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$NAD(P)^+$ decomposition and antioxidant defense of the cell

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1. Introduction

Reactive oxygen species (ROS) are known to induce opening of some permeability transition pores (PTP) in the inner mitochondrial membrane, whereby the membrane then becomes permeable for low molecular mass compounds including $NAD(P)^+$. When released from mitochondria, $NAD(P)^+$ is decomposed by the ROS-activated NADase localized to the outer mitochondrial membrane (see the accompanying article by F. Di Lisa and M. Ziegler, this issue). Among the $NAD(P)^+$ decomposition products, cyclic ADP ribose and nicotinic acid adenine dinucleotide phosphate are formed. These compounds induce an increase in cytosolic $[Ca^{2+}]$ which promotes further PTP opening. Thus, the above events represent a self-activating process resulting in exhaustion of the $NAD(P)^+$ pool. Here, I review evidence suggesting that such an effect (in cooperation with ROS-dependent inactivation of aconitase) represents a line of antioxidant defense preventing respiratory substrate oxidation and, hence, reduction of the respiratory chain electron carriers competent in the $O_2^{\bullet-}$ formation. This antioxidant mechanism is likely actuated when less radical measures fail to prevent an increase in the ROS level.

In the eukaryotic cell, the main portion of O_2 is known to be consumed by mitochondria. The major product of O_2 reduction is H_2O. However, some O_2 is reduced to $O_2^{\bullet-}$. The $O_2^{\bullet-}$ formation is strongly activated if the respiratory chain is inhibited by antimycin A or if succinate is oxidized by complex I carrying out reverse electron transfer at the expense of the respiratory or ATP energy. Smaller but measurable amounts of $O_2^{\bullet-}$ are produced by complex III in the resting state [1–4].

2. Mitochondria and protection against ROS

To avoid intoxication by $O_2^{\bullet-}$ and products of $O_2^{\bullet-}$ conversions (reactive oxygen species, ROS), mitochondria are equipped with a multilevel defense system preventing ROS formation and removing the ROS formed [3,4].

The list of mitochondrial anti-ROS mechanisms includes:

1. Very active *cytochrome oxidase*. It operates at a high rate even at low $[O_2]$, hence it can effectively decrease $[O_2]$. This may result in maintaining a safely low oxygen level that is not sufficient to obtain a high rate of $O_2^{\bullet-}$ formation by respiratory chain enzymes [3,4]. In tissues such as skeletal muscle, mitochondrial clusters are localized just below the plasmalemma and form proton potentials that may be transmitted along mitochondrial filaments to the cell core to be utilized for ATP synthesis [5].
2. Mechanisms of *mild uncoupling* by free fatty acids, mediated by mitochondrial anion carriers such as the ATP/ADP antiporter (ANT), the aspartate/glutamate antiporter, uncoupling proteins, etc. Mild uncoupling keeps the mitochondrial proton potential below a threshold level required to stimulate $O_2^{\bullet-}$ production by complexes I and III [2–4,6].
3. *Cytochrome c* catalyzing oxidation of $O_2^{\bullet-}$ back to O_2 if the cytochrome is desorbed from the outer surface of the inner mitochondrial membrane into the intermembrane space [2,4].
4. Mitochondrial *superoxide dismutase*, localized in the matrix, converts $O_2^{\bullet-}$ to H_2O_2, which can escape from mitochondria because it is much more permeant than $O_2^{\bullet-}$.
5. Mitochondrial *glutathione peroxidase* and *catalase*, which decompose H_2O_2 in the matrix.
6. *Tocopherol, $CoQH_2$, ascorbate* and some other low molecular mass antioxidants that directly quench ROS (for review, see [3]).

It should be stressed that all the above mechanisms do not interfere with performance of the main mitochondrial function, i.e. phosphorylating respiration.

