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Review

The fairytale of the GSSG/GSH redox potential ☆

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ABSTRACT

Background: The term GSSG/GSH redox potential is frequently used to explain redox regulation and other biological processes.
Scope of review: The relevance of the GSSG/GSH redox potential as driving force of biological processes is critically discussed. It is recalled that the concentration ratio of GSSG and GSH reflects little else than a steady state, which overwhelmingly results from fast enzymatic processes utilizing, degrading or regenerating GSH.
Major conclusions: A biological GSSG/GSH redox potential, as calculated by the Nernst equation, is a deduced electrochemical parameter based on direct measurements of GSH and GSSG that are often complicated by poorly substantiated assumptions. It is considered irrelevant to the steering of any biological process. GSH-utilizing enzymes depend on the concentration of GSH, not on $[GSH]^2$, as is predicted by the Nernst equation, and are typically not affected by GSSG. Regulatory processes involving oxidants and GSH are considered to make use of mechanistic principles known for thiol peroxidases which catalyze the oxidation of hydroperoxides by GSH by means of an enzyme substitution mechanism involving only bimolecular reaction steps.
General significance: The negligibly small rate constants of related spontaneous reactions as compared with enzyme-catalyzed ones underscore the superiority of kinetic parameters over electrochemical or thermodynamic ones for an in-depth understanding of GSH-dependent biological phenomena. At best, the GSSG/GSH potential might be useful as an analytical tool to disclose disturbances in redox metabolism. This article is part of a Special Issue entitled Cellular Functions of Glutathione.

1. Introduction

The provocative title of this short commentary was imposed by the guest editors of the present issue. I had to accept it, because I am guilty of having opened up a debate on the relevance of some physicochemical parameters to biology at training courses and meetings, thereby seemingly challenging established scientific laws. The sin committed, though, was little else than questioning if physicochemical parameters such as the electrochemical potential ΔE, as defined by the Nernst equation, or the equivalent free energy ΔG of Gibbs or Helmholtz, respectively, can adequately describe phenomena of real life. These fundamental equations were developed to describe closed chemical systems, while life, in biochemical terms, is defined as an open and extremely metastable condition that owes its persistence to a dense network of physical and chemical barriers that prevent ΔE-driven shortcuts or ΔG-driven implosions. In short, I advocated the priority of kinetics over thermodynamics. This exercise may appear superfluous in view of the evidence that any kind of living organism, as long as it is alive, is far off any equilibrium and that its life-sustaining strategy consists in the kinetic control of life-terminating equilibration. Accordingly, I hardly felt tempted to address this triviality, if not as introductory remarks in enzymology training of undergraduate students. However, the controversial and emotional response to similar statements in front of seniors suggests the need for clarification.

2. De-mystification of the thiol/disulfide potential

The electrochemical potential of a compound E simply describes its tendency to become oxidized, whereby the oxidation of hydrogen to a proton under 'standard conditions' serves as reference standard (E = 0). The difference of two half-reaction potentials (also called E_{hc}; see below), e.g. for the oxidation of GSH to GSSG (Eq. (1)) and the reduction of H_2O_2 to water (Eq. (2)), which sum up to the reduction of H_2O_2 by GSH (Eq. (3)), yields an electromotive force ΔE. The latter can be transformed into the Gibbs free energy ΔG by Eq. (4).

$$2\,GSH - 2\,e^- - 2\,H^+ \leftrightharpoons GSSG \quad (1)$$

$$H_2O_2 + 2\,e^- + 2\,H^+ \leftrightharpoons 2\,H_2O \quad (2)$$

$$2\,GSH + H_2O_2 \leftrightharpoons GSSG + 2\,H_2O \quad (3)$$

$$\Delta G = -nF\Delta E \quad (4)$$

ΔE or ΔG, respectively, reveals in which direction an oxidation–reduction reaction would go, if it is not physically, sterically or by

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whatever means prevented from doing so. Quantitatively, ΔE is calculated by the Nernst equation (Eq. (5)):

$$\Delta E = \Delta E^0 - \frac{RT}{nF} \ln Q \quad (5)$$

Therein ΔE^0 is the electrochemical force under standard condition, *i. e.* a constant characterizing the redox reaction under consideration, R is the gas constant, T the absolute temperature, n the number of electrons involved, F the Faraday constant, and Q the mass law quotient with the actual concentrations of reaction partners, in our example those of the backward reaction over the ones of the forward reaction in Eq. (3) (with the omission of water; Eq. (6)):

$$Q = [GSSG]/[GSH]^2[H_2O_2] \quad (6)$$

The importance of thiol/disulfide ratios, related ΔEs or reduction capacities for explaining biological phenomena is highlighted in countless publications. A representative example is the highly quoted review article by Freya Schafer and Garry Buettner [1]. It is here selected as representative of the more serious examples. It properly addresses critical issues such as the need for clear definitions in redox biology, the value of quantitative data, difficulties in precise potential calculations under consideration of pH, subcellular redox compartmentalization, the requirement to overcome activation energy barriers and the integration of redox couples in the metabolic context. The article's basic message shall be quoted from the summary: "*There are many redox couples in a cell that work together to maintain the redox environment*; *the GSSG/2GSH couple is the most abundant redox couple in a cell. Changes of the half-cell reduction potential* (E_{hc}) *of the GSSG/2GSH couple appear to correlate with the biological status of the cell*: *proliferation* $E_{hc} \sim -240$ *mV differentiation*; $E_{hc} \sim -200$ *mV*; *or apoptosis* $E_{hc} \sim -170$ *mV*." There is nothing wrong with underscoring an intriguing correlation. However, the last sentence of this summary sounds like a patent application to explain all secrets of life: "*These are the first steps toward a new quantitative biology*, *which hopefully will provide a rationale and understanding of the cellular mechanisms associated with cell growth and development*, *signaling, and reductive or oxidative stress*." When reading this sentence for the first time, I became concerned that the addressed correlation of the GSSG/2GSH potentials and biological phenomena might be misunderstood as a cause/effect relationship, and such concern proved to be justified indeed. When rolling through the publications quoting the Schafer and Buettner review, one stumbles across examples manifesting the misunderstanding already in the title [2,3]. But there is little, if any, experimental evidence nor any conceivable theoretical basis suggesting that biological events might be *caused* by changes in thiol/disulfide ratios or potentials calculated there from.

