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Research article

Oxidative metabolism, ROS and NO under oxygen deprivation

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ABSTRACT

Oxygen deprivation, in line with other stress conditions, is accompanied by reactive oxygen (ROS) and nitrogen species (RNS) formation and is characterised by a set of metabolic changes collectively named as the 'oxidative stress response'. The controversial induction of oxidative metabolism under the lack of oxygen is necessitated by ROS and RNS signaling in the induction of adaptive responses, and inevitably results in oxidative damage. To prevent detrimental effects of oxidative stress, the levels of ROS and NO are tightly controlled on transcriptional, translational and metabolic levels. Hypoxia triggers the induction of genes responsible for ROS and NO handling and utilization (respiratory burst oxidase, non-symbiotic hemoglobins, several cytochromes P450, mitochondrial dehydrogenases, and antioxidant-related transcripts). The level of oxygen in the tissue is also under metabolic control via multiple mechanisms: Regulation of glycolytic and fermentation pathways to manage pyruvate availability for respiration, and adjustment of mitochondrial electron flow through NO and ROS balance. Both adaptive strategies are controlled by energy status and aim to decrease the respiratory capacity and to postpone complete anoxia. Besides local oxygen concentration, ROS and RNS formation is controlled by an array of antioxidants. Hypoxic treatment leads to the upregulation of multiple transcripts associated with ascorbate, glutathione and thioredoxin metabolism. The production of ROS and NO is an integral part of the response to oxygen deprivation which encompasses several levels of metabolic regulation to sustain redox signaling and to prevent oxidative damage.

1. Introduction

Almost two decades of oxidative stress studies in plants under diverse biotic and abiotic stress factors, has yielded a concept of ROS being ubiquitous stress markers and signaling species [12,89,113,182]. Oxygen deprivation stress stands somewhat apart in the universal and quite concise picture of stress responses. The intrinsic contradiction between low oxygen concentration from one hand, and the well documented ROS production under these conditions on the other hand, have promoted studies on the biochemistry underlying ROS formation under oxygen deprivation and during reoxygenation [18,19,21–23,27,57,156,190]. Such biochemical adjustments are closely related to plant survival under hypoxia and inherently connected with the activation of remaining oxygen. The response to oxygen deprivation can differ significantly depending on the surrounding oxygen concentration. Under complete anoxia the direct formation of ROS is abolished by the absence of oxygen, although several anoxia-specific metabolic alterations can promote the oxidative response upon transfer to hypoxia (micro-aerobic conditions) or after re-admission to atmospheric oxygen [19,21,26,27,148]. Among the main processes relevant for the occurrence of oxidative stress under hypoxia are the preservation of energy [68] and the regulation of the internal O_2 concentration via metabolic control of respiration [31,194]. Moreover, during the last years a vast amount of evidence has accumulated on the regulatory role of reactive nitrogen species (RNS) in hypoxic metabolism [85,145]. These metabolic adjustments have a common crosspoint – mitochondrial metabolism affected by the lack of oxygen and by hypoxic metabolites [13,15,86].

Multiple routes for hypoxic ATP production and consumption have been elucidated recently: Pyrophosphate-dependent glycolysis [80], anaerobic nitrite-dependent ATP synthesis [167], and a reverse reaction for ATP synthesis – anaerobic ATP hydrolysis in mitochondria [169]. The abovementioned NO can exert a dual effect on cell energetics, depending on the target and localization: Inhibition of key mitochondrial enzymes cytochrome oxidase,

Abbreviations: AA, ascorbic acid; AA–GSH cycle, ascorbate–glutathione cycle; AAO, ascorbic acid oxidase; ADH, alcohol dehydrogenase; AOX, alternative oxidase; COX, cytochrome oxidase; DHA, dehydroascorbic acid; ETC, mitochondrial electron transport chain; FFA, free fatty acids; LP, lipid peroxidation; NO, nitric oxide; $^1O_2$, singlet oxygen; $O_2^{-\bullet}$, superoxide anion; $ONOO^-$, peroxynitrite; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; UCP, mitochondrial uncoupling protein.

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aconitase and upregulation of alternative oxidase, sustaining of NADH turnover via hemoglobin-dependent reaction cascade, and termination of the lipid peroxidation cascade [25,51,85,128,188].

Two hypotheses on the regulation of internal O_2 concentration in the cells through the control of respiration rely on regulation of the glycolytic pathway and pyruvate availability [59,194] and on the regulation of mitochondrial ETC by NO and ROS balance [31]. Both adaptive strategies aim to decrease the respiratory capacity and to postpone complete anoxia. They also emphasize the key role of adenylate energy charge of the tissue [13]. These routes are inherently connected with mitochondria and hypoxic oxidative stress. However, several non-mitochondrial enzyme reactions are capable of producing ROS (and RNS) in a stress-dependent manner [21]. Ascorbic acid oxidase (AAO) may have a role in controlling local O_2 concentration without production of ROS and in the creation of steep O_2 gradients [45]. The phenomenon of oxidative stress under hypoxia has found its affirmation on the metabolic level: ROS production, ethane emission and lipid peroxidation, acetaldehyde formation via non-fermentative route and activation of antioxidant systems [18,19,21,26,27,156]. The occurrence of an oxidative component in response to oxygen deprivation has been also confirmed by microarray studies on the whole genome level. Indeed, expression of a wide range of ROS- and redox-related transcripts is induced by the lack of oxygen [34,35,95,109,183].

