Nitric oxide biosynthesis in plants – the short overview

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ABSTRACT

In the past two decades, many pathways of nitric oxide biosynthesis have been described. This review offers the general knowledge of mechanisms of plant nitric oxide biosynthesis.

Keywords: nitrate reductase; NOS like enzyme

With the finding of a number of roles of the gaseous free radical nitric oxide (NO) in animal cells, many studies have reported its presence in the plant kingdom and its diverse function in plant cells. In plants, NO first came to prominence within the context of regulating defence during pathogen infection (Mur et al. 2013b). Afterwards, it was described that NO is involved in many plant physiological processes, e.g. in stimulation of seed (Beligni and Lamattina 2000) and pollen (Šírová et al. 2011) germination, floral regulation (He et al. 2004), senescence (Jasid et al. 2009), stomatal closure (Neill et al. 2008), root development (Pagnussat et al. 2003, Correa-Aragunde et al. 2004) etc.

Unfortunately, NO studies in plants lag behind the studies in animal kingdom. One of the questions, which was acceptably clarified in animal cells but remains unclear in plants, is NO biosynthesis. In animal organisms, it is nitric oxide synthase (NOS) which converts L-arginine to L-hydroxyarginine and subsequently to nitric oxide and citrulline with participation of O_2 and NADPH. Three independent animal NOS types are known: neuronal NOS, inducible NOS which was originally isolated from macrophages, and endothelial NOS.

On one hand the indisputable evidence of such enzyme in plant cells is missing but on the other hand many other pathways were suggested. This review tries to offer the general knowledge of mechanisms of plant NO biosynthesis.

Enzymatic production

Nitrate reductase. The best-characterized production pathway for NO in plants is nitrate reductase (NR, EC 1.7.1.1.) pathway. This enzyme was found both as a cytosolic form and as a plasma membrane-bound form (Planchet and Kaiser 2006). In *Arabidopsis*, NR is encoded by two homologous genes, *Nia1* and *Nia2* (Wilkinson and Crawford 1993).

NR normally reduces nitrate to nitrite at the expense of NAD(P)H but also catalyzes 1-electron transfer from NAD(P)H to nitrite resulting in NO formation (Planchet and Kaiser 2006) *via* the reaction:

$$NAD(P)H + 3\,H_3O^+ + 2\,NO_2^- \rightarrow NAD(P)^+ + 2\,NO + 5\,H_2O$$

The reduction efficiency is low – about 1% of NR activity (Rockel et al. 2002) but the importance

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of NR as a NO source was demonstrated by using NR-deficient mutants which produced significantly lower levels of NO (Planchet et al. 2005).

NR is more pronounced in a low-oxygen environment and requires nitrite levels to be in excess of the natural substrate nitrate. For example for maize NR, the K_m for nitrite is 100 μmol, and nitrate is a competitive inhibitor with a K_i of 50 μmol (Rockel et al. 2002). The enzyme is activated by a decrease in the cellular pH (Kaiser and Brendle-Behnisch 1995).

Nitrite:NO reductase. A plasma membrane-bond nitrite:NO reductase (NiNOR), distinct from the plasma membrane NR, was shown to convert nitrite to NO in tobacco (Stöhr and Stremlau 2006, Moreau et al. 2010). The K_m (nitrite) for NiNOR reaction is 175 μmol for plant mitochondria (Gupta et al. 2005). It appears to use cytochrome *c* as an electron donor *in vitro*, but it has yet to be cloned and fully identified (Wilson et al. 2008).

The plasma membrane-bound NR:NiNOR system was suggested to be involved in the sensing of nitrate availability in the soil (Meyer and Stöhr 2002). Furthermore, evidence has recently been provided that NiNOR mediated NO production has a role in the regulation of root infection by mycorrhizal fungi (Moche et al. 2010).

NOS like enzyme. As plants appear able to grow and to complete their life cycle in the absence of nitrate and nitrite, e.g., with ammonium as the only source of nitrogen, they must possess nitrite-independent, oxidative pathways for NO production (Rümer et al. 2009). Indeed, in analogy to animal NOS (EC 1.14.13.39), plants appear to have an enzyme, which is completely independent of nitrite and whose function consists in deamination of L-arginine into L-citrulline and NO using NADPH and O_2 and requiring Ca^{2+}/calmodulin:

$$\text{L-arginine} + \text{NAD(P)H} + O_2 \rightarrow \text{L-citrulline} + \text{NAD(P)}^+ + H_2O + NO$$

NOS activity was measured in pea by ozone chemiluminiscence, using commercial neuronal NOS as a positive control (Corpas et al. 2006). This activity was also detected using electron paramagnetic resonance spin-trapping technique in soybean chloroplasts (Simontacchi et al. 2004) and in sorghum seed embryonic axes (Jasid et al. 2006). In addition, immunological evidence for NOS occurrence in pea and maize tissues was obtained with antibodies against animal NOS (Barroso et al. 1999, Ribiero et al. 1999).

