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ARTICLE

Grigory G. Martinovich · Sergey N. Cherenkevich
Heinrich Sauer

Intracellular redox state: towards quantitative description

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Abstract Redox state is a widely used term for the description of redox phenomena in biological systems. The regulating mechanisms responsible for maintaining the redox state are not yet fully known. But it was shown that changes in the redox state might lead to a cascade of intracellular events, beneficial or deleterious to the cell. There are several methods for the description of the intracellular redox state. These methods are based on using measured intracellular concentrations of reduced and oxidized glutathione in the Nernst equation. However, glutathione is not always a basic redox component in biological fluids, organelles, cells or tissues. As a result, changes in the intracellular redox state are not always accompanied by considerable changes of glutathione concentration. In this work it was proposed to use the concept of effective reduction potential for the quantitative characteristic of the intracellular redox state. The effective reduction potential was substantiated on the basis of a thermodynamic description. A new equation for the calculation of the effective reduction potential was derived. This equation summarizes the contribution of different oxidizing and reducing agents in the formation of an effective redox potential. The theoretical estimation of the effective reduction potential values for the different biological fluids and cells was carried out with the use of a method developed.

Keywords Reduction potential · Redox state · Oxidizing agent · Reducing agent · Nernst equation

G. G. Martinovich (✉) · S. N. Cherenkevich
Department of Biophysics, Belarus State University,
Fr. Skorina Ave., 4, 220050 Minsk, Belarus
E-mail: martinovichgg@bsu.by
Tel.: +375-172-095440
Fax: +375-172-095445
E-mail: cherenkevich@bsu.by

H. Sauer
Institute of Physiology, Aulweg 129,
35392 Giessen, Germany
E-mail: heinrich.sauer@physiologie.med.uni-giessen.de

Introduction

Redox processes, involving the transfer of electrons, are central processes of energy conversion in respiratory organisms. Furthermore, it is now realized that many cellular processes depend on the intracellular redox state. In generally, the redox state is defined as a result of the balance between the levels of oxidized and reduced species of redox couples. Cells maintain the reduced intracellular state in the face of the highly oxidizing extracellular environment.

During recent years, considerable evidence has been accumulated to show that the intracellular redox state can regulate cellular growth and development (Sauer et al. 2001; Droge 2002). The redox state also plays a crucial role in cell proliferation (Hutter et al. 1997; Aw 2003). Agents altering the intracellular redox state initiate differentiation in dividing oligodendrocyte type-2 astrocyte progenitor cells (Smith et al. 2000). Moreover, cardiac differentiation of embryonic stem cells is critically regulated by exogenous as well as endogenous reactive oxygen species (ROS) (Sauer et al. 1999, 2000).

Changes in the intracellular redox state initiate various signaling pathways and regulate the transcriptional and post-transcriptional events that control gene expression (Haddad2002). It was shown that changes in the endothelial cell redox state cause transcription-independent and transcription-dependent surface expression of different endothelial cell adhesion molecules, which leads to neutrophil–endothelial adhesion (Kokura et al. 1999). Very often, reduction or oxidation of protein redox-active groups is a key event in enzymatic reactions. The function of most proteins crucially depends on the redox state of the solution (Ullmann and Knapp 1999).

Now the term ''redox state'' is used widely in free-radical biology. Unfortunately, at present the term is not exactly defined. Historically, the term redox state has been used to describe the concentration ratio of the oxidized and reduced form of a specific redox couple. For example, Krebs (1967) defined the redox state of the

NAD^+/NADH couple in a cell to be the ratio of the concentration free NAD^+ to free NADH. But cells contain many different redox couples. Moreover, various redox couples are linked to each other to form a set of related couples. The intracellular redox state is a reflection of the state of these couples. In recent years, the term redox state has been used not only to describe the state of a particular redox couple, but also more generally to describe the redox environment of a cell (Schafer and Buettner2001). This more general use of the term is also not very well defined and differs considerably from historical use.

There are various methods of monitoring redox state changes in cells, including the measurement of the intracellular concentrations of reduced and oxidized glutathione (GSH and GSSG), the measurement of the intracellular concentrations of $NAD(P)^+$ and NAD(P)H (Adams et al. 2001), the use of fluorescent indicators such as RedSensor Red CC-1 (Chen and Gee 2000) or engineered green fluorescent proteins (Ostergaard et al. 2001). However, these methods use different approaches to the redox state description and give different quantitative results that hamper the comparison of the data obtained.

