Unravelling the potential of hydrogen in biological systems

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Molecular hydrogen (H$_2$) and oxyhydrogen (66% H$_2$/33% O$_2$) gases have been demonstrated to remediate the effects of numerous diseases in adults$^{[1-4]}$. By acting as an anti-inflammatory and antioxidative agent, it is reported that H$_2$ can improve recovery through mitigating hyperinflammatory responses and reducing oxidative stress$^{[5-7]}$. As the precise mechanisms of H$_2$ activity are currently undefined, the lack of primary target identification, coupled with difficulties regarding administration methods (e.g., dosage and dosage frequencies, and long-term effects of treatments), there is a requirement for H$_2$ research to evidence how it can reasonably and effectively, be incorporated into healthcare.

H$_2$ forms through the amalgamation of two hydrogen atoms, resulting in an H-H bonding energy of 107 kcal/mol (4.64 eV)$^{[8]}$ and a redox potential (2H + 2e$^-$ → H$_2$) of -0.421 eV at pH 7 relative to the standard hydrogen electrode$^{[9]}$. While the primary mechanism of H$_2$ activity remains unclear, a prevalent hypothesis posits that H$_2$ can mitigate excessive reactive oxygen and nitrogen species (ROS/RNS) through direct interaction with radical and ionic species such as hydroxyl radicals (·OH) and peroxynitrite (ONOO$^-$). However, the favourability of these reaction kinetics in vivo is still a matter of debate$^{[10-12]}$.

Despite the documented clinical benefits of H$_2$ therapies$^{[13-16]}$, significant questions remain about the distribution and molecular mechanisms of H$_2$ within biological systems. Key questions include how H$_2$ reaches target tissues, what the primary physiological targets of H$_2$ interactions are, and how H$_2$ maintains its influence over time. In response, international research institutions are dedicating resources to understanding the effects of H$_2$ on cellular and systemic physiology. This editorial assesses emerging research on H$_2$ distribution and molecular activity of H$_2$ in biological systems.

The physical and chemical characteristics of H$_2$ (e.g., electrochemically neutral, lightweight, non-polarity, etc.), should permit the molecule to diffuse through biological fluids, the extra-cellular matrix, cellular membranes and cytosolic compartments$^{[17,18]}$. However, it is undetermined whether this mechanism of...
dispersal would be able to explain the effects seen in distal organs. For example, when hydrogen-rich saline (HRS) is administered via intraperitoneal delivery, substantial amounts of H\textsubscript{2} are recorded in proximal sites such as the pancreas and spleen, whilst stepwise reductions, progressing radially, are shown for the distal organs\textsuperscript{[19]}. Interestingly, when H\textsubscript{2} is delivered via inhalation, although the primary beneficiary organs are typically in close proximity (e.g., the brain, respiratory system and heart), significant increases in H\textsubscript{2} concentration are also seen in the spleen and skeletal muscle 30 minutes after application. Although this research\textsuperscript{[20]} identified that intraperitoneal injection with HRS resulted in H\textsubscript{2} elevation in the majority of tissues analysed, this method was not effective in delivering capacious amounts of H\textsubscript{2} into the bloodstream or the wider cardiovascular system but was highly effective in delivering H\textsubscript{2} to proximal organs including the intestines, liver, pancreas and spleen. Such results suggest a preference for distribution by simple diffusion, however, this still does not explain the elevated levels of H\textsubscript{2} in distal organs such as the brain and kidneys, when elevated H\textsubscript{2} levels in the blood are not detected. However, this factor could be explained if H\textsubscript{2} were to transitorily reside within micropores, or pockets, formed within haemoglobin of red blood cells\textsuperscript{[21-23]}; or if H\textsubscript{2} could be temporarily retained by molecules suspended in the serum such as carbohydrates (e.g., glycogen), or inorganic ions, calcium, potassium, or phosphates, as examples. This potential for temporary retention and residence within biological structures might contribute to observed biological effects of H\textsubscript{2} administration.

While evidence supporting the mitigation of cellular component oxidation and peroxidation by H\textsubscript{2} is clear\textsuperscript{[24-28]}, evidence that this diatom acts as a direct antioxidant remains sparse. Although seminal calculations conducted by Kim et al. (2022)\textsuperscript{[29]} suggest antioxidant activity through interactions between H\textsubscript{2} and protohaem, kinetic modelling indicates that H\textsubscript{2} is unlikely to react with other reactive gases \textit{in vivo}, Table 1. To elucidate, the superoxide (O\textsubscript{2}\textsuperscript{•\textminus}) anion, the principal molecule for reactive oxygen and reactive nitrogen species formation, has a spontaneous dismutation rate constant of \(8 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}\) (pH 7.8), albeit, this is a second-order rate reaction\textsuperscript{[30]}. The first-order rate reaction is calculated to be \(10^6\)-fold more expeditious\textsuperscript{[31]}. Additionally, both the hydroxyl radical and the peroxynitrite ion, which are significant contributors to oxidative stress\textsuperscript{[32,33]}, have reaction kinetics that far surpass the reactions that may occur with H\textsubscript{2}. The reaction kinetics of 'OH with H\textsubscript{2} are calculated to be \(4.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\)\textsuperscript{[34]}, whilst ONOO\textsuperscript{–} is reported not to have a spontaneous dismutation rate of 1.1 - 1.3 M\textsuperscript{−1} s\textsuperscript{−1}, with or without the addition of H\textsubscript{2}\textsuperscript{[35]}.

