Possible role of free oxidation processes in the regulation of reactive oxygen species production in plant mitochondria

V.N. Popov

Department of Plant Physiology and Biochemistry, Voronezh State University, Voronezh 394693, Russia

Abstract

The non-coupled substrate oxidation mediated by components of the electron transport chain that are not coupled to energy accumulation (such as plant alternative oxidase and rotenone-insensitive NADH dehydrogenases) and uncoupled respiration are peculiar features of plant mitochondria. The physiological significance of such energy-wasting oxidation processes is still debated. It is proposed that non-coupled oxidation could regulate the level of reduction of components of the electron transport chain and the rate of one-electron reduction of oxygen, thereby affecting the rate of formation of reactive oxygen species.

With the development of photosynthesis by the ancestors of the present cyanobacteria some 3.5 billion years ago, molecular oxygen appeared in the atmosphere, so that aerobic respiration became possible. However, molecular oxygen is an active and fairly strong oxidant, and can permeate into most (micro)environments. Whereas four electrons are required for complete reduction of an oxygen molecule to water, the components of the electron transfer chain include many one-electron carriers. Some of these carriers react with molecular oxygen to produce the superoxide anion; other ROS (reactive oxygen species) such as hydrogen peroxide and hydroxyl radicals can be derived from superoxide, again without the involvement of enzymes [1].

The occurrence of photosynthesis in plant cells promotes ROS formation under conditions of high illumination, due to the high levels of reduction of components of the photosynthetic electron transport chain in chloroplasts, the evolution of molecular oxygen, and increases in membrane potentials and in NADH/NAD\(^+\) and ATP/ADP ratios. Plant mitochondria are considered to be an important source of superoxide and peroxide when electron transport through the cytochrome part of the respiratory chain is restricted due to stress-induced changes in the membrane components [2,3]. Under such conditions, both the high level of reduction of respiratory chain components before the cytochromes and the increased oxygen level in the cell due to the lower respiratory rate will enhance the production of superoxide and H\(_2\)O\(_2\). A non-linear relationship between the changes in membrane potential across the mitochondrial membrane, the level of reduction of cytochrome b\(_{556}\) and the rates of superoxide and H\(_2\)O\(_2\) generation was clearly demonstrated in experiments with rat heart mitochondria [1].

A similar relationship has been obtained with cauliflower mitochondria (V.N. Popov, unpublished work).

The plant cell has developed detoxification systems to cope with free radicals. Enzymic detoxification by superoxide dismutases, ascorbate peroxidase, monoascorbate or dehydroascorbate reductases, glutathione peroxidase, glutathione reductase, catalase and ‘guaiacol’ peroxidase, either individually or co-operatively, removes ROS. In the normally growing plant, where there is relatively little production of ROS, the enzymic and antioxidant detoxification capacity is sufficient to keep the amount of ROS under control. Several stress conditions, however, such as wounding or other mechanical influences (radiation, drought, flooding, osmotic and temperature effects) disturb the balance between pro- and anti-oxidative potentials [2]. An important increase in the production of ROS is caused by conditions that result in the inhibition of electron transport chains near their termini [3,4].

One of the many mechanisms that mitochondria are proposed to use to decrease ROS generation is uncoupling between mitochondrial respiration and membrane potential, resulting in increased respiratory rates (Figure 1) (for review, see [5]). Protonophores are well known to decrease mitochondrial ROS generation [1], and mitochondrial uncoupling proteins, discovered in various plant tissues [6], have been demonstrated to exert the same effect. Based on these observations, Skulachev [1] formulated the hypothesis that mild uncoupling of mitochondria may be an effective mechanism to decrease mitochondrial ROS generation without seriously compromising cellular energetics. A decrease in mitochondrial membrane potential due to uncoupling induced by non-esterified fatty acids through PUMP (plant uncoupling mitochondrial protein) activity inhibits the mitochondrial generation of ROS at the level of the semiquinone forms of CoQ (coenzyme Q) in potato tuber mitochondria [6]. It is important to note that accumulation of superoxide radical stimulates linoleic acid-induced proton...
Figure 1 | Interrelationships between ROS and pathways of non-coupled and uncoupled respiration in plant mitochondria

The bold arrows show pathways, participating in limitation of ROS formation [1,6,8,14]. ? indicates that the function is only hypothesized [1]. NADH DH, rotenone-insensitive NADH dehydrogenases; AO, alternative oxidase; bc, and aa3, complexes III and IV respectively of the electron transport chain, ΔΨ, membrane potential.

leak in potato mitochondria that is related to the activity of uncoupling protein [7]. An ATP/ADP antiporter could also result in a decrease in the mitochondrial membrane potential in the presence of non-esterified fatty acids in potato and pea mitochondria.

Another possible mechanism of ROS regulation in plant mitochondria is the so-called alternative oxidase (Figure 1). This cyanide-resistant oxidase is localized in the inner membrane of plant mitochondria. It catalyses the four-electron reduction of O2 by two ubiquinols with no conservation of energy, in spite of the fact that in the same membrane there are CoQH2–cytochrome c reductase and cytochrome c oxidase, which catalyse this reaction in an energy-coupled fashion. Although the alternative oxidase is probably the most studied system of non-coupled respiration in plant mitochondria, the functional significance of this pathway remains unclear [3,5]. It is not likely to be thermoregulation, which in plants is inherent only in the flowers of a few species [3,5].

In 1993, Purvis and Shewfelt [8] hypothesized that the alternative oxidase acts in anti-oxygen defence by transporting to oxygen the excess of reducing equivalents. In accordance with this proposal, the alternative oxidase was shown to be operative at much higher CoQH2/CoQ ratios than the energy-coupled CoQH2–cytochrome c reductase [3].

Independently, it was suggested [1] that the alternative oxidase may be involved in the anti-oxygen defences of plant mitochondria by decreasing the concentrations of O2 and its one-electron reductants in these organelles. It was proposed that the alternative oxidase, in co-operation with the non-coupled NAD(P)H–CoQ reductases also found in plant mitochondria, form a respiratory chain that bypasses all three energy-coupling sites of the main respiratory chain, and hence can perform a defensive function without being restricted by ADP availability. It is also notable that the non-coupled chain does not include the superoxide-producing components that become long-lived in state 4, such as CoQH2* [1].

In 1995 and 1996, Wagner [9] and Vanlerberghe and McIntosh [10] demonstrated induction of the alternative oxidase by H2O2. This explains findings that such induction in plant [11] and yeast [12] cells can also be achieved by adding antimycin A, which is known to markedly increase H2O2 production by mitochondria. In the same way, i.e. by accumulation of H2O2, salicylate [13], an inhibitor of catalase, could induce the alternative oxidase. The hypothesis that a non-coupled alternative oxidase in plant mitochondria operates as an anti-oxygen defence mechanism [1,3,8] has been confirmed in our experiments on isolated soybean and pea cotyledon mitochondria [14]. It was shown that salicyl hydroxamate and propyl gallate, inhibitors of the alternative oxidase, strongly stimulated H2O2 production by these mitochondria oxidizing succinate. The effective concentrations of these inhibitors proved to be the same as those that decreased the cyanide-resistant respiration. The inhibitors proved to be ineffective in stimulating H2O2 formation in rat liver mitochondria, which lack the alternative oxidase. Using EPR analysis, it was shown that salicyl hydroxamate is an effective inductor of superoxide production by plant mitochondria [15].

I thank the CRDF (Civilization Research and Development Foundation) for support (grant N V2-010-02, Annex 03).

References


Received 24 June 2003