constitutive NOS isoforms in mammals, NOS activity in lupin nodules was Ca^{2+}-independent thus resembling mammalian inducible isoforms (Cueto et al., 1996). On the other hand, an enzyme induced in plants by pathogenic infection was Ca^{2+}-dependent (Delledonne et al.,

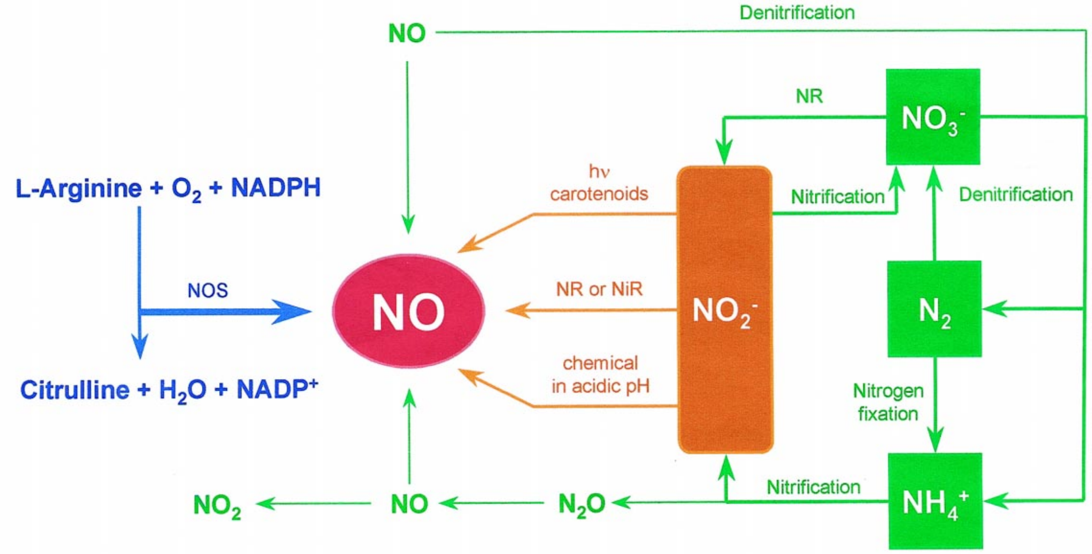

Fig. 1. Possible sources of nitrogen monoxide. NO is generated by the action of nitric oxide synthase (NOS). Other major origins of NO are the reactions utilising NO_2^-: non-enzymatic reductions either at acidic pH or light-driven in the presence of carotenoids, and enzymatic catalysed by NAD(P)H-dependent nitrate (NR) or nitrite (NiR) reductases. It could also be a by-product of denitrification, nitrate assimilation and/or respiration. Nitrification of NH_4^+ is the major source of N_2O emitted to the atmosphere where it might be further oxidised to NO and NO_2.

1998). Differentiated sensitivity of plant and mammalian enzymes to NOS inhibitors (Durner et al., 1998) made the analyses of plant enzyme even more complicated. Moreover, the existence of multiple calmodulin isoforms affecting differently NOS activity opens the possibility of more complex regulatory circuits and variable branching of signalling pathways (Cho et al., 1998).

4. Role of nitrogen monoxide in plants

4.1. Molecular targets

Due to differentiated chemical reactivity of NO species, there are many potential targets of NO action. From the biological point of view, the best characterised are the reactions of $NO^{\cdot}$ and NO^{+}. Nitric oxide reacts with $^{\cdot}O_2^-$ yielding peroxynitrite ($ONOO^-$), the major toxic reactive nitrogen oxide species (Stamler et al., 1992). As the apoplastic route for NO transport has been proposed (Leshem, 1996), and superoxide dismutase (SOD) is a major regulator of $^{\cdot}O_2^-$ levels, recent demonstration of exocellular SOD isoforms in plants (Schinkel et al., 1998) suggested the existence of an exocellular system preventing the formation of $ONOO^-$. Similarly, mitochondrial alternative oxidase was implicated in restriction of $^{\cdot}O_2^-$ generation under conditions of cytochrome *c* oxidase inhibition by NO again limiting oxidative damage of the cells (Millar and Day, 1997).