However, Lo et al. (2000) demonstrated that these antibodies are rather unspecific. Likewise, the response of NOS activity in barley root mitochondria to inhibitors, substrates and cofactors was atypical when compared to iNOS, hence the existence of NOS root mitochondria was implausible (Gupta and Kaiser 2010). Another point, calling the existence of NOS in plants into question, is the necessity of tetrahydrobiopterin in mammalian NOS. This molecule seems to promote and/or stabilize the active dimeric form of the enzyme (Alderton et al. 2001). The presence of tetrahydrobiopterin in cells of higher plants is unclear. Nevertheless, its function could be carried out by tetrahydrofolate, whose metabolism was described sufficiently in higher plants (Sahr et al. 2005, Corpas et al. 2009). In addition, no gene or protein with sequence similar to the large animal NOS proteins was found even in the sequenced *Arabidopsis* genome (Crawford and Guo 2005).

Nevertheless, *Arabidopsis* has a gene with 16% sequence similarity to the gene from snail *Helix pomatia* which is implicated in NO synthesis and which, when expressed in *Escherichia coli*, increases NO synthesis in crude cytosolic fractions from particular snail organs (Huang et al. 1997). This *Arabidopsis* gene was identified as a member of GTP-binding family, encoding NOS-like protein *AtNOS1* (Guo et al. 2003). *AtNOS1* protein cross-reacts with antibodies against nNOS (Guo et al. 2003). As *AtNOS1* might indirectly affect NO synthesis, because it might serve as GTPase, Crawford et al. (2006) suggested that *AtNOS1* should be rename nitric oxide associated 1 (*AtNOA1*). However, the relationship between *AtNOA1* function and NO accumulation is rather unclear (Moreau et al. 2010).

So far, two locations for *AtNOA1* were reported: chloroplasts (Flores-Pérez et al. 2008) and mitochondria (Guo and Crawford 2005). Apart from its role in NO production, *AtNOA1* might act in binding RNA/ribosomes (Sudhamsu et al. 2008).

In algae, namely in *Ostreococcus tauri* and *O. lucimarinus*, two NOS sequences were found (Foresi et al. 2010). In the case of *O. tauri* it was found that the amino acid sequence of the NOS is 45% similar to that of a human NOS. It is close to the mammalian inducible NOS isoform because (a) its folding was likely to be similar to that of human inducible NOS and (b) this algae enzyme lacks the autoregulatory control element indicating that it is close to the mammalian inducible NOS isoform.

On the other hand, *Ostreococcus* genome has been completely sequenced (Derelle et al. 2006) and it lacks the genes encoding for the enzymes that synthetize tetrahydrobiopterin, suggesting that *Ostreococcus* NOS may bind another cofactor for catalytic activity (Correa-Aragunde et al. 2013).

However, it is still premature to declare that plant NOS was found because this organism belongs to a primitive class within the green plant lineage, the Prasinophyceae (Chlorophyta), so we cannot assume that higher plants have retained this gene (Hancock 2012).

Xanthine oxidoreductase. In addition to O_2 reduction, xanthine oxidase is also capable of reducing organic nitrates as well as inorganic nitrate and nitrite releasing NO (Godber et al. 2000). Xanthine oxidoreductase, the ubiquitous molybdenum-containing enzyme, occurs in two convertible forms: the superoxide-producing xanthine oxidase (form O, EC 1.1.3.22) and xanthine dehydrogenase (form D, EC 1.1.1.204) (Palma et al. 2002). Xanthine oxidoreductase was found present in pea leaf peroxisomes where the major form of the enzyme is xanthine oxidase and only 30% is present as xanthine dehydrogenase (Sandalio et al. 1988, Corpas et al. 1997, del Río et al. 2004). Wang et al. (2010) suggested a probable role for xanthine oxidoreductase in the production of NO upon phosphate deficiency in cluster roots of lupine (*Lupinus albus*).

Another enzymatic sources. Wimalasekera et al. (2011) suggested that polyamine oxidases and copper containing amine oxidases might directly or indirectly contribute to NO synthesis. This is in agreement with the studies of Tun et al. (2006), who reported that polyamines spermine and spermidine were able to trigger NO production in plants.

The production of NO and ATP *via* cytochrome *c* oxidase and/or reductase and possibly by alternative oxidase was described at the inner membrane of mitochondria isolated from the barley roots (Stoimenova et al. 2007). NO production by this mechanism occurs below 1% oxygen (Gupta and Igamberdiev 2011, Mur et al. 2013a).

