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Smart antioxidant molecular hydrogen: a new paradigm for mitigating oxidative stress in mitochondrial redox biology

Yusuke Ichikawa*, Bunpei Sato, Yoshiyasu Takefuji, Fumitake Satoh

Mitochondria play a central role in cellular energy production through tightly regulated redox reactions coupled with electron transport. However, they are also a major source of reactive oxygen species (ROS), and dysregulation of this process leads to oxidative damage associated with aging and a wide range of diseases.

Molecular hydrogen (H_2) selectively reduces highly toxic hydroxyl radicals (·OH) without interfering with mitochondrial function or donating electrons under physiological conditions. Given its largely unreactive nature within biological systems, excessive intake of hydrogen is unlikely to cause harm. Owing to these properties, hydrogen should be regarded as a "smart antioxidant" that fundamentally differs from conventional antioxidants.

Both basic research and clinical reports on the antioxidant effects of hydrogen have rapidly increased in recent years. However, most of these studies have focused primarily on the efficacy and safety of hydrogen itself, while few have provided a systematic comparison with conventional antioxidants, such as vitamin C and vitamin E.

In this perspective, we discuss the antioxidant properties of H_2 in the context of mitochondrial redox biology and compare its potential effects with those of conventional antioxidants. By reviewing existing literature, we aim to clarify the specificity of hydrogen toward hydroxyl radicals and highlight considerations for its potential use as a selective antioxidant.

Electrons—a foundation of mitochondrial function: Mitochondria, often referred to as the "powerhouses of the cell," depend on electrons for their electron transport and redox systems.[1] These organelles generate significant quantities of adenosine triphosphate (ATP), the essential energy currency for cellular activities, through electron movement, which is indispensable for this process.[1]

The electron transport chain (ETC) is composed of four main enzyme complexes (I–IV) embedded in the inner mitochondrial membrane and cofactors such as coenzyme Q and cytochrome c.[2] Electrons derived from the reduced form of nicotinamide adenine dinucleotide and flavin adenine dinucleotide are sequentially transferred from complex I or II to coenzyme Q to complex III to cytochrome c, and finally to complex IV (**Additional Figure 1**).[2] This electron flow pumps protons (H^+) across the inner membrane, thereby generating a membrane potential that drives ATP synthase.[2] Ultimately, electrons reduce oxygen to form water. Without this electron movement, ATP cannot be synthesized, leading to cellular energy deficiency and death.

Oxidative stress in mitochondria and its clinical effect: In normal mitochondrial respiration, electrons are transferred step-by-step to oxygen, producing water. However, if this transfer is incomplete, electrons may leak and react with oxygen prematurely, generating ROS such as the superoxide anion ($O_2\cdot^-$). Among the complexes that constitute the mitochondrial ETC, complexes I, II, and III are major sources of superoxide. Electron transfer within these complexes is not perfectly efficient. As a result, some electrons leak to molecular oxygen, which is partially reduced to form superoxide (**Additional Figure 1**). Superoxide is then converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (**Additional Figure 1**). While H_2O_2 can be further decomposed to water and oxygen by catalase and glutathione peroxidase, the presence of reduced ferrous iron (Fe^{2+}) can cause the Fenton reaction, generating ·OH (Equation 1).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- \quad (1)$$

·OH are highly reactive and indiscriminately oxidize lipids, proteins, and DNA, contributing to oxidative stress and triggering cell death. In particular, ferroptosis, a form of iron-dependent cell death, has gained attention for its association with ·OH generation.[3]

Mitochondria contain various electron carriers with heme or iron-sulfur clusters, making them the most iron-rich organelles in cells. Consequently, these elements play a pivotal role in iron metabolism. Disruption of mitochondrial iron homeostasis or dysfunction of iron-transporting proteins can lead to iron accumulation, enhancing the Fenton reaction and ·OH production. This exacerbates oxidative stress and promotes ferroptosis, which is implicated in aging, cancer, Parkinson's disease, amyotrophic lateral sclerosis, and more.

Mitochondria play a pivotal role in oxygen metabolism and exhibit a structural tendency to generate oxidative stress, developing antioxidants that selectively and efficiently eliminating ·OH within mitochondria is crucial for preventing and treating oxidative stress-related diseases.

Reductive stress caused by conventional antioxidants: Oxidative stress arises when ROS, especially ·OH, accumulate and harm biological systems. As described above, ROS superoxide anion is converted to H_2O_2 by the enzyme superoxide dismutase, and H_2O_2 is subsequently decomposed into water and oxygen by catalase. However, no antioxidant enzymes exist for hydroxyl radicals; therefore, their elimination relies solely on non-enzymatic antioxidants. Non-enzymatic antioxidants work by donating electrons to neutralize ·OH, typically through their reducing activity. Common non-enzymatic antioxidants include vitamin C, vitamin E, glutathione. For example, vitamin C donates electrons to convert ROS into water, but these electrons may also interfere with other metabolic processes.[4]

Ideally, the action of antioxidants should be limited to harmful ROS, such as hydroxyl radicals. In reality, however, their reducing activity can affect mitochondrial electron transport and redox systems, causing "reductive stress," which hinders ATP production.

