Mutation Research 773 (2017) 274–281

Contents lists available at ScienceDirect

Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Mini-review

Potentiation of hydrogen peroxide toxicity: From catalase inhibition to stable DNA-iron complexes

Tulip Mahaseth, Andrei Kuzminov*

Department of Microbiology, University of Illinois at Urbana-Champaign, USA

ARTICLE INFO

Article history:
Received 22 April 2016
Accepted 29 August 2016
Available online 30 August 2016

Keywords:
Hydrogen peroxide
Fenton's reaction
Iron metabolism
Nitric oxide
Cyanide
Catastrophic chromosomal fragmentation

ABSTRACT

Hydrogen peroxide (H_2O_2) is unique among general toxins, because it is stable in abiotic environments at ambient temperature and neutral pH, yet rapidly kills any type of cells by producing highly-reactive hydroxyl radicals. This life-specific reactivity follows the distribution of soluble iron, Fe(II) (which combines with H_2O_2 to form the famous Fenton's reagent),—Fe(II) is concentrated inside cells, but is virtually absent outside them. Because of the immediate danger of H_2O_2, all cells have powerful H_2O_2 scavengers, the equally famous catalases, which enable cells to survive thousand-fold higher concentrations of H_2O_2 and, in combination with adequate movement of H_2O_2 across membranes, make the killing H_2O_2 concentrations virtually impractical to generate in vivo. And yet, low concentrations of H_2O_2 are somehow used as an efficient biological weapon. Here we review several examples of how cells potentiate H_2O_2 toxicity with other chemicals. At first, these potentiators were thought to simply inhibit catalases, but recent findings with cyanide suggest that potentiators mostly promote the other side of Fenton's reaction, recruiting iron from cell depots into stable DNA-iron complexes that, in the presence of elevated H_2O_2, efficiently break duplex DNA, pulverizing the chromosome. This multifaceted potentiation of H_2O_2 toxicity results in robust and efficient killing.

1. Life-specific reactivity of H_2O_2

Hydrogen peroxide, H_2O_2, is a metastable oxygen species and an important intermediate in the redox pathway linking molecular oxygen, O_2, to the fully reduced oxide in water, H_2O. The complete pathway [1], $O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow (2)OH^\bullet \rightarrow (2)H_2O$, also includes a relatively unstable superoxide (O_2^-), as well as the extremely reactive hydroxyl radical (OH·), which is the species responsible for the "reactivity" of all reactive oxygen species [2]. Paradoxically, hydrogen peroxide is generally non-reactive with organic compounds [3–5]; in everyday life, 3% (~0.8 M) hydrogen peroxide solution is a common household antiseptic, stored in plastic bottles for years. H_2O_2 does not directly interact with pure biopolymers (nucleic acids, demetallated proteins, polysaccharides, lipids); perhaps, the only exceptions are thiol-based sensor proteins (like OxyR in bacteria), reacting to the presence of micromolar levels of H_2O_2 with formation of disulfide bonds that are used for signaling [6]. At the same time, hydrogen peroxide is a surprisingly potent bio-toxin, as the same 3% H_2O_2 solution kills all kinds of cells within several minutes [7,8], that is, as soon as H_2O_2 penetrates the cell wall barrier.

The main reason H_2O_2 is generally stable in abiotic environment, yet becomes so reactive upon contact with life, is the differential availability of soluble iron. Since H_2O_2 by itself does not react with organic compounds (with the above exception of select thiol groups in a few signaling proteins), it would be completely innocuous, if not for the fact that iron is broadly employed by all types of cells in catalysis of many essential metabolic reactions and transitions [9]. Iron can be found in two forms: the soluble Fe(II) and the practically insoluble Fe(III) [10]. In the current oxidative atmosphere of the Earth, at least in the oxic environments, the trace amounts of soluble Fe(II) iron are rapidly oxidized by usually more abundant H_2O_2 to Fe(III) iron [11], which does not react with the remaining H_2O_2, explaining the general H_2O_2 stability in abiotic environment. But exactly due to this reason, the only soluble iron in the environment outside cells is represented by the (highly variable) trace amounts of Fe(III) complexed with natural organic ligands [12–14]. The limited and unpredictable availability of Fe(III) iron forces cells of all types to actively procure and stockpile iron to maintain their metabolism and support multiplication [15], accumulating 0.1–1.0 mM total iron [10,16–18]. But even inside

* Corresponding author at: B103 C&LSL, 601 South Goodwin Ave., Urbana, IL 61801-3709, USA.
E-mail address: kuzminov@life.illinois.edu (A. Kuzminov).

http://dx.doi.org/10.1016/j.mrrev.2016.08.006
1383-5742/

the cell, the availability of free Fe(II) iron is limited and tightly controlled [15], because when free Fe(II) and H_2O_2 meet, Fenton's reaction occurs.

In this famous reaction [19–21] (Fig. 1), soluble Fe(II) iron donates one electron to a hydrogen peroxide molecule, causing its decomposition: $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\bullet} + OH-$ that produces hydroxyl radical capable of reacting with any organic compound at diffusion rates [5,22]. Hydroxyl radicals kill via DNA damage, as indicated by the exquisite H_2O_2 sensitivity of DNA repair mutants [23–25]. Fenton's reaction is the reason why otherwise relatively innocuous extracellular H_2O_2 becomes a potent poison once inside the cell. At the same time, due to its small size and lack of charge, H_2O_2 shows substantial permeability through the membrane barrier [26,27], so exposed cells cannot simply block the entry of this "conditional" poison. This life-specific reactivity in combination with substantial membrane permeability makes hydrogen peroxide a popular weapon in bio-warfare: our immune cells use it to kill invading microbes [28–30], bees use it as a honey preservative [31], lactic acid bacteria generate it to kill off the competition [32] (and are one of the few life forms that learned to do without iron in their metabolism [33]), ants (if given a choice) take it as an anti-fungal medication [34], while plants employ hydrogen peroxide to reduce grazing by herbivores [35].

