ROS

REVIEW ARTICLES

Cellular Redox Environment and Its Influence on Redox Signaling Molecules

John T. Hancock[1] and Matthew Whiteman[2]

[1]Department of Applied Sciences, University of the West of England, Bristol, UK; [2]University of Exeter Medical School, University of Exeter, Exeter, UK

Correspondence: john.hancock@uwe.ac.uk (J.T.H.)

Hancock JT and Whiteman M. Reactive Oxygen Species 5(14):78–85, 2018; ©2018 Cell Med Press
http://dx.doi.org/10.20455/ros.2018.815
(Received: December 2, 2017; Revised: December 16, 2017; Accepted: December 17, 2017)

ABSTRACT | The redox potential of a cell's internal environment is well recognized as important for controlling cellular activities. Both animal and plant cells generate and are exposed to a range of reactive molecules involved in cell signaling, including reactive oxygen species and reactive nitrogen species, such as hydrogen peroxide and nitric oxide. Redox active molecules exist in different oxidation states, with the ratio of the states being able to be determined using the Nernst equation. Therefore, influence of redox environments of cells on the likelihood of the persistence of a particular redox state of a molecule can be estimated, and this might have a profound effect on whether molecules can act as signals. Although the cellular redox may have little influence on some molecules, for others there may be a significant impact from the redox environment. Furthermore, cellular redox environments fluctuate and as they become more oxidizing some signaling molecules may become more persistent while the moderating effect of others may be lessened. Such influence of redox environment needs to be taken into account if the role of such molecules in cell signaling is to be understood.

KEYWORDS | Reactive oxygen species; Redox environment; Redox potential; Redox signaling

ABBREVIATIONS | cySS, cystine; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; LMW, low-molecular weight; NADH, reduced form of nicotinamide adenine dinucleotide; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSNO/RSH, S-nitrosothiol/corresponding thiol; TRX_{ox}, oxidized form of thioredoxin; TRX_{re}, reduced form of thioredoxin

CONTENTS

1. INTRODUCTION

The redox environment inside cells has been the subject of considerable discussion over many years [1–3]. It is important to understand as it is used for the maintenance of reduced compounds and for cell signaling. The intracellular reduction potential has been estimated to be relatively reducing [2] (normally lower than –200 mV relative to a standard hydrogen electrode), therefore giving an ideal environment for the production and maintenance of reduced co-factors such as the reduced form of nicotinamide adenine dinucleotide (NADH) and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). However, the actual concentrations of such co-factors in cells will also be influenced by their binding to other cellular components [1, 4]. It is important to also understand that the redox environment of cells is not fixed but has a dynamic nature. Schafer and Buettner [2] estimated that the redox environment may become significantly more oxidizing, changing by as much as 70 mV as cells move from a proliferative state to one of apoptosis. Such changes can have profound effects on cellular components such as proteins, and therefore redox signaling is now recognized as a major influence in the control of cellular function [5].

One of the most significant influences on the redox environment is both the amount and reduction state of the tri-peptide glutathione [2]. Intracellular concentrations may be greater than ten millimolar. Its influence on the redox is determined by its mid-point potential [6], but also by its overall concentration because the reaction relates to a squared ratio in the Nernst equation [2]. Cells can therefore manipulate their intracellular redox by the generation [7] or loss of glutathione [8] as well as the ratio of the oxidized to reduced states [2]. Therefore, glutathione can be measured as an estimate of the intracellular redox state [9] and its influence has been linked to health and disease [10] especially as it can also alter protein function through glutathionylation [11].

The presence, or accumulation, of other redox molecules also influences intracellular redox states, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS encompasses superoxide anions, hydrogen peroxide (H_2O_2) and the hydroxyl radical while RNS includes nitric oxide and peroxynitrite. Both ROS and RNS are known to be major signaling molecules in both plants and animals [12, 13] and can cause post-translational modifications of proteins and so control cellular function: oxidation and S-nitrosylation respectively [14].