In view of the central role of redox chemistry in many cellular functions we suggest that the development of a quantitative redox state description is of fundamental importance to understanding life processes in respiratory organisms. Here, we derive a new equation for the description of the redox state in different biological media. The equation summarizes the contribution of different redox couples in the process of redox state formation. It may be used for a description and an understanding of the cellular redox mechanisms associated with cell growth and development.

Results and discussion

Schafer and Buettner (2001) proposed the use of the term "redox environment" for the description of a linked set of redox couples. According to their definition, the redox environment is the summation of the reduction potentials of redox couples found in biological fluids, organelles, cells or tissues, multiplied by the concentration of reduced species in the redox couple. In mathematical terms, the redox environment is represented by

$$\text{Redox environment} = \sum_{i=1}^{n} E_i \cdot [\text{reduced species}]_i, \qquad (1)$$

where E_i is the reduction potential of the given redox pair and $[\text{reduced species}]_i$ is the concentration of the reduced species in that redox pair. However, the units of the results of the calculation in Eq. 1 do not allow easy interpretation of the results obtained. Consequently, Hancock et al. (2004) suggested that the redox environment should be calculated as a mean of the redox contributions of reduced and oxidized couples. Equation 1 was modified to

$$\text{Redox environment} = \frac{\sum_{i=1}^{n} E_i \cdot [\text{total concentration of couple}]_i}{\sum_{i=1}^{n} [\text{total concentration of couple}]_i}, \qquad (2)$$

where E_i is the reduction potential of the given redox pair and $[\text{total concentration of couple}]_i$ is the concentration of the reduced and oxidized species of the couple. The values of E_i in Eq. 2 are calculated using the Nernst equation:

$$E_i = E_\mathrm{m} - \frac{RT}{zF} \ln \frac{[A]}{[A^+]}, \qquad (3)$$

where R is the gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature in kelvin, F is the Faraday constant (9.6485×10^4 C mol^{-1}), z is the number of electrons which transfer to the oxidized species of the couple in one redox reaction, $[A^+]$ is the concentration of the oxidized species of the couple, $[A]$ is the concentration of the reduced species of the couple. E_m is the midpoint potential, at which the concentration of the reduced species and concentration of the oxidized species for any given redox couple are equal.

However, this approach does not take into consideration the number of electrons transferred in redox reactions. Moreover, in both foregoing approaches the authors suggest using the glutathione disulfide–glutathione couple (GSSG/2GSH) for a description of the intracellular redox state. The reduction potential of the GSSG/2GSH couple calculated with the Nernst equation is used most often for the quantitative description of changes in redox state (Kirlin et al. 1999; Hoffman et al. 2001; Jones et al. 2002; Rebrin et al. 2003; Nunez et al. 2004). However, the intracellular redox state is formed as a result of the interaction of many redox couples. Changes of the redox state of these couples do not necessarily induce changes in the reduction potential of the GSSG/2GSH couple. Recently, it was demonstrated that the ROS generated from spermine nonoate in human endothelial cells decrease intracellular ascorbate concentrations, although GSH was not affected (May and Qu2004). It was also shown that nitrogen dioxide depletes uric acid and ascorbic acid but not glutathione in respiratory tract lining fluid (Kelly and Tetley1997). Moreover, the GSSG/2GSH couple in biological fluids, organelles, cells or tissues is not always a basic redox couple in the medium. Quantitative analysis of ascorbate and glutathione contents in developing rat cortex and hippocampus studies supplemented with appropriate intracellular and extracellular volume fraction data revealed that ascorbate predominates in neurons, whereas glutathione is predominant in glia (Rice and Russo-Menna1998). The glutathione concentration in blood is much less than the concentration of uric acid and ascorbic acid (Jones et al. 2000).

Given that animal tissues contain approximately 99% GSH, and rarely approach even 25% GSSG under the most oxidizing conditions, the approximate full cellular ranges of the reduction potential of the GSSG/2GSH couple lie between −140 and −250 mV in the cytosol (Dalton et al. 2004). On other hand, the reduction potentials of different redox couples in biological systems lie between −320 [HAD(P)$^+$/HAD(P)H couple] and +820 mV (O_2/H_2O couple).

