Bioenergetics: Open Access
ISSN: 2167-7662

Piloni and Puntarulo, Bioenergetics 2016, 5:2
DOI: 10.4172/2167-7662.1000e125

Editorial Open Access

A Simple Kinetic Model to Estimate Ascorbyl Radical Steady State Concentration in Rat Central Nervous System. Effect of Subchronic Fe Overload

Natacha E Piloni and Susana Puntarulo*
Institute of Biochemistry and Molecular Medicine, University of Buenos Aires, Conicet, Physical Chemistry, Faculty of Pharmacy and Biochemistry, Junín, Buenos Aires, Argentina

***Corresponding author:** Puntarulo S, Fisicoquímica, Institute of Biochemistry and Molecular Medicine, Faculty of Pharmacy and Biochemistry Junín 956, CAAD1113, Buenos Aires, Argentina, Tel: 54-11-5-287-4233; E-mail: susanap@ffyb.uba.ar

Rec date: Jul 13, 2016; **Acc date:** Nov 10, 2016; **Pub date:** Nov 14, 2016

Abstract

All biological systems, contain many antioxidants; including water-soluble compounds, such as ascorbic acid. The one-electron oxidation of ascorbate (AH^-produces the ascorbyl radical ($A^\bullet$), that has a relatively long lifetime compared to other species, such as hydroxyl radical, peroxyl, alkoxyl, and carbon-centered lipid free radicals. $A^\bullet$ has been proposed as a marker of oxidative stress either *in vitro*, or *in vivo* in numerous systems. The application of a simple kinetic analysis allowed us to estimate the steady state concentration of $A^\bullet$. These data were successfully compared to experimental values obtained using Electron Paramagnetic Resonance (EPR) in rat brain under physiological conditions. The model was also applied to estimate the response to stress due to subchronic Fe overload.

Keywords: Ascorbyl radical; Brain; Iron; Kinetic model; Ascorbyl radical steady state concentration; Oxidative stress

Abbreviations AH^-: Ascorbate; $A^\bullet$: Ascorbyl Radical; EPR: Electron Paramagnetic Resonance; DHA: Dehydroascorbate; AO: Ascorbate Oxidase; $^\bullet OH$: Hydroxyl Radical; $ROO^\bullet$: Peroxyl Radical; α-$T^\bullet$: Tocopheryl Radical; MDAR: Monodehydroascorbate Reductase; LIP: Labile Iron Pool; FW: Fresh Weight

Editorial

There is an increasing interest in the use of ascorbyl radical content ($A^\bullet$) in biological tissues as an informative, non-invasive and natural indicator of oxidative stress [1] in the hydrophilic medium. It was also showed that $A^\bullet$ steady state concentration in tissues may be considered as an objective but not a universal indicator characterizing oxidative stress intensity, since ascorbate (AH^-) oxidation rate depends on AH^- concentration, pH and non-disproportionation mechanisms of $A^\bullet$ decay. A basic kinetic approach was applied to calculate $A^\bullet$ steady state concentration in the rat brain under physiological conditions. Ascorbic acid is an especially significant component, due to the fact that its low redox potential, allows it to donate one single electron to almost any free radical occurring in a biological system or to reduce oxidized biological radical scavengers, such as α-tocopherol [1]. The $A^\bullet$ is the intermediate in the oxidation of AH^- to dehydroascorbate (DHA) [2]. It has an unpaired electron in a highly delocalized π-system, giving stability to it as the terminal small-molecule antioxidant [1]. The concentration of $A^\bullet$ is a dynamic value, which is determined by the rates of generation and decay of this species. $A^\bullet$ is formed by AH^- oxidation processes. In cells from the nervous system, AH^- oxidation could be catalyzed either by the activity of the enzyme ascorbate oxidase (AO), detected in the plasmatic membrane of the synaptic terminals in rat brain [3], O_2, Fe^{2+}, Fe^{3+}, Cu^{2+} [1], $O2^{\bullet-}$, hydroxyl radical ($^\bullet OH$) [4], and other radicals such as peroxyl radical ($ROO^\bullet$), and tocopheryl radical (α-$T^\bullet$) [5]. The decay of $A^\bullet$ is related to the activity of the enzyme monodehydroascorbate reductase (MDAR), the spontaneous dismutation of $A^\bullet$ to AH^- and DHA, and other reactions (Figure 1). Applying a simple kinetic analysis, the following rate expression can be formulated for the velocity of generation of $A^\bullet$ ($vA^\bullet$generation) (equation 1).