Other signaling molecules here include hydrogen sulfide (H_2S) [15] and hydrogen gas (H_2) [16]. H_2S can lead to S-sulfhydration [17], altering protein function, perhaps in competition with other redox active molecules [15], while H_2 can influence cellular redox by manipulating antioxidant levels [18].

The present dogma is that ROS and other redox molecules influence the redox environment and that this leads to the process of oxidative stress, leading to cellular damage [19]. To some extent, this is probably true, with considerations of compartmentalization being taken into account. However, it is argued here that the opposite is also true, that the redox environment of the cell will be a major influence on whether redox signaling molecules persist in the cell and whether they are able to have effects often assigned to them.

2. MAINTENANCE AND INFLUENCE OF REDOX ENVIRONMENTS

The redox environment will be dictated by the major redox-capable components of the cellular location; the cytoplasm is commonly studied. It is considered that intracellular glutathione is a good indicator of redox poise [2], with values being derived using the Nernst equation (Equation 1: bearing in mind the squared ratio needed for the GSSG/2 GSH couple; GSSG and GSH denote oxidized form and reduced form of glutathione, respectively).

Equation 1: The Nernst equation (redox equation) assuming an intracellular pH of 7.4.

$$E_h = E_{m(pH7.4)} + (RT/nF) \times 2.303\log([\text{oxidized}]/[\text{reduced}])$$

Where E_h is the redox potential; $E_{m(pH7.4)}$ is the mid-point potential of redox couple at pH 7.4; R is the gas constant; T is the temperature in Kelvin; F is the Faraday constant; n is the number of electrons used in oxidation/reduction.

However, the redox environment will also be determined by the presence of other abundant low-molecular weight (LMW) thiols (**Table 1**) [20], including cysteine (Cys), cysteinyl-glycine (Cys-Gly)

TABLE 1. Redox couples which are instrumental in controlling the redox environment

Redox couple	Notes	Mid-point potential (mV)	Reference
GSH/GSSG	$E^{0\prime}$ (pH = 7.0)	–240	[6, 20]
GSH/GSSG	$E_{\text{pH = 7.4}}$	–264	[2]
GSH/GSSG	$E_{\text{pH = 8}}$	–299	[2]
Cys-bis-Gly/2 Cys-Gly	$E^{0\prime}$	–226	[20, 21]
Cysteine/2 Cys	$E^{0\prime}$	–226	[20, 21]

TABLE 2. Redox potentials of various cell environments [2]

Cell type (proliferating)	$E_{h\ \text{pH 7.4}}$ (mV)	Reference
Normal fibroblasts	–247	[22]
Fibrosarcoma	–238	[22]
Murine hybridoma	–235	[23, 24]
Human lymphocytes	–237	[25]
Jurkat	–240	[25]
Murine hybridoma	–257	[26]
Average	–242	
Cells proliferating	–242	[2]
Cells differentiating	–200	[2]
Cells under apoptosis	–170	[2]
Liver cytosol	–390	[4]

and γ-glutamyl-cysteine (γ-Glu-Cys). It was found that in non-aged seeds non-GSH thiols contributed to approximately 15% of the redox which involved thiol-disulfide reactions ($E_{\text{thiol-disulphide}}$), while this increased to approximately 25% in 10-week-old seeds. A shift in this redox couple was correlated to the loss of seed viability, showing that there was a real biological effect [20]. Methods for measuring the couples for glutathione (GSSG/2 GSH), cysteine/cystine (cys/cySS), thioredoxins (TRX_{red}/TRX_{ox}) and the oxidation states of proteins have been described [3] while Schafer and Buettner [2] suggested that the equation to calculate the redox environment should include all redox influencing species (Equation 2).

Equation 2: $\text{Redox environment} = \sum_{i-1}^{n\,(\text{couple})} E_i \times [\text{reduced}]_i$

Where E_i is the half-cell reduction potential of the redox couple of interest [2].