Nonenzymatic production

NO formation under acidic conditions. A nonenzymatic mechanism for the synthesis of NO from NO_2^- under acidic conditions is described by the following reaction schemes, where through a series of reactions, two molecules of HNO_2 interact and give rise to NO and NO_2, and NO_2 can be converted to NO and oxygen (Stöhr and Stremlau 2006, Moreau et al. 2010):

$$2\ NO_2^- + 2\ H^+ \leftrightarrow 2\ HNO_2 \leftrightarrow NO + NO_2 + H_2O \leftrightarrow$$
$$\leftrightarrow 2\ NO + ½\ O_2 + H_2O$$

At acidic pH, an apoplastic non-enzymatic conversion of nitrite to NO occurring in the presence of reductants such as ascorbic acid was described (Bethke et al. 2004). In addition, simultaneous exposure of carotenoids to NO_2 and light resulted in the release of NO into the gas phase (Cooney et al. 1994).

NO production from hydroxylamine and salicylhydroxamate. Rümer et al. (2009) described another form of oxidative NO formation: when hydroxylamine was applied to tobacco cell culture which was deficient in NR, NO was emitted. However, because the natural existence of hydroxylamines in plants was not confirmed, the physiological significance of this pathway remains unclear (Gupta et al. 2011). Similarly salicylhydroxamate, an inhibitor of alternative oxidase, was oxidised to NO (Rümer et al. 2009). The diagram describing both oxidative and reductive pathway of NO production is on Figure 1.

NO scavenging

To avoid cell damage caused by excess of NO, NO production and scavenging must be in the poise. For example, under hypoxic condition, there is a very efficient NAD(P)H- and non-symbiotic hemoglobin-dependent NO-scavenging system (Igamberdiev et al. 2004). In this system, oxygenated ferrous (Fe^{2+}) hemoglobin converts NO to NO_3^- and becomes metamoglobin (Fe^{3+}) form which is then reduced to oxygenated ferrous (Fe^{2+}) by metamoglobine reductase (Hill 2012). The other enzyme through which NO content is reduced is *S*-nitrosoglutathione reductase (GSNOR). *S*-nitrosoglutathione results from the reaction between NO and reduced glutathione. GSNOR can regulate the cellular level of GSNO content *via* the NADH-dependent reduction of GSNO to glutathione disulphide and ammonia (Liu et al. 2001). This way, GSNOR represents a means through which NO content may be regulated as was demonstrated using GSNOR mutants (Feechan et al. 2005, Mur et al. 2013a). Alternative oxidase was also reported to modulate tobacco leaf mitochondria concentration of NO *via* leaking electron flow from the electron

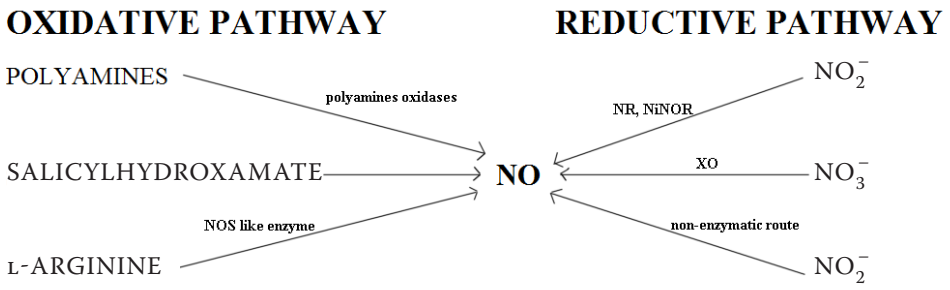

Figure 1. Two pathways for NO production: oxidative, where NO results from the oxidation of polyamines, salicylhydroxamate and arginine, and reductive pathway, where NO is produced by the reduction of nitrate (NO_3^-) or nitrite (NO_2^-). NR – nitrate reductase; NiNOR – nitrite:NO reductase; NOS – nitric oxide synthase

transport chain to terminal electron acceptor oxygen or nitrite in the cytochrome pathway (Cvetkovska and Vanlerberghe 2012, Mur et al. 2013a).

In conclusions, the studies concerning NO production seem to be a great promise for the future. The next studies revealing NOS in plants will be necessary. One of the methods for the clear evidence of NOS occurrence in plants would be to measure the incorporation of radiolabel from radiolabelled L-arginine into L-citrulline as operational evidence of the correct NO synthesizing pathway (Wilson et al. 2008). As NOS is eagerly studied drug target in animal cells (Joubert and Malan 2011), we can expect the same afford in plant biology. Promising approach seems to be also the gene manipulation. For example, increased NO content in *A. thaliana* by expressing rat nNOS improved plant salt and drought tolerance (Shi et al. 2012).

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