$$vA^\bullet \text{ generation} = d[A^\bullet]/dt = (d[A^\bullet]/dt)AO + (d[A^\bullet]/dt)O_2 + (d[A^\bullet]/dt)Fe^{3+} + (d[A^\bullet]/dt)O_2^{\bullet-} + (d[A^\bullet]/dt)\,{}^\bullet OH^+ + (d[A^\bullet]/dt)ROO^\bullet + (d[A^\bullet]/dt)\alpha\text{-}T^{\bullet+} \text{other radicals} \quad (1)$$

According to Martín-Romero et al. [3], the activity of AO in the synaptic terminals of rat brain is 40 nmol $A^\bullet$ min^{-1} (mg protein)$^{-1}$. Thus, taking into account that in brain tissues there are 72 mg protein (g fresh weigh, FW)$^{-1}$, and that 1 g FW represents 0.82 mL of water, the rate of generation of $A^\bullet$ by this reaction could be estimated as $58.5 \times 10^{-6} Ms^{-1}$.

The non-enzymatic autoxidation of AH^- to generate $A^\bullet$ [6], is a very slow reaction at physiological pH ($k = 6 \times 10^{-7} s^{-1}$) [4] and could be discarded. In aqueous solutions, the rate of generation of $A^\bullet$ catalyzed by either metals or other radicals could be estimated by a second rate reaction (equation 2) .

$$V = k\,[AH^-]\,[\text{metal or radical}] \quad (2)$$

The k value for $O2^{\bullet-}$ is 2.7×10^5 $M^{-1}s^{-1}$ [7], for $^\bullet OH$ is 7.2×10^9 $M^{-1}s^{-1}$ [8], and for $ROO^\bullet$ is 1 to 2×10^6 $M^{-1}s^{-1}$ [9]. Piloni et al. [10] reported by HPLC measurements, an AH^- concentration of 0.29 ± 0.02 nmol/mg FW or 2.9×10^{-6} M steady state concentration in rat control brain, taking into account the protein and water content indicated in the brain tissue. Thus, according to the estimated k values and the steady state concentration of free radicals [11], only the generation rate catalyzed by $ROO^\bullet$ (0.01×10^{-6} Ms^{-1}) is comparable to the generation rate of $A^\bullet$ catalyzed by the activity of AO in rat brain.

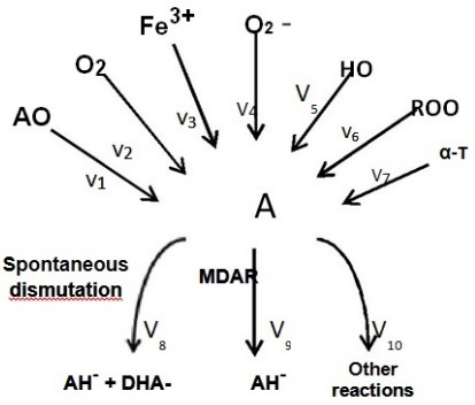

Figure 1: The decay of A• is related to the activity of the enzyme monodehydroascorbate reductase (MDAR), the spontaneous dismutation of A• to AH^- and DHA, and other reactions.