Given that the GSSG/2 GSH couple alone could be millimolar [2, 27] these thiol couples (**Table 1**) will be the overriding factors keeping the intracellular redox environment stable. Given also that 25% of the environment could be influenced by other LMW thiols [20] the total thiol concentration maintaining redox poise in cells is significant. To influence this the concentrations of ROS and RNS added to make an appreciable difference would have to be considerable.

The most studied ROS is H_2O_2, with effects reported at low levels, such as 10 µM in work on *C. elegans* [27], and 1–20 µM in a study of synaptic plasticity [28]. Although some organisms such as *Streptococcus* and *Enterococcus* bacteria can produce H_2O_2 to higher levels, such as 2 mM [29], very high levels in human tissues would be considered to be 600 µM, as in eye aqueous humor [27]. The influence on redox environment through Equation 2 must be limited if H_2O_2 is considerably lower than the 10 mM of glutathione. It is hypothesized here that the influence will be the other way around, that is, the redox environment will have a major impact on the [oxidized]/[reduced] ratio of the signaling molecule. There is a caveat. Intracellular redox environment studies usually measure the overall redox state, but as with other signals, redox components will be compartmentalized [30] and actual levels of LMW thiols, ROS and RNS may be different to those measured. Having said that, there have been reports

TABLE 3. Theoretical values of various redox ratios at proposed redox environments

Redox couple $E^{o\prime}$ (mV)	e^- (n=)	At –390 mV (liver cytosol)[a]	At –242 mV (proliferating)[b]	At –200 mV (differentiating)[c]	At –170 mV (apoptotic)[c]	Comment/reference for mid-point potential
$NAD^+/NADH$						
–320[d]	2	4.3×10^{-3}	431.6	1.1×10^{4}	1.1×10^{5}	Probably ~1:100. Bound to cytosolic binding sites[e]
$O_2/O_2^{\bullet -}$						
–160	1	1.3×10^{-4}	4.1×10^{-2}	0.21	0.68	[31]
O_2/H_2O_2						
+300	2	4.9×10^{-24}	4.9×10^{-19}	1.3×10^{-17}	1.3×10^{-16}	[31]
$O_2^{\bullet -}/H_2O_2$						
+940	1	3.4×10^{-23}	1.1×10^{-20}	5.5×10^{-20}	1.8×10^{-19}	[31]
$O_2^{\bullet -}/H_2O$						
+1200	3	1.9×10^{-81}	6.3×10^{-74}	8.6×10^{-72}	2.9×10^{-70}	Quoted as $[O_2^{\bullet -}]/[H_2O]^2$ [31]
H_2O_2/H_2O						
+1320	2	9.1×10^{-49}	9.1×10^{-44}	2.4×10^{-42}	2.5×10^{-41}	Quoted as $[H_2O_2]/[H_2O]^2$ [31]
Dehydroascorbic acid/ascorbic acid						
+80	2	1.3×10^{-16}	1.3×10^{-11}	3.5×10^{-10}	3.6×10^{-9}	[32, 33]
Cytochrome c (ferric/ferrous)						
+220	1	5.0×10^{-11}	1.6×10^{-8}	8.0×10^{-08}	2.6×10^{-7}	[32]
$2H^+/H_2$						
–420 (30ºC)	2	10.3	1.0×10^{6}	2.7×10^{7}	2.8×10^{8}	Values quoted as $[H^+]^2/H_2$ [32, 34]
–413 (25ºC)	2	6.0	6.0×10^{5}	1.6×10^{7}	1.6×10^{8}	[35]
$OH^{\bullet}/H_2O$						
+2310	1	2.5×10^{-46}	7.8×10^{-44}	4.0×10^{-43}	1.3×10^{-42}	[36]
H_2O_2/OH^- (H_2O)						
+320	1	1.0×10^{-12}	3.2×10^{-10}	1.6×10^{-9}	5.3×10^{-9}	[36]
NH_3^+/NH_3						
+2130	1	2.7×10^{-43}	8.6×10^{-41}	4.4×10^{-40}	1.4×10^{-39}	[37]
$NO^+/NO^{\bullet}$						
+1210	1	9.4×10^{-28}	3.0×10^{-25}	1.5×10^{-24}	4.9×10^{-24}	[36]
$NO^{\bullet}/NO^-$ (singlet)						
–350	1	0.21	66.7	341.8	1097.8	[36]
$NO^{\bullet}/NO^-$ (triplet)						
+390	1	6.7×10^{-14}	2.1×10^{-11}	1.0×10^{-10}	3.5×10^{-10}	[36]
$2NO^-/N_2O_2^-$						
+650	1	2.7×10^{-18}	8.6×10^{-16}	4.4×10^{-15}	1.4×10^{-14}	Value quoted as $[NO^-]^2/[N_2O_2^-]$ [36]
$^1O_2/O_2^{\bullet -}$						
+830	1	2.5×10^{-21}	7.8×10^{-19}	4.0×10^{-18}	1.3×10^{-17}	[36]