Therefore, a new fundamental equation taking into account the contribution of different redox couples in the formation of the redox state in cells is required to describe quantitatively the redox environment in different media. In this article a new equation for the calculation of the redox state on basis of a thermodynamic description of natural processes is derived.

Consider a medium that contains a linked set of k redox couples. According of the first law of thermodynamics the free-energy change in a reversible transition at constant (p, T) is equal to the work done. Thus, electrical work (W_{el}) carrying out the moving of a charge (Q) through an electrical potential difference (E) is equal to the free-energy change ($-\Delta G$). The transferred charge is equal to the Faraday constant, the molar value of the electronic charge, multiplied by the valence of the charge (z) and the number (n) of moles of electrons transferred in the redox species. Thus,

$$W_{el} = -\Delta G = QE = znFE. \tag{4}$$

We suggest that the redox state of a linked set of redox couples should be described by the effective reduction potential (E^{eff}), which can be defined as

$$E^{eff} = \frac{W_{el}}{Q}. \tag{5}$$

In the equilibrium state of a multicomponent system the resulting free-energy change is

$$\Delta G = \sum_{i=1}^{k} G_i, \tag{6}$$

where G_i is free energy for a given reaction. The charge transferred in redox reactions for reduction of species in k redox couples is

$$Q = \sum_{i=1}^{k} z_i n_i F, \tag{7}$$

where z_i is the number of electrons which transfer to the oxidized species of a given couple in one redox reaction and n_i is the number of moles of electrons transferred in reactions for a given species. The electrical work carried out for the transfer of electrons in all reactions is

$$W_{el} = -\sum_{i=1}^{k} G_i = \sum_{i=1}^{k} z_i n_i F E_i, \tag{8}$$

where E_i is the reduction potential of the given redox pair. Then, the effective reduction potential of the system is defined by

$$E^{eff} = \frac{W_{el}}{Q} = \frac{\sum_{i=1}^{k} z_i n_i F E_i}{\sum_{i=1}^{k} z_i n_i F} = \frac{\sum_{i=1}^{k} z_i n_i E_i}{\sum_{i=1}^{k} z_i n_i}. \tag{9}$$

Using the expression $c_i = n_i/V_s$, where V_s is the volume of the solution and c_i is the molar concentration of the reduced species in a given redox couple, we obtain

$$E^{eff} = \frac{\sum_{i=1}^{k} z_i (n_i/V_s) E_i}{\sum_{i=1}^{k} z_i (n_i/V_s)} = \frac{\sum_{i=1}^{k} z_i c_i E_i}{\sum_{i=1}^{k} z_i c_i}. \tag{10}$$

Equation 10can be reorganized to form

$$E^{eff} = \sum_{i=1}^{k} a_i E_i, \tag{11}$$

where

$$a_i = \frac{c_i z_i}{\sum_{j=1}^{k} c_j z_j} \tag{12}$$

is the "specific charge" transferred in a given reaction. The specific charge is equal to the ratio of the charge transferred in a given reaction to the total charge transferred in all reactions. In the special case for one-electron redox the reaction coefficient a_i is the mole fraction of reduced species in the redox couple.

This definition allows us to consider the "effective reduction potential" as quantitative parameter of an intracellular redox state characterizing the total reductive capacity of the medium. The more negative the value of the effective reduction potential (E^{eff}) the larger the reductive capability of the medium.

The exploitable reduction potentials of GSSG/2GSH and NAD(P)$^+$/NAD(P)H couples result in different values of the reduction potential in a medium that hamper the comparison of the data obtained. For example, the reduction potentials of the GSSG/2GSH and NAD(P)$^+$/NAD(P)H couples in mouse striatum are −233 and −327 mV, respectively (Adams et al. 2001). The reduction potentials of the GSSG/2GSH and NAD(P)$^+$/NAD(P)H couples in rat hepatocyte cytoplasm are −250 and −280 mV, respectively (Eberle et al. 1981; Marques-da-Silva et al. 1997). On the other hand, the value of the effective reduction potential summarizes the contribution of different redox couples in the formation of the redox state in cells.