Other potential targets of $NO^{\cdot}$ action include proteins containing transition metal ions either as haem or non-haem complexes, mainly iron–sulphur centres in the latter case. NO^{+} is involved in addition and substitution reactions with nucleophiles, and most often nitrosations occur at −S, −N, −O, and −C centres of organic molecules (Stamler et al., 1992). This multiplicity of potential reactions has dual consequences. Firstly, depending on the location, structure, and reactivity of the product formed, it could be regarded either as an end result of NO degradation or as an element of NO-transporting/storage pool. Secondly, especially due to reactions with other free radicals, NO could be treated as a molecule breaking the propagation of radical-mediated oxidation chains thus limiting cellular damage (Beligni and Lamattina, 1999).

4.2. Regulatory interplay

Despite the multiplicity of potential targets, only few proteins have as yet been demonstrated to be regulated by interactions with NO. The most interesting candidate is guanylate cyclase and the resulting activation of cGMP-dependent signalling pathway. This relation was demonstrated in TMV-infected tobacco and an involvement of another NO-dependent signalling molecule: cyclic ADP-ribose was also detected (Durner et al., 1998). As NO easily forms iron–nitrosyl complexes with haem iron, it is not surprising that NO effect on the functioning of plant peroxidases participating in wall lignification has been recently revealed (Ferrer and Ros Barcelo, 1999).

The most interesting observations are those on the involvement of NO in regulation of plant defence responses to pathogen infection (Noritake et al., 1996; Delledonne et al., 1998; Durner et al., 1998). There seems to exist a regulatory interplay between NO and ROS, and salicylic acid is also playing its role. Direct reactions of NO and ROS might be involved as well as indirect through activation/repression of respective signalling pathways leading to changes at the gene expression and protein activity levels (reviewed by Bolwell, 1999; Durner and Klessig, 1999). However, as we are at the beginning of the road in this “web of signals” there are many more questions to be answered and care should be taken to avoid misinterpretations. It is thus worth reminding that the biochemistry of reactive NO and oxygen species might be intimately intertwined as e.g. under conditions of arginine depletion, NOS is synthesising H_2O_2 (Stamler et al., 1992). On the other hand, diphenylene iodonium — an inhibitor considered specific for the ROS-generating NADPH oxidase is also an inhibitor of H_2O_2-generating activity of peroxidases and moreover it inhibits NOS activity (reviewed by Bolwell and Wojtaszek, 1997; Bolwell, 1999). Similarly, some inhibitors of guanylate cyclase — a major NO target, acting through oxidation of thiols and transition metals, are even more potent NOS inhibitors (Hausladen and Stamler, 1998). Finally, on the basis of many pharmacological data, and in continuation of previous suggestions (Wojtaszek, 1997), the role of phenolic compounds as regulators of the extent of NO action should also be not overlooked.

4.3. Potential functions

Relatively little is known about the biological functions of NO in plants. Some of them might be inferred from the data gathered on mammalian cells, but, mainly due to differences in developmental processes, some of them would be probably unique to plants. Thus, like in mammalian cells there are clear indications that NO is involved in signalling events during interactions with microorganisms, either symbiotic (Cueto et al., 1996; Mathieu et al., 1998) or pathogenic (Noritake et al., 1996; Delledonne et al., 1998; Durner et al., 1998). On the other hand, NO is probably a regulatory molecule during plant development affecting growth (Gouvea et al., 1997), and activity of enzymes involved in cell differentiation (Ferrer and Ros Barcelo, 1999). Endogenous NO is even sometimes considered as a plant growth regulator (Leshem, 1996), and the evidence for an interplay between NO and ethylene — a hormonal system unique to plants — in the regulation of maturation and senescence of plant tissues has been already evidenced (Leshem et al., 1998).

The field of chemical biology of nitric oxide in plants is rapidly expanding, and it is hoped that the near future will bring some more answers. Even now a quick survey through the literature reveals other potential analogies with mammalian systems offering a promising prospectus for future studies in plants, and two examples will be mentioned here. Plant oligosaccharides are able to stimulate the release of NO from murine effector cells (Hirazumi and Furusawa, 1999) suggesting potential interplay between NO and cell wall-derived oligosaccharins. The identification of plant homologues of NRAMP1 protein provides an indication that plants might be equipped with a system similar to the animal one where NRAMP1 has been identified as a controller of innate immunity and is speculated to function as a nitrite transporter supplying substrate for NO synthesis (reviewed by Nathan, 1995).

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