TABLE 3. (*continued*)

Redox couple $E^{o\prime}$ (mV)	e^- (n=)	At –390 mV (liver cytosol) [a]	At –242 mV (proliferating)[b]	At –200 mV (differentiating)[c]	At –170 mV (apoptotic)[c]	Comment/reference for mid-point potentials
$ONOO^-/NO_2$						
+1400	1	5.8×10^{-31}	1.8×10^{-28}	9.4×10^{-28}	3.0×10^{-27}	[36]
NO_2/NO_2^-						
+990	1	4.9×10^{-24}	1.5×10^{-21}	7.9×10^{-21}	2.5×10^{-20}	[36]
+1040	1	7.0×10^{-25}	2.2×10^{-22}	1.1×10^{-21}	3.6×10^{-21}	[38]
$RO^\bullet/ROH$						
+1600	1	2.4×10^{-34}	7.7×10^{-32}	3.9×10^{-31}	1.3×10^{-30}	[36]
$RS^\bullet/RSH$						
+900	1	1.6×10^{-22}	5.1×10^{-20}	2.6×10^{-19}	8.4×10^{-19}	[36]
$RSNO/RSH, NO^\bullet$						
–400	1	1.4	466.5	2389.9	7676.0	[36]
S/H_2S						
–230	2	3.9	0.4	10.3	106.4	[39]

Note: [a], [2, 4, 40, 41]; [b], Table 1; [c], [2]; [d], [34]; [e], [1, 4].

of intracellular redox values (**Table 2**) with an average value of approximately –242 mV. Taking these data, using published data for the mid-point potentials for redox couples which could be important for cell signaling and using the Nernst equation (Equation 1) estimates of the [oxidized]/[reduced] value for a range of redox couples can be obtained (**Table 3**). Furthermore, as a cell moves from a proliferative state to one of apoptosis [2] how a change of redox environment may influence the [oxidized]/[reduced] of signaling couples can be calculated (**Table 3**).

For many redox couples there is no tangible influence of the redox environment on the likely biological activity of those signaling molecules. At –242 mV the O_2/H_2O_2 couple will vastly favor the presence of H_2O_2, enabling H_2O_2 to act as a cellular signal. A change of intracellular redox of approximately 70 mV will make little difference to this. Many of the redox couples listed (**Table 3**) have mid-point potentials significantly more positive than the redox environment, so changes of ~70 mV makes no difference; there is little effect on important couples such as $RO^\bullet/ROH$ and $RS^\bullet/RSH$ for example, although local peptide environments may influence here. There will be little influence on some non-protein couples, such as $NO^+/NO^\bullet$, favoring $NO^\bullet$ at all cellular redox potentials. As NO^+ and $NO^\bullet$ will react in different ways [42], and $NO^\bullet$ being the species associated with intracellular signaling, this is important.