2. ROS and NO under oxygen deprivation stress

2.1. Chemistry and sources of ROS and NO

Due to the paramount significance of ROS and NO in physiology, pathology and signaling, issues on ROS and NO formation, chemistry of their interconversion and utilization have been studied intensively and are covered in several reviews [20,71,72,115, 144,164]. In the process of ROS formation energy is required only for the first step, i.e. one-electron reduction of molecular oxygen to yield superoxide $O_2^{-\bullet}$. All subsequent steps can proceed spontaneously, provided appropriate electron donors and acceptors are present. The low energy cost of ROS formation, redox active cellular environment and ubiquitous distribution of O_2 prior to activation events, make ROS a universal species in signaling cascades [89] and in destructive processes. Oxygen tends to accumulate in the hydrophobic membrane environment due to the favourable partitioning between water/lipid phase [50], increasing the probability of membrane lipid peroxidation. Charged superoxide, trapped within the cellular compartment where it has been formed, is further processed by specific superoxide dismutase (SOD) isoforms to H_2O_2 [32,146]. Restricted intermembrane mobility and short lifetime of radical species favour the reaction with the local oxidation targets and might bring about the specificity of the ROS signal. Non-radical nature of H_2O_2, its ability to cross biological membranes and longer lifetime and chemical reactivity make H_2O_2 a good candidate for intercompartmental signaling. The hydroxyl radical $OH^{\bullet}$, the most reactive oxidant in the ROS family, is formed in the Fenton reaction catalyzed by transition metal ions and is considered as one of initiation radicals for lipid peroxidation [134,144].

Changing the spin of an electron on the outer electron sheath of triplet state oxygen leads to the formation of singlet oxygen ($^1O_2$). In biological systems $^1O_2$ is produced under UV stress or in chloroplasts due to photosensitisation of chlorophyll molecules [178].

In biological systems both H_2O_2 and superoxide can be formed in a number of enzymatic reactions (by lipoxygenases, peroxidases, plasma membrane NADPH oxidase, xanthine oxidase and amine oxidase), during photorespiration, in the Mehler reaction in chloroplasts and via iron catalyzed Haber–Weiss and Fenton reactions [20,21,28,29,144]. Under oxygen deprivation several enzymes have been shown to produce ROS and, in some cases, this ability was potentiated by the accumulation of anoxic metabolites and/or cytoplasmic acidification. Xanthine oxidoreductase can use acetaldehyde accumulating under hypoxia (fermentation product) and hypoxanthine (ATP catabolism product) as an electron donor to perform primary activation of O_2 and, hence, produce $O_2^{-\bullet}$ and H_2O_2 [62,73].

The chemical properties of nitric oxide, NO, make this gas a good candidate for a signaling molecule. NO can freely penetrate the lipid bilayer and, hence, can be transported within the cell. NO is quickly produced on demand via inducible enzymatic or non-enzymatic routes. Due to its free radical nature (one unpaired electron) NO has a short half-life (in order of seconds), and can be removed easily when no longer needed [16,97,145,165]. NO can also react either with O_2 or $O_2^{-\bullet}$. The end products, NO_2, N_2O_2, and peroxynitrite $ONOO^-$ all have deleterious consequences and signaling roles in biological systems [188].

Metabolic alterations brought about by oxygen deprivation provide a possibility for increased NO levels in hypoxic tissues. At low oxygen tensions NO-generating activity of xanthine oxidoreductase is enhanced. Interestingly, under normoxic conditions xanthine oxidoreductase is capable of both NO and $O_2^{-\bullet}$ formation with consequent production of $ONOO^-$ [62]. It has been shown that under oxygen deprivation the accumulated NO_2^- can be converted to NO by several hypoxically induced enzymes, namely by cytosolic nitrate reductase (can use nitrite as a substrate and convert it to NO, induced several fold under hypoxia) [49,150,189], and by plasma membrane-bound nitrite–NO reductase (acidic conditions under anoxia are favourable for this enzyme with optimum at pH 6.1 while hemoglobin (Hb) can act as a physiological e^- donor for this reaction) [166]. Indeed, accumulation of NO has been shown in hypoxic alfalfa root cultures and maize cell cultures [48]. Anoxic mitochondria are capable of nitrite reduction to NO, in a process specific for root mitochondria and elevated under the lack of O_2 [69,140].

The direct effects of NO on biological targets include the reduction of free metal ions or oxidation of metals in protein complexes such as hemoglobin, and Fe-nitrosyl formation resulting in the activation of guanylate cyclase and hemoxygenase, inhibition of P450, cytochrome *c* oxidase, aconitase and catalase, and down-regulation of ferritin [188]. NO exerts an inhibitory effect on aconitase, a TCA cycle enzyme which catalyses the reversible conversion of citrate to isocitrate. A cytoplasmic isoform of aconitase is also affected: NO promotes the release of iron–sulphur cluster from the active center of the enzyme [128]. The possible release of ferrous ions from this cluster, e.g. upon cytoplasmic acidification, can enhance ROS production. NO intervention with TCA cycle enzyme would also have an impact on the energy status of the cell, already affected by cytochrome oxidase (COX) inhibition. The NO inhibitory effect on COX in potato tuber mitochondria has been shown recently [42]. Therefore, not only the TCA cycle enzymes but also ETC are targets for NO regulation under oxygen deprivation stress.

On the other hand, reactions of NO with hemoglobin allow the maintenance of NAD^+ levels for the needs of glycolysis under hypoxic conditions [82,84]. Overexpression of hypoxically induced class 1 hemoglobin in maize cell cultures has resulted in the maintenance of the energy status, and these cells showed less induction of ADH and were able to maintain the redox status of the cells. Transformed alfalfa roots overexpressing hemoglobin have lower levels of NO [48]. Hence, the regulation of NO level under oxygen deprivation can be achieved in plants via interaction with class 1 non-symbiotic hemoglobins through several routes. In