More radical measures can be applied if the ROS concentration in mitochondria increases in spite of the operation of these lines of defense. The ANT contains some amino acid residue(s) that are easily oxidized by ROS [7,8]. Such oxidation modifies the ANT from a nucleotide antiporter to a nonspecific channel (permeability transition pore, PTP) in the inner mitochondrial membrane that becomes permeable to substances of molecular mass < 1.5 kDa. As a result, osmotic disbalance across the inner mitochondrial membrane arises causing swelling of the matrix. This, in turn, causes disruption of the outer mitochondrial membrane, since its area is much smaller than the area of the inner membrane. This is why cytochrome *c* is released from the intermembrane space into the cytosol, an event actuating more lines of antioxidant defense: (i) extramitochondrial cytochrome *c* scans the cytosol searching for superoxide and reoxidizing it to O_2 and (ii) this cytochrome *c* oxidizes cytochrome b_5 localized on the outer surface of the outer mitochondrial membrane and endoplasmic reticulum in liver, kidney and some other tissues. The system composed of NADH–cytochrome b_5 reductase and cytochrome b_5 avoids the initial and middle complexes of the main respiratory chain where $O_2^{\bullet-}$ can be formed (reviewed in [4]).

Keywords: NAD; NADase; Reactive oxygen species; Mitochondrion; Ca^{2+}; Permeability transition pore

Abbreviations: ANT, ATP/ADP antiporter; cADPR, cyclic ADP ribose; NAADP, nicotinic acid adenine dinucleotide phosphate; PTP, permeability transition pore; ROS, reactive oxygen species

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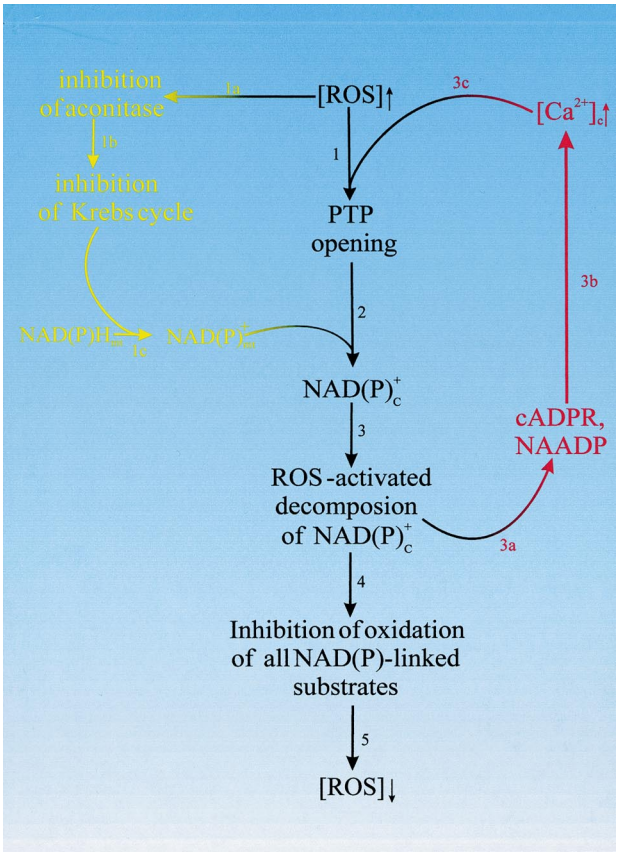

Fig. 1. PTP opening followed by $NAD(P)^+$ decomposition as a mechanism preventing ROS formation. 1: ROS-induced PTP opening; 1a: ROS-induced aconitase inactivation resulting in inhibition of the Krebs cycle (1b) and, hence, oxidation of mitochondrial NAD(P)H (1c); 2: release of $NAD(P)^+$ from mitochondrial matrix; 3: decomposition of released $NAD(P)^+$ by ROS-activated NADase of the outer mitochondrial membrane; 3a: among the $NAD(P)^+$ decomposition products, cADPR and NAADP are formed; 3b: the cADPR- and NAADP-induced increase in the cytosolic Ca^{2+} level; 3c: more PTPs are opened due to the cytosolic $[Ca^{2+}]$ increase; 4: inhibition of substrate oxidation due to $[NAD(P)^+]$ lowering; 5: cessation of mitochondrial ROS formation due to complete oxidation of the respiratory chain, which in turn results in a [ROS] decrease. mt, mitochondrial; c, cytosolic.