2. H_2O_2 is impossible to concentrate *in vivo*

However, this strategy of production of a diffusible life-specific source of hydroxyl radicals suffers from the problems of targeting and is also undermined by efficient detoxification. Because superoxide and hydrogen peroxide in sub-micromolar concentrations are byproducts of aerobic metabolism [36], and because both H_2O_2 and O_2^- are commonly employed in pathogen-defense mechanisms, the cells are equipped with powerful catalases [37] (Fig. 1) and superoxide dismutases [38]. Both are rare examples of diffusion-limited enzymes—the fastest enzymes possible [39]—capable of scavenging up to low millimolar concentrations of hydrogen peroxide during acute exposures without adverse consequences for the cell, once H_2O_2 is removed. Even though 3 mM concentrations of H_2O_2 will eventually kill during prolonged exposures [40], the H_2O_2 concentrations that kill cells within minutes start around 30 mM [24,41]. This creates a classic engineering problem: besides the obvious caveat that such high H_2O_2 concentrations will be dangerous to the producing cell itself, no known cells are actually capable of producing a short burst of 30 mM H_2O_2, or of maintaining a several-hour 3 mM levels of H_2O_2 (perhaps with the exception of lactobacilli [42]).

In fact, for our leucocytes, the real problem appears several orders of magnitude greater. Due to the abovementioned substantial H_2O_2 permeability, it is almost impossible to achieve a significantly higher concentration of H_2O_2 in any cellular compartment relative to the rest of the cytoplasm, especially if its production is slow and indirect, which is thought to be the case in the phagosome [43,44]. That is, if a leucocyte targets superoxide production (that generates H_2O_2 by dismutation) exclusively to the phagosome around a captured bacterium, the continuous escape of H_2O_2 from the phagosome will keep the maximal H_2O_2 concentration ~3 μM [43,45,46], about 1000 times lower than that required for slow killing, not to mention the fast killing. So, how do the cells solve this problem of creating lethal local concentrations of a readily-diffusible toxin?

3. Potentiated toxicity of H_2O_2

Theoretically, this engineering problem has an elegant chemical solution. Indeed, the cellular systems that negate H_2O_2 effectiveness (for example, H_2O_2 scavengers or DNA repair pathways) could be inactivated with a different agent, to either increase the effective intracellular H_2O_2 concentrations or to make DNA damage irreparable. In particular, catalases have heme in their active centers [37], so any simple chemical that binds heme iron tightly (NO, CN, H_2S [47,48]) will inhibit catalases and thus will reduce the killing concentrations of H_2O_2. In fact, catalases *could have been* the original target of the evolutionary arms race, as the cells have a second hydrogen peroxide scavenging enzyme, called

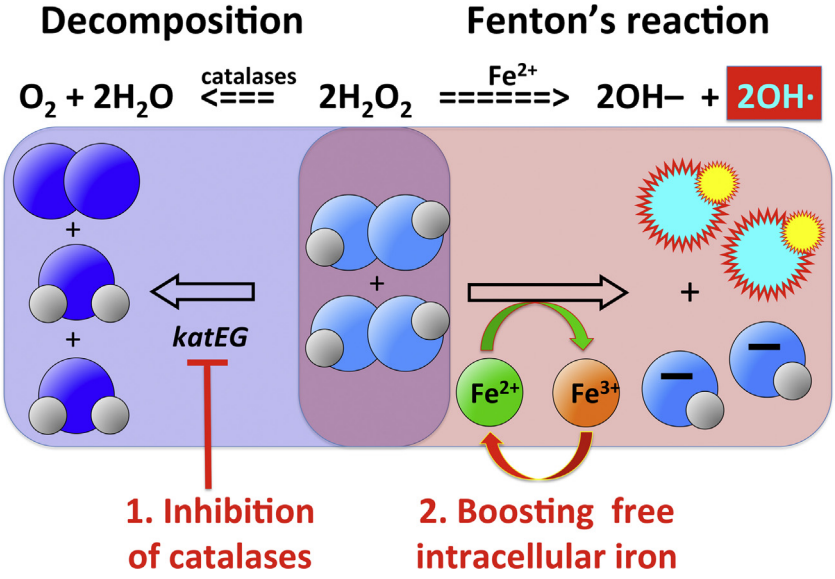

Fig. 1. Hydrogen peroxide scavenging by catalases versus "radicalization" by soluble iron (Fe(II)), and the two obvious strategies to potentiate H_2O_2 toxicity. Hydrogen atoms are small gray spheres. Oxygen atoms are spheres of various shades of blue: the darker the blue, the more stable the oxygen atom. At the top, in black font, the two opposite in vivo fates of hydrogen peroxide are shown as formulas. At the bottom, in red font and symbols, the corresponding potentiation strategies are indicated: 1) inhibition of H_2O_2 decomposition (or DNA repair); 2) increasing the concentration of soluble intracellular iron.