The other terms in equation 1 could be discarded, since they are more than two orders of magnitude lower as compared to the enzymatic catalyzed generation rate. On the other hand, the labile iron pool (LIP) in rat brain, assessed by a fluorescent measurement [10], is 3.6 ± 0.9 pmol (mg FW)$^{-1}$, or approximately 1.8 × 10^{-6} M. Since the k value for the reaction catalyzed by Fe^{3+} is 30 $M^{-1}s^{-1}$ [12], the rate of generation of A• described by the equation 2 is 1.6 × 10^{-10} Ms^{-1}.

Finally, the total rate of generation of A• in rat brain could be estimated as 58×10^{-6} Ms^{-1} from the addition of the rate of the reactions catalyzed by the activity of AO, ROO• and Fe^{3+}.

The rate of disappearance of A• could be calculated with equation 3,

vA• disappearance = -d[A•]/dt = (- d[A•]/dt)dismutation + (- d[A•]/dt)MDAR + (d[A•]/dt)other radicals (3)

The rate of disappearance of A• by dismutation could be estimated assuming a value for k of 1.5 × 10^6 $M^{-1}s^{-1}$ [10,13,14]. In rat brain, the activity of MDAR is 13 × 10^{-3} U NADH min^{-1} (mg protein)$^{-1}$, where 1 U is the amount of enzyme that uses 1 µmol of substrate [15].

Thus, the rate of disappearance of A• is 26 × 10^{-9} moles A• min^{-1} (mg protein)$^{-1}$ or approximately 38 × 10^{-6} Ms^{-1}. The expression (d[A•]/dt)other radicals includes free radical dependent termination reactions or reactions of A• that occur in the plasmatic membranes probably due to the activity of cytochrome b [16], and are understood as minor reactions that could be discarded for the purpose of this simple analysis.

Under physiological control conditions there should be no significant changes in A• concentration over time (d[A•]/dt = 0) and, thus, generation and disappearance rate of A• are equal. Using the kinetic model shown here, the [A•] could be estimated as 2.6 µM for rat brain.

The direct detection of A• employing Electron Paramagnetic Resonance (EPR) in biological systems is possible since both, AH^- and DHA, are spectroscopically silent [6]. Moreover, A• has an extraordinary long lifespan (50 s) as compared to other radical species [4], rendering its identification at room temperature feasible. EPR spectra from rat brain homogenates presented a duplet with the following spectral parameters: g = 2.005 and A*h* = 1.8 G, consistent with the EPR spectrum of A•, and the experimentally measured steady state concentration was 3.2 µM, considering previous data shown by Piloni et al. [10]. The adjustment between the experimental value and the data estimated by this simple kinetic analysis is quite good, even though the contribution of some reactions was excluded. The agreement between these values suggested that the approximations and estimations done conducted to a feasible description of the physiological processes occurring in rat brain (Table 1).

	A• steady state concentration	
	EPR measurements	Calculated estimation
Control	3.2 ± 0.4 µMa	2.6 µM
Subchronic Fe overload		
2 h post-treatment	2.7 ± 0.3 µMa	3.1 µM
4 h post-treatment	5.4 ± 0.5µMa,*	4.4 µM

[a]Taken from Piloni et al. (2016) [17].

*significantly different from control values. ANOVA ($p < 0.05$).

Table 1: A•steady state concentration in rat brain.

Moreover, this model could be successfully applied to estimate A• steady state concentration in rat brain under conditions of subchronic Fe overload developed by the administration ip of six doses of 50 mg (kg body weight)$^{-1}$ of Fe-dextran. The LIP increased to 17.1 and 13.5 µM after 2 and 4 h of the injection of the 6th dose of Fe-dextran, respectively [17]. The AH^- concentration was 1.6 and 1.5 µM, after 2 and 4h, respectively. Applying the kinetic model reported here under these conditions, the A• steady state concentration resulted as 3.1 and 4.4 µM after 2 and 4 h, respectively. These data showed no changes after 2 h and an increase of 70% after 4 h, as compared to control values. Meanwhile, EPR measurements resulted in an A• steady state concentration of 2.7 and 5.4 µM after 2 and 4 hours, respectively, showing an increase of 86% after 4 h as compared to control values, in good agreement with the estimated values.