The previously described respiratory chain mechanisms activate electron transfer or divert it to another pathway. An alternative possibility, as suggested by Gardner and Fridovich [9] and Kim Lewis (personal communication), consists of ceasing the electron supply to the respiratory chain (resulting in complete oxidation of the chain) making the reduction of O_2 to $O_2^{\bullet-}$ in mitochondria impossible. According to Gardner and Fridovich, such an effect can be achieved by means of the $O_2^{\bullet-}$-induced inactivation of aconitase, an enzyme initiating the Krebs cycle. Aconitase is very sensitive to $O_2^{\bullet-}$, which causes oxidation and release of one of the Fe^{2+} ions present in the aconitase 4Fe–4S cluster [9]. Inhibition of the Krebs cycle must entail exhaustion of its intermediates serving as respiratory substrates and, hence, oxidation of the respiratory chain components. One more consequence of the aconitase inhibition should be accumulation of citrate which forms an autoxidizable complex with Fe^{2+}. As a result, Fe^{2+} is oxidized to Fe^{3+}, an effect preventing the Fe^{2+}-dependent conversion of H_2O_2 to $OH^{\bullet}$, the most aggressive ROS. The Fe^{3+} obtained remains bound to citrate^{3-} since its binding to citrate^{3-} is much stronger than that of Fe^{2+} [3].

3. $NAD(P)^+$ decomposition and antioxidant defense

In the review by Di Lisa and Ziegler in this issue [10], the authors summarize data that seem to point to the existence of an additional line of mitochondrial antioxidant defense. It has been determined that a ROS-activated NADase (NAD^+ glycohydrolase) [11] is localized in the outer mitochondrial membrane, which catalyzes the decomposition of NAD^+ and $NADP^+$ released from the mitochondrial matrix via PTP. Among the reaction products, cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) are obtained from NAD^+ and $NADP^+$, respectively. cADPR triggers Ca^{2+} release from the endoplasmic reticulum whereas NAADP releases Ca^{2+} from some other sources [12]. It is important in this context that (i) NADH and NADPH cannot, in contrast to NAD^+ and $NADP^+$, be decomposed in the above described fashion [11] and (ii) an increase in the cytosolic Ca^{2+} level potentiates the PTP opening (reviewed in [7,13]). The scheme in Fig. 1 summarizes these observations.

It should be stressed that exhaustion of the cellular $NAD(P)^+$ pool must stop ROS formation in mitochondria independently of the mechanism of its formation (energized complexes I and III or antimycin A-inhibited complex III). Moreover, it stops generation of ROS by the endoplasmic reticulum in tissues possessing cytochrome P450 (which can form $O_2^{\bullet-}$ in a NAD(P)H-dependent fashion [14]), as well as by some extramitochondrial NAD(P)H oxidases present in the plasma membrane, endoplasmic reticulum, or cytosol of certain types of cells (for reviews, see [15,16]).

The events described in Fig. 1 are reversible and the situation can be normalized when [ROS] decreases and PTP is closed. However, if this is not the case and the ROS level appears to be permanently high, the mitochondrion commits suicide [6,17]. In fact, a mitochondrion with open PTP will perish just like a ship with open Kingstons. Several processes that are of vital importance for the mitochondrion require ion and electrical gradients across the inner membrane to be maintained. In particular, collapse of the mitochondrial membrane potential makes the electrophoretic import of precursors of mitochondrial proteins impossible, as well as preventing their proper arrangement in the inner membrane. Thus, repair of the PTP-bearing mitochondrion ceases.

I coined the word *mitoptosis* for mitochondrial suicide by analogy with apoptosis [6]. This analogy was quite recently supported by the observation that mitoptosis is accompanied by decomposition of mitochondrial nucleic acids just as apoptosis results in splitting of nuclear DNA. It was found that addition of H_2O_2 to HA-1 cells causes degradation of mitochondrial DNA and RNA in 8–20 min whereas nuclear DNA and cytosolic RNAs remain unaffected 5 h after the H_2O_2 treatment [18].

In fact, ROS-induced mitoptosis may be regarded as a process that purifies the intracellular population of mitochondria from those that have become dangerous for the cell because their ROS production exceeds their ROS-scavenging capacity.

Massive ROS-linked mitoptosis increases cytosolic concentrations of proapoptotic proteins hidden in the intermembrane spaces such as cytochrome *c*, apoptosis-inducing factor, second mitochondrial apoptosis-activating protein (Smac; anoth-

er name, DIABLO), and some procaspases (reviewed in [19]). As a result, ROS-overproducing cells commit suicide and a tissue is purified of such cells [3].

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