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Short communication

Electrochemistry of Redox-active Mn Porphyrin-based SOD Mimic MnTnBuOE-2-PyP^{5+} - Study of Redox Species Involved in ROS/RNS Scavenging

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Abstract

Manganese ortho tetrakis(N-n-butoxyethylpyridinium-2-yl)porphyrin, MnTnBuOE-2-PyP^{5+}, is a third-generation redox-active compound currently undergoing preclinical exploration. This work is intended to complement the already extensive research of its chemical and biological properties by a simple electrochemical study. The thermodynamic parameters related to the Mn(IV) porphyrin species of MnTnBuOE-2-PyP^{5+} determined in this work support its observed reactivity as an efficient scavenger of peroxynitrite. The corresponding driving forces for the possible single-electron or two-electron reductions of ONOO$^-$ have been estimated as well.

Keywords

metalloporphyrins, oxidative stress, superoxide, peroxynitrite, cyclic voltammetry.

Introduction

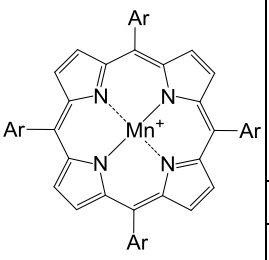
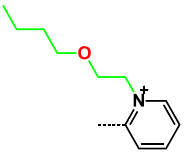
Therapeutic potential of metalloporphyrin-based mimics of superoxide dismutase (SOD) enzyme has been well documented [1-5]. Briefly, a true SOD mimic should be able to dismutate superoxide (*i.e.* oxidize or reduce $O_2^{\bullet -}$) at a catalytic rate, $k_{cat}(O_2^{\bullet -})$, higher than the rate of $O_2^{\bullet -}$ self-dismutation (5×10^5 $M^{-1}s^{-1}$ at pH = 7). The formal reduction potential of the SOD mimic should be optimally midway between the single-electron formal reduction potentials for oxygen ($E^{0'}$ = – 0.16 V *vs*. NHE) and superoxide ($E^{0'}$ = + 0.89 V *vs*. NHE) [6-7]. Over the last 15 years, a rational approach to the design of potential SOD mimics led to the development of several manganese porphyrin complexes (MnPs) with unique electronic properties and high reactivity towards $O_2^{\bullet -}$, but also other reactive species such as peroxynitrite, ONOO$^-$ [2-5]. Indeed, a structure-activity relationship (half-wave reduction potential, $E_{1/2}$, *vs.* log $k_{cat}(O_2^{\bullet -})$) has been developed and found to be valid for any other class of SOD mimics as well [1].

Recent efforts have been directed towards increasing the bioavailability and reducing the toxicity of MnPs. The original structure of a potent first-generation SOD mimic, manganese *ortho* tetrakis(*N*-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP^{5+}) was modified *via* lengthening the alkyl chains of pyridyl

substituents, yielding the more lipophilic compound, the manganese *ortho* tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin (MnTnHex-2-PyP^{5+}) [8]. This second-generation compound showed much higher brain and mitochondrial distribution than MnTE-2-PyP^{5+}, but was also found to be toxic at higher concentrations and could have a potentially limited use [9-10]. The third-generation compound, manganese *ortho* tetrakis(*N*-n-butoxyethylpyridinium-2-yl)porphyrin (MnTnBuOE-2-PyP^{5+}), was designed *via* introduction of oxygen atoms into the alkyl chains on the pyridyl substituents. This compound preserved high redox-activity and lipophilicity but exhibited significantly reduced toxicity relative to MnTnHex-2-PyP^{5+} [11].

Comparison of MnTE-2-PyP^{5+} and MnTnBuOE-2-PyP^{5+} with regards to their redox properties ($E_{1/2}$ for Mn^{III}P/Mn^{II}P redox couple), reactivities towards superoxide, log $k_{cat}(O_2^{\bullet -})$, and peroxynitrite, log $k_