In fact, a thiol/disulfide potential cannot electrochemically be assessed in a complex biological system. Any kind of electrode will respond to the redox-active compounds it senses most easily, and thiols or disulfides do by no means belong to this category. This implies that the thiol/disulfide potentials of biological samples are throughout calculated from chemically determined concentrations of the redox partners by means of the Nernst equation. Hereby, the effect of the concentrations of reactants on ΔE is considered by the mass law term Q (Eqs. (5) and (6)). The justification of this approximation depends on two pivotal assumptions: i) the reaction has to proceed as formulated and ii) it has to be reversible. Both requirements are usually met by electrochemical cells made up from inorganic components, but are more or less problematic even for simple organic system. The oxidation of GSH by H_2O_2 (Eq. (3)) may serve as a revealing example for a comparatively simple but biologically relevant redox reaction: i) As commonly formulated (Eq. (3)), this reaction does not proceed at all; it is the thiolate form that reacts with H_2O_2. But this complication is easily considered in the Nernst equation [1]. ii) The forward reaction, as formulated, requires a ternary collision of two GSH molecules with H_2O_2, which is the basis of the $[GSH]^2$ term in Q (Eq. (6)). Such ternary collision is, however, quite unlikely and I am not aware of any measurement suggesting that it happens at any relevant frequency. Whenever the oxidation of GSH by any kind of oxidant was measured, the velocity depended on [GSH] and not on $[GSH]^2$. iii) The backward reaction, finally, would be the regeneration of GSH and H_2O_2 from GSSG and water, a possibility that, to my knowledge, has never been considered or documented to happen. In short, for the chosen example at least, the transformation of reactant concentrations into ΔE is based on two paper chemistry reactions that have never been demonstrated to occur in real life. Instead, GSH oxidation follows a two-step scheme: first the thiolate is oxidized to a sulfenic acid (Eq. (7))

$$GS^- + H_2O_2 \rightarrow RSOH + OH^- \quad (7)$$

and the latter then dissociates and reacts with the second thiol to form the disulfide.

$$GSO^- + H^+ + GSH \rightarrow GSSG + H_2O \quad (8)$$

Since the second reaction (Eq. (8)) is faster than the first one (Eq. (7)), it remains kinetically silent and the overall reaction depends on [GSH]. The rate constant for the reaction has been determined and did not exceed 30 $M^{-1}s^{-1}$, neither for GSH nor for any other physiologically relevant low molecular mass thiol, even when the values were extrapolated to full dissociation of the thiol groups [4].

These values correspond to 3–5 $M^{-1}s^{-1}$ under physiological conditions and, thus, fall short by 5–7 orders of magnitude when compared to corresponding values for the oxidation of the peroxidatic cysteines (Eq. (9)) or selenocysteines (Eq. (10)) in peroxiredoxins (Prx) [5] or glutathione peroxidases (GPx) [6], respectively.

$$Prx - S^- + H_2O_2 \rightarrow Prx - SOH + OH^- \quad (9)$$

$$GPx - Se^- + H_2O_2 \rightarrow GPx - SeOH + OH^- \quad (10)$$

The oxidized enzymes are then stepwise reduced, typically by thioredoxin in case of Prxs and by GSH in case of the selenium-containing GPxs [7]. For GSH oxidation, the highly unlikely reaction of one H_2O_2 molecule and 2 GSH molecules by means of a ternary collision is replaced by an extremely fast bimolecular reaction of the enzyme's selenocysteine residue with H_2O_2 (Eq. (10)) followed by bimolecular reactions of each of the GSH molecule with the modified (oxidized and Se-glutathionylated, respectively) enzyme. This catalytic trick, which is shared by Prxs and most oxido-reductases, is known as 'enzyme substitution mechanism' and, historically, can be traced back to the beginning of the last century when Wilhelm Ostwald described the acceleration of sluggish chemical processes by '*Zwischenstoffkatalyse*' (catalysis by formation of intermediates) [8]. The corresponding rate equation for a typical selenium-containing GPx [9,10] (Eq. (11))

$$[E_0]/v_0 = 1/k_{+1} \cdot [H_2O_2] + 1/k'_{+2} \cdot [GSH] \quad (11)$$

reveals that the enzymatic reaction, like the spontaneous one, depends on [GSH] and $[H_2O_2]$, but not $[GSH]^2$, and the dependence on [GSH], incidentally, holds true for the realm of enzymatic processes using GSH [11]. Moreover, the GPx reaction is not affected by physiological concentrations of GSSG. The latter has anyway no realistic chance to take part in a backward reaction, as it is rapidly reduced by the glutathione reductase at the expense of NADPH or excreted, extracellularly degraded by γ-glutamyl transpeptidase and recycled for *de novo* synthesis of GSH [11,12].