The values of E^{eff} in different biological fluids, organelles and cells estimated using Eq. 11 are presented in Table 1. The effective reduction potentials were calculated with the use of the mean concentrations of basic reducing agents [GSH, ascorbic acid, NAD(P)H and uric acid] and the values of its reduction potentials at pH 7. In the articles cited the concentrations of basic reducing agents were generally determined using high-performance liquid chromatography with electrochemical detection (for uric acid and ascorbic acid) or fluorescence detection [for NAD(P)H and GSH].

The values of E^{eff} in Table 1 indicate only the contribution of basic reducing agents in the formation of the

Table 1 The average concentration of the main reducing agents in biological fluids, organelles and cells and the values of the effective reduction potential

	GSH (mM)	AscH (mM)[a]	NAD(P)H (mM)	UH_2^- (mM)[b]	Reference	Calculated value of E^{eff} (mV)
Human respiratory tract lining fluids	0.001	0.07	0.001	0.01	(Ames et al. 1981; Sies 1982; Van der Vliet et al. 1999)	+200
Rat β-cell cytoplasm	1.5	4	0.04	0.01	(Steffner et al. 2004)	+55
Human monocyte cytoplasm	2	5	0.03	0.01	(Evans et al. 1982; Levy et al. 1993)	+50
Human neuron cytoplasm	2.5	5	0.03	0.001	(Rice and Russo-Menna 1998; Toien et al. 2001)	+20
Human erythrocyte cytoplasm	2	0.3	0.03	0.01	(Beckar et al. 1994)	−200
Human astrocyte cytoplasm	8	0.8	0.03	0.001	(Rice and Russo-Menna 1998; Toien et al. 2001)	−215
Rat bile	1	0.05	0.001	0.1	(Ballatori and Truong 1992)	−180
Rat hepatocyte cytoplasm	7	1	0.03	0.01	(Bellomo et al. 1992)	−205
Rat hepatocyte mitochondria	10	1	6	0.01	(Costantini et al. 1996)	−260

[a]Ascorbic acid
[b]Uric acid

redox state and do not take into account the contribution of oxidants, the protein's thiols and other redox components. However, even such an approximate approach indicates that the value of E^{eff} for different media may serve as a new characteristic of the redox capability of the medium.

As indicated, the data presented for the concentrations of basic reducing agents in different biological fluids, organelles and cells vary over a broad range and this results in different values of the effective reduction potential in biological media. The data obtained show that E^{eff} is greatest in astrocytes and hepatocytes and is less in other cells. E^{eff} in human respiratory tract lining fluids is the lowest.

Recently, considerable evidence has been accumulated to show that the reduction potential of the GSSG/2GSH couple ($E_{GSSG/2GSH}$) is also tissue-dependent. The reduction potential of the GSSG/2GSH couple has been calculated for various tissue from mice (Dalton et al. 2004). It was shown that the reduction potential of GSSG/2GSH changes between −229 (for liver) and −35 mV (for plasma). However, the values of the effective reduction potential in different tissues lie in a wider range (Table 1). This difference between the range of the $E_{GSSG/2GSH}$ values and the E^{eff} values indicates that the magnitude of E^{eff} is more effective as a redox indicator.

For example, under conditions of oxidative stress the concentration of oxidants is increased, which results in shifting of the $E_{GSSG/2GSH}$ value and the E^{eff} value to a positive one. The $E_{GSSG/2GSH}$ value in liver reported by Dalton et al. (2004) was −229 mV (GSH 7.942 mM; GSSG 0.149 mM) and the E^{eff} value in liver obtained in this work was −205 mV. The oxidation of hepatocyte