For instance, long-term use of high-dose vitamin C has been reported to impair mitochondrial function and reduce ATP levels (**Figure 1**).[4] Vitamin E, while protective against lipid peroxidation, may disrupt membrane potential and structure when in excess.

Moreover, vitamin C (ascrbic acid; AscH2) may act as a pro-oxidant by reducing ferric iron (Fe^{3+}) to ferrous ion (Fe^{2+}) (Equations 2 and 3 and **Additional Figure 2**), thereby enhancing ·OH formation via the Fenton reaction (Equation 1 and **Additional Figure 2**).[5] Thus, despite being antioxidants, such compounds may paradoxically increase oxidative stress. Ideally, an antioxidant should avoid reducing activity, not interfere with mitochondrial function, and selectively react only with ·OH.

$$AscH_2 \rightarrow \text{Dehydroascorbic acid} + 2H^+ + 2e^- \quad (2)$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (3)$$

H_2—a smart antioxidant without biomolecular interference: While it serves as a reducing agent in chemical synthesis under high temperature or with catalysts, it is chemically stable under physiological conditions and does not react with biomolecules. The relative electron-donating capacity of representative reducing antioxidants can be ranked as follows: vitamin C (strong) > reduced glutathione (strong) > vitamin E (moderate) > H_2 (low).[6,7] Importantly, this ranking does not represent an absolute numerical comparison based on redox potentials, which are thermodynamic parameters measured using electrodes. H_2, although considered a strong reducing agent in terms of redox potential ($E° = -420$ mV), exhibits little observable reductive activity within biological systems. This apparent discrepancy arises from factors related to bond dissociation energy and molecular orbital theory. The H–H bond is exceptionally strong (436 kJ/mol), with electrons tightly bound in a highly stable molecular state, thereby rendering H_2 structurally resistant to electron transfer with other biomolecules. Consequently, under normal physiological conditions, H_2 rarely reacts with biological molecules and exhibits extremely low electron-donating capacity. Furthermore, humans lack enzymes that can activate or metabolize H_2, further limiting its ability to donate electrons to biomolecules. As a result, hydrogen exerts minimal reductive activity and does not contribute to reductive stress. Therefore, H_2 remains stable and does not trigger redox reactions or additions with lipids, proteins, or other macromolecules.[8]

Importantly, hydrogen does not interact with electron carriers in the mitochondrial ETC (**Figure 2**).[9] Therefore, unlike conventional antioxidants, it does not interfere with mitochondrial function. Moreover, hydrogen selectively reacts with highly reactive and cytotoxic ·OH, converting them into harmless water molecules (**Figure 2**).[10]

The selective action of hydrogen on ·OH has been quantitatively and kinetically demonstrated experimentally.[8] In cell-free experiments using the Fenton reaction, the initial rate of hydroxyphenyl

fluorescein fluorescence, an indicator of ·OH, was significantly reduced in a dissolved H_2 concentration-dependent manner. This provides kinetic evidence that H_2 suppresses ·OH generation and rapidly scavenges ·OH. Furthermore, in cellular analyses, electron spin resonance measurements using hydroxyphenyl fluorescein fluorescence and 5,5-dimethyl-1-pyrroline N-oxide showed that H_2 treatment significantly decreased intracellular ·OH levels. In contrast, indicators of other major physiological ROS, such as superoxide ($O_2\cdot^-$) and H_2O_2, showed little change, quantitatively confirming that H_2 exhibits high selectivity toward ·OH. This specificity is the basis of hydrogen's smart antioxidant behavior.

Animal studies and clinical trials in humans have confirmed the exceptional safety of hydrogen, with virtually no adverse effects even at high concentrations or with prolonged exposure.[11] Furthermore, hydrogen does not interact with enzymes due to the lack of specific hydrogen-metabolizing enzymes in humans, with the exception of certain microbial hydrogenases. Its low affinity for biological macromolecules due to its small molecular size further supports its inertness.[10] Another study also suggests hydrogen may help maintain mitochondrial homeostasis by preserving membrane potential, normalizing ATP production, and suppressing oxidative damage to ETC complexes.[12]

In summary, hydrogen provides pinpoint antioxidant effects by selectively neutralizing ·OH without reducing activity or interfering with essential cellular functions. This makes it a promising strategy for redox homeostasis with both safety and efficacy.