Cellular redox does influence redox ratios however. For the $O_2^{\bullet-}/H_2O_2$ couple H_2O_2 is favored, which would aid signaling where a molecule has to persist and move to have influence. However, for the $O_2^{\bullet-}$ H_2O and H_2O_2/H_2O couples cellular redox would favor the conversion to H_2O: not good for signaling. The presence of the signaling species is also not favored for the 2 H^+/H_2 couple: the proton to gas ratio being ~1000; the gas being important for signaling [16]. For the $ONOO^-/NO_2$ couple, peroxynitrite may not be persistent in cells although it is relatively stable and known to have biological effects [43].

The reduction of cytochrome c is favored. The oxidation of cytochrome *c*, as affected by ROS, may have a role in the activation of cell death programs [44]. It may be expected, therefore, that the oxidation of cytochrome c and its protein interactions would have to be compartmentalized to avoid immediate re-reduction.

Along with the influence of average cellular redox it can be determined if changes in redox have an influence, that is, oxidation by approximately 70 mV [2]. The $O_2/O_2^{\bullet-}$ couple sees a significant lowering of $O_2^{\bullet-}$ concentrations, so diminishing the bioavailability of $O_2^{\bullet-}$ and lowering the possible H_2O_2 concentrations resulting from dismutation. For the

H^+/H_2 couple the preference for the gaseous (signaling) form would be lowered, whereas for the $NO^{\bullet}/NO^-$ couple the preference moves to the $NO^{\bullet}$ (signaling) form. The S-nitrosothiol/corresponding thiol (RSNO/RSH) couple will favor the RSNO form, helping to drive, or prolong, RSNO signaling. The S/H_2S couple will lower the H_2S concentration: H_2S may keep other redox signaling under control [15] so the influence of H_2S goes down, the influence of RSNO goes up, so allowing redox signaling to continue, or even increase.

3. CONCLUSIONS AND PERSPECTIVES

The redox environment of the cell is extremely important and is maintained at a relatively reducing potential by a range of small thiol compounds. This reduction potential will have little influence on many biological-relevant redox couples but for some it may be important. The presence of H_2O_2 and $NO^{\bullet}$ may be favored, both which are important for signaling, while the presence of H_2 may be low. However, the redox of the cell is not static and as it becomes oxidizing this may have an influence on redox couples: $O_2^{\bullet-}$ presence may be lowered, as may that of H_2S while $NO^{\bullet}$ may be favored. Therefore, the influence of intracellular redox on redox-sensitive signaling molecules needs to be considered.

Future work needs to fully understand the redox environment at a local level to get a complete understanding of the effect on redox couples in cells. As with many signaling processes compartmentalization is important to consider and will give a better understanding of the prevalence of the oxidation state of important signaling molecules in cells.

ACKNOWLEDGMENTS

This work was funded by the University of the West of England, Bristol, UK, who financed the authors' time and literature sourcing for the preparation of this manuscript.