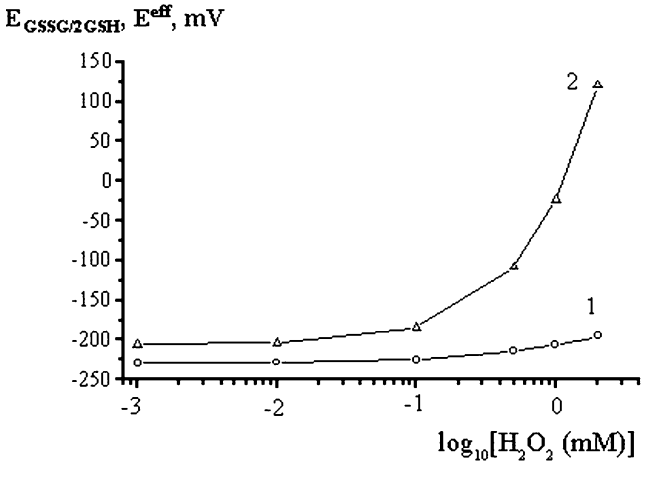

Fig. 1 Theoretical reduction potential of the glutathione disulfide–glutathione (*GSSG/2GSH*) couple and the effective reduction potential in hepatocytes as a function of the intracellular H_2O_2 concentration. *1* $E_{GSSG/2GSH}$, *2* E^{eff}

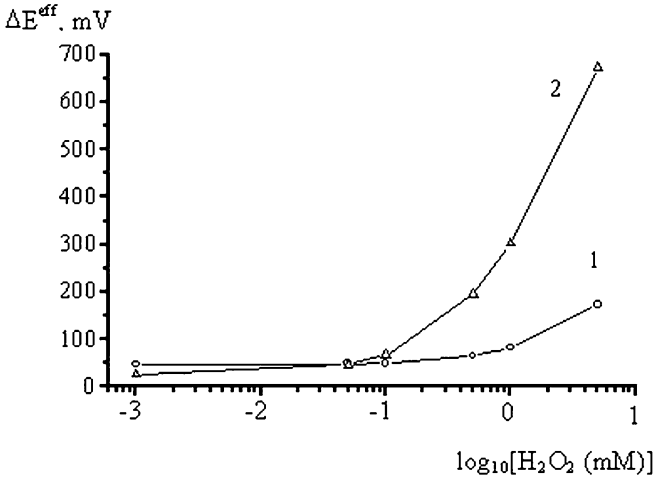

Fig. 2 The dependence of the transmembrane difference of the effective reduction potential on the concentration of H_2O_2. *1* ΔE^{eff} on the mitochondrial membrane, *2* ΔE^{eff} on the plasmatic membrane

GSH by 0.5 mM H_2O_2 will change the $E_{GSSG/2GSH}$ value to −214 mV (GSH 7.442 mM; GSSG 0.399 mM), i.e., the shifting of the $E_{GSSG/2GSH}$ value induced by 0.5 mM H_2O_2 is 15 mV. On other hand, the shifting of the E^{eff} value induced by 0.5 mM H_2O_2 is 97 mV (from −205 to −108 mV, calculated using Eq. 11). The theoretical reduction potentials of the GSSG/2GSH couple and the effective reduction potentials in liver as a function of the intracellular H_2O_2 concentration are presented in Fig. 1.

As different cellular compartments have different concentrations of basic reducing agents (Table 1), the effective reduction potential is organelle-dependent. E^{eff} in the hepatocyte cytoplasm is typically −205 mV, whereas E^{eff} in the mitochondrial matrix is −260 mV. Thus, there is a transmembrane difference of the effective reduction potentials (ΔE^{eff}) between cellular cytoplasm and mitochondria, and also between cellular cytoplasm and extracellular fluids.

The changes in ΔE^{eff} on the mitochondrial and the plasmatic membrane with an increase in the H_2O_2 concentration were estimated using Eq. 11 (Fig. 2). It is proposed that the H_2O_2 concentration in mitochondria, the cytoplasm and the extracellular medium increases equally. ΔE^{eff} between cellular cytoplasm and extracellular fluids changed more quickly than ΔE^{eff} between cellular cytoplasm and mitochondria. That is, the changes in E^{eff} in cells depend on the reductant's concentration and its type. It may be important in the regulation of transmembrane transport processes under the redox state changes.

The proposed thermodynamic approach shows new possibilities of redox mechanism description. The use of the characteristic effective reduction potential allows us to interpret a number of intracellular and extracellular redox signaling phenomena. Changes in the effective redox potentials may control the oxidation or reduction of protein thiol groups that will be accompanied by the conformation alteration of these proteins and thus modulate their function. The more accurate definition of protein redox potentials and medium redox potentials permits us to determine whether the cellular processes are activated or not at the respective redox state. The understanding of cellular redox processes will allow us to develop new methods for the control of cellular functions.

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