Conclusion: We discussed oxidative stress caused by ROS, the limitations of conventional antioxidants, and the potential of H_2 as a novel antioxidant. Traditional antioxidants like vitamins C and E eliminate ROS through reduction but may disrupt mitochondrial ETC and cause reductive stress, impairing ATP production and cellular function. In contrast, H_2 is chemically inert *in vivo*, does not interfere with mitochondrial function, and selectively reacts with ·OH to form water, suppressing oxidative stress safely. Therefore, H_2 can be regarded as a side-effect-free, smart antioxidant and a promising alternative that addresses the limitations of conventional antioxidants.

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YI, BS and FS are employees of MiZ Company limited. YT declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

During the preparation of this work, the authors used ChatGPT to check grammar and refine English expressions. After consulting ChatGPT, the authors reviewed and edited the content as necessary and take full responsibility for the content of the publication. The following text was used as a prompt for the AI.

Yusuke Ichikawa*, Bunpei Sato, Yoshiyasu Takefuji, Fumitake Satoh
Research and Development Department, MiZ Company Limited, Kamakura, Kanagawa, Japan (Ichikawa Y, Sato B, Satoh F)
Faculty of Data Science, Musashino University, Tokyo, Japan; Keio University, Tokyo, Japan (Takefuji Y)
***Correspondence to:** Yusuke Ichikawa, PhD, y_ichikawa@e-miz.co.jp.
https://orcid.org/0000-0002-2526-4681 (Yusuke Ichikawa)

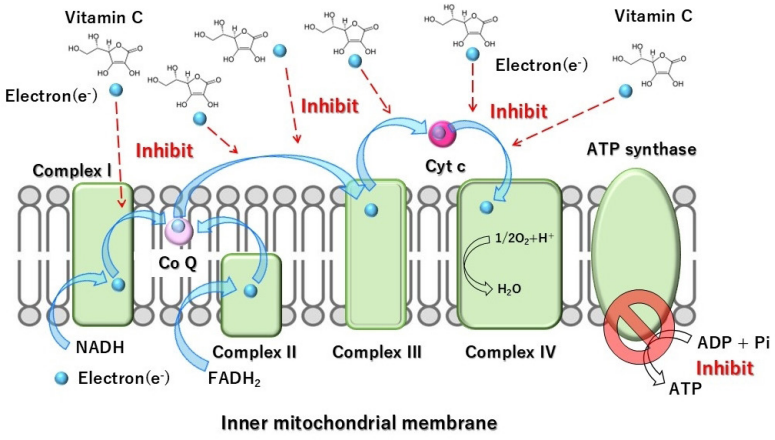

Figure 1 | Excessive reduction of antioxidants disrupts mitochondrial electron transport and inhibits ATP production.
Excessive administration of electron-donating antioxidants, such as vitamin C, may disrupt the mitochondrial electron transport chain by the electrons supplied from the antioxidants, potentially impairing mitochondrial function and reducing ATP production. Created with Microsoft PowerPoint Office 2019. ADP: Adenosine diphosphate; ATP: adenosine triphosphate; Cytc:???; $FADH_2$: flavin adenine dinucleotide; NADH: nicotinamide adenine dinucleotide; Pi: phosphoric acid.

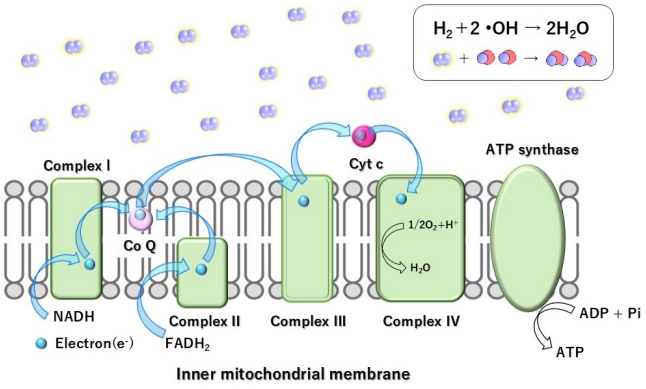

Figure 2 | Non-interfering nature of molecular hydrogen.
Molecular hydrogen is an antioxidant that does not interfere with biomolecules and can specifically react with hydroxyl radicals to convert them into water without affecting the electron transport chain (ETC) across the mitochondrial inner membrane. Created with Microsoft PowerPoint Office 2019. ADP: Adenosine diphosphate; ATP: adenosine triphosphate; Cytc:???; $FADH_2$: flavin adenine dinucleotide; NADH: nicotinamide adenine dinucleotide; Pi: phosphoric acid.

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Additional files:
Additional Figure 1: *The healthy mitochondrial electron transport chain.*
Additional Figure 2: *Vitamin C acts as a pro-oxidant by promoting hydroxyl radical formation.*

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