REFERENCES

1. Bucher T, Brauser B, Conze A, Klein F, Langguth O, Sies H. State of oxidation-reduction and state of binding in the cytosolic NADH-system as disclosed by equilibration with extracellular lactate-pyruvate in hemoglobin-free perfused rat liver. *Eur J Biochem* 1972; 27(2):301–17.
2. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001; 30(11):1191–212.
3. Harris C, Hansen JM. Oxidative stress, thiols, and redox profiles. *Methods Mol Biol* 2012; 889:325–46. doi: 10.1007/978-1-61779-867-2_21.
4. Bücher T, Sies H. Metabolic interaction of mitochondrial and cytosolic systems in rat liver. In: *Cell Compartmentation and Metabolic Channeling* (L Nover, F Lynen, K Mothes). Fischer Verlag, Jena, Germany.1980, pp. 279-302.
5. Forman HJ, Ursini F, Maiorino M. An overview of mechanisms of redox signaling. *J Mol Cell Cardiol* 2014; 73:2–9. doi: 10.1016/j.yjmcc.2014.01.018.
6. Rost J, Rapoport S. Reduction-potential of glutathione. *Nature* 1964; 201:185.
7. Forman HJ. Glutathione: from antioxidant to post-translational modifier. *Arch Biochem Biophys* 2016; 595:64–7. doi: 10.1016/j.abb.2015.11.019.
8. Ghibelli L, Coppola S, Rotilio G, Lafavia E, Maresca V, Ciriolo MR. Non-oxidative loss of glutathione in apoptosis via GSH extrusion. *Biochem Biophys Res Commun* 1995; 216(1):313–20. doi: 10.1006/bbrc.1995.2626.
9. Hwang C, Lodish HF, Sinskey AJ. Measurement of glutathione redox state in cytosol and secretory pathway of cultured cells. *Methods Enzymol* 1995; 251:212–21.
10. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr* 2004; 134(3):489–92.
11. Ghezzi P, Chan P. Redox proteomics applied to the thiol secretome. *Antioxid Redox Signal* 2017; 26(7):299–312. doi: 10.1089/ars.2016.6732.
12. Heinrich TA, da Silva RS, Miranda KM, Switzer CH, Wink DA, Fukuto JM. Biological nitric oxide signalling: chemistry and terminology. *Br J Pharmacol* 2013; 169(7):1417–29. doi: 10.1111/bph.12217.
13. Noctor G, Foyer CH. Intracellular redox

compartmentation and ros-related communication in regulation and signaling. *Plant Physiol* 2016; 171(3):1581–92. doi: 10.1104/pp.16.00346.
14. Hancock JT. The role of redox mechanisms in cell signalling. *Mol Biotechnol* 2009; 43(2):162–6. doi: 10.1007/s12033-009-9189-1.
15. Hancock JT, Whiteman M. Hydrogen sulfide and cell signaling: team player or referee? *Plant Physiol Biochem* 2014; 78:37–42. doi: 10.1016/j.plaphy.2014.02.012.
16. Dai C, Cui W, Pan J, Xie Y, Wang J, Shen W. Proteomic analysis provides insights into the molecular bases of hydrogen gas-induced cadmium resistance in Medicago sativa. *J Proteomics* 2017; 152:109–20. doi: 10.1016/j.jprot.2016.10.013.
17. Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, et al. Hydrogen sulfide-linked sulfhydration of NF-kappaB mediates its antiapoptotic actions. *Mol Cell* 2012; 45(1):13–24. doi: 10.1016/j.molcel.2011.10.021.
18. Ohta S. Molecular hydrogen as a novel antioxidant: overview of the advantages of hydrogen for medical applications. *Methods Enzymol* 2015; 555:289–317. doi: 10.1016/bs.mie.2014.11.038.
19. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol* 2014; 24(10):R453–62. doi: 10.1016/j.cub.2014.03.034.
20. Birtic S, Colville L, Pritchard HW, Pearce SR, Kranner I. Mathematically combined half-cell reduction potentials of low-molecular-weight thiols as markers of seed ageing. *Free Radic Res* 2011; 45(9):1093–102. doi: 10.3109/10715762.2011.595409.
21. Jones DP, Carlson JL, Mody VC, Cai J, Lynn MJ, Sternberg P. Redox state of glutathione in human plasma. *Free Radic Biol Med* 2000; 28(4):625–35.
22. Hutter DE, Till BG, Greene JJ. Redox state changes in density-dependent regulation of proliferation. *Exp Cell Res* 1997; 232(2):435–8. doi: 10.1006/excr.1997.3527.
23. Kirlin WG, Cai J, Thompson SA, Diaz D, Kavanagh TJ, Jones DP. Glutathione redox potential in response to differentiation and enzyme inducers. *Free Radic Biol Med* 1999; 27(11–12):1208–18.
24. Jones DP, Maellaro E, Jiang S, Slater AF, Orrenius S. Effects of *N*-acetyl-L-cysteine on T-cell apoptosis are not mediated by increased cellular glutathione. *Immunol Lett* 1995; 45(3):205–9.
25. Cai J, Wallace DC, Zhivotovsky B, Jones DP. Separation of cytochrome c-dependent caspase activation from thiol-disulfide redox change in cells lacking mitochondrial DNA. *Free Radic Biol Med* 2000; 29(3–4):334–42.
26. Hwang C, Sinskey AJ, Lodish HF. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* 1992; 257(5076):1496–502.
27. Bhatla N, Horvitz HR. Light and hydrogen peroxide inhibit *C. elegans* feeding through gustatory receptor orthologs and pharyngeal neurons. *Neuron* 2015; 85(4):804–18. doi: 10.1016/j.neuron.2014.12.061.
28. Kamsler A, Segal M. Hydrogen peroxide modulation of synaptic plasticity. *J Neurosci* 2003; 23(1):269–76.
29. Bolm M, Jansen WT, Schnabel R, Chhatwal GS. Hydrogen peroxide-mediated killing of *Caenorhabditis elegans*: a common feature of different streptococcal species. *Infect Immun* 2004; 72(2):1192–4.
30. Go YM, Jones DP. Redox compartmentalization in eukaryotic cells. *Biochim Biophys Acta* 2008; 1780(11):1273–90. doi: 10.1016/j.bbagen.2008.01.011.
31. Koppenol WH, Butler J. Energetics of interconversion reactions of oxyradicals. *Adv Free Rad Biol Med* 1985; 1:91–131.
32. Holme DJ, Peck H. *Analytical Biochemistry*. Longman Technical Scientific, Harlow, London, UK. 1983.
33. Wells WW, Xu DP. Dehydroascorbate reduction. *J Bioenerg Biomembr* 1994; 26(4):369–77.
34. Nicholls DG, Ferguson SJ. *Bioenergetics 2*. Academic Press, London, UK. 1992.
35. Goodwin TW, Mercer EI. *Introduction to Plant Biochemistry*. Pergamon Press, Oxford, UK. 1983.
36. Koppenol WH. The chemical reactivity of radicals. In: *Free Radical Toxicology* (KB Wallace). Taylor and Francis, London, UK. 1997, pp. 3–14.
37. Stanbury DM. Reduction potentials involving inorganic free radicals in aqueous solution. *Adv*