Thus, even though further studies are required to corroborate that this model could be successfully employed under other situations of

oxidative stress, it seems that the basic considerations applied to the mechanism of the reactions involved in the keeping of a controlled $A^{\bullet}$ steady state concentration in rat brain could be described by this simple kinetic model (or a variation of it). An analytical approach of this nature was not performed for rat brain up to the present, and intents to be an initial step to understand the complex scenario of the network of free radical reactions in the brain cells.

Acknowledgments

Supported by grants from the University of Buenos Aires, ANPCyT and CONICET. S.P. is career investigator from CONICET, and N.P. is a member of the career of professional technical assistance from CONICET.

References

1. Malanga G, Aguiar MB, Puntarulo S (2012) Oxidative stress in aquatic ecosystems. (1st edn.). In: Abele D, Zenteno-Savín T, Vázquez-Medina JP (eds.). Wiley- Blackwell, US.
2. González PM, Abele D, Puntarulo S (2012) A kinetic approach to assess oxidative metabolism related features in the bivalve Mya arenaria. Theory Biosci 131: 253-264.
3. Martín-Romero FJ, Gutiérrez-Martín Y, Henao F, Gutiérrez-Merino C (2002) The NADH oxidase activity of the plasma membrane of synaptosomes is a major source of superoxide anion. J Neurochem 82: 604-614.
4. Buettner GR, Jurkiewicz BA (1993) Ascorbate free radical as a marker of oxidative stress: an EPR study. Free Radic Biol Med 14: 49-55.
5. Bielski BHJ (1982) Chemistry of ascorbic acid radicals. American Chemical Society, Washington DC.
6. Heber U, Miyake C, Mano J, Ohno C, Asada K (1996) Monodehydroascorbate radical detected by electron paramagnetic resonance spectrometry is a sensitive probe of oxidative stress in intact leaves. Plant Cell Physiol 37: 1066-1072.
7. Nishikimi M (1975) Oxidation of ascorbic acid with superoxide anion generated by the xanthine-xanthine oxidase system. Biochem Biophys Res Commun 63: 463-468.
8. Simic MG (1991) Oxidative damage and Repair. Pergamon Press, Oxford.
9. Sharma MK, Buettner GR (1993) Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. Free Radic Biol Med 14: 649-653.
10. Piloni NE, Fermandez V, Videla LA, Puntarulo S (2013) Acute iron overload and oxidative stress in brain. Toxicology 314: 174-182.
11. Boveris A1 (1998) Biochemistry of free radicals: from electrons to tissues. Medicina 58: 350-356.
12. Galleano M, Aimo L, Puntarulo S (2002) Ascorbyl radical/ascorbate ratio in plasma from iron overloaded rats as oxidative stress indicator. Toxicol Lett 133: 193-201.
13. Roginsky VA, Stegmann HB (1994) Ascorbyl radical as natural indicator of oxidative stress: quantitative regularities. Free Radic Biol Med 17: 93-103.
14. Arroyo A, Navarro F, Gómez-Díaz C, Crane FL, Alcaín FJ, et al. (2000) Interactions between ascorbyl free radical and coenzyme Q at the plasma membrane. J Bioenerg Biomembr 32: 199-210.
15. Nishino H, Ito A (1986) Subcellular distribution of OM cytochrome b-mediated NADH-semidehydroascorbate reductase activity in rat liver. J Biochem 100: 1523-1531.
16. Horemans N, Asard H, Caubergs RJ (1994) The role of ascorbate free radical as an electron acceptor to cytochrome b-mediated trans-plasma membrane electron transport in higher plants. Plant Physiol 104: 1455-1458.
17. Piloni NE, Perazzo JC, Fernandez V, Videla LA, Puntarulo S (2016) Sub-chronic iron overload triggers oxidative stress development in rat brain: implications for cell protection. Biometals 29: 119-130.