\textbf{Table 1.} Data extracted and adapted from Zhang et al. (2019)\textsuperscript{[36]}. Lifetime: natural log2 divided by the sum of the products of rate constant and concentrations for all molecules that ROS react with. Diffusion distance: calculated with the formula \(x = (6Dt)^{1/2}\) (x, D, t stand for diffusion distance, diffusion coefficients and lifetime, respectively).
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<table>
<thead>
<tr>
<th>Substance</th>
<th>Chemical Nomenclature</th>
<th>Theoretical Distance Travelled (meters)</th>
<th>Theoretical Longevity (seconds)</th>
<th>Primary Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Peroxide</td>
<td>H₂O₂</td>
<td>10⁻³ m</td>
<td>10⁻³ s</td>
<td>Metal groups and thiols</td>
</tr>
<tr>
<td>Hydroxyl Radical</td>
<td>‘OH</td>
<td>10⁻⁹ m</td>
<td>10⁻⁹ s</td>
<td>Indiscriminate – all organic macromolecules</td>
</tr>
<tr>
<td>Nitric Oxide Radical</td>
<td>NO'</td>
<td>10⁻¹ m</td>
<td>10⁻¹ s</td>
<td>Metal groups and thiols</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>ONOO⁻</td>
<td>10⁻⁶ m</td>
<td>10⁻⁶ s</td>
<td>CO₂, Cys, Trp, Met and metal groups</td>
</tr>
<tr>
<td>Superoxide</td>
<td>O₂⁻</td>
<td>10⁻⁹ m</td>
<td>10⁻³ s</td>
<td>Fe–S clusters and NO‘</td>
</tr>
</tbody>
</table>

Given the favourable reaction rates of >1 x 10⁹ M⁻¹ s⁻¹ for most radical interactions, the neutralisation of ROS/RNS in the milieu of amino acids, lipids and other reactive gases, by H₂, appears biologically insignificant. Therefore, while H₂ shows promise as an antioxidant, it is unlikely to be due to direct interactions with highly reactive biological compounds.

Offering another explanation into the antioxidant activity of H₂, *in silico* research infers that H₂ can bind to the iron (Fe²⁺) in haem via asymmetric dihydrogen bonds and symmetric bilateral electron transfer, known as Kubas bonding[^37]. The authors report that symmetric binding is more favourable under physiological conditions due to a moderately lower activation energy (2.04 eV vs. 2.14 eV). Wherein, Fe²⁺ is hypothesized to reduce the dissociation energy of the H-H bond in H₂, forming an Fe²⁺/H⁺ complex that can reduce highly reactive ions and radicals. The dissociation energy of Fe²⁺/H⁺ was calculated to be 2.78 eV, allowing the bound H⁺ radical to neutralise other reactive species[^38]. However, the kinetics of this proposed mode of action remain unclear.

Given these complexities, it is conceivable that the therapeutic potential of H₂ extends beyond simple antioxidant activity. The effects of H₂ on signaling pathways and gene expression modulation might offer alternative explanations for its observed benefits. For example, the downregulation of the NADPH oxidase (NOX-1) enzyme noted in H₂-treated models suggests a regulatory role that could mitigate oxidative stress indirectly, by reducing the production of superoxide anions[^39]. Additionally, H₂ has been observed to influence cell signalling pathways involved in inflammation and oxidative stress, hinting at a broader regulatory function within cellular systems. Empirical studies demonstrate that H₂ can modulate signal transduction pathways such as the Nrf2 pathway, which is crucial for cellular defence against oxidative stress[^40-42]. This may be important as activation of Nrf2 leads to the expression of antioxidant enzymes including haem oxygenase-1 (HO-1) and superoxide dismutase (SOD), which can enhance the cell’s intrinsic ability to manage oxidative damage[^43]. This indirect mechanism of reducing oxidative stress through upregulation of endogenous antioxidant systems could be a significant aspect when considering the therapeutic efficacy of H₂.

As we delve deeper into the mechanisms and biomedical applications of H₂, we stand at the cusp of unlocking new therapeutic strategies that could significantly impact the treatment of oxidative stress-related diseases. While the antioxidant properties of H₂ are well-documented, its therapeutic potential likely extends...
beyond direct radical scavenging\cite{44}. The modulation of signaling pathways, gene expression, and inflammatory responses represents promising avenues through which H₂ may exert its beneficial effects. As the scientific community continues to explore the potential of H₂, several key areas warrant further investigation. Firstly, the development of advanced analytical techniques to accurately measure H₂ concentration and distribution in biological tissues will be pivotal. Secondly, understanding the interaction of H₂ with cellular components at the molecular level will provide insights into its precise mechanisms of action. And lastly, clinical trials assessing the efficacy and safety of H₂ in various disease models will help establish its therapeutic potential and inform clinical guidelines. By creating a robust, interdisciplinary, evidence-based library of research, it is possible that H₂ therapeutics may herald new, innovative treatments for chronic inflammatory and oxidative stress-related diseases.

**Conflict of interest**

The authors declare that they have a financial and professional affiliation with Water Fuel Engineering, a company that designs and manufactures oxyhydrogen-producing electrolyzers.

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