Inorgan Chem 1989; 33:69–138.
38. Bartberger MD, Liu W, Ford E, Miranda KM, Switzer C, Fukuto JM, et al. The reduction potential of nitric oxide (NO) and its importance to NO biochemistry. *Proc Natl Acad Sci USA* 2002; 99(17):10958–63. doi: 10.1073/pnas.162095599.
39. Li Q, Lancaster JR, Jr. Chemical foundations of hydrogen sulfide biology. *Nitric Oxide* 2013; 35:21–34. doi: 10.1016/j.niox.2013.07.001.
40. Krebs HA, Veech RL. Pyridine-nucleotide interrelations. In: *The Energy Level and Metabolic Control in Mitochondria* (S Papa, JM Tager, SC Slater). Adriatica Editrice, Bari, Italy. 1969. pp. 329–82.
41. Moyle J, Mitchell P. The proton-translocating nicotinamide-adenine dinucleotide (phosphate) transhydrogenase of rat liver mitochondria. *Biochem J* 1973; 132(3):571–85.
42. Orenha RP, Galembeck SE. Molecular orbitals of NO, NO^+, and NO^-: a computational quantum chemistry experiment. *J Chem Educ* 2014; 91:1064–9. doi: 10.1021/ed400618j.
43. Hughes MN. Relationships between nitric oxide, nitroxyl ion, nitrosonium cation and peroxynitrite. *Biochim Biophys Acta* 1999; 1411(2–3):263–72.
44. Hancock JT, Desikan R, Neill SJ. Does the redox status of cytochrome c act as a fail-safe mechanism in the regulation of programmed cell death? *Free Radic Biol Med* 2001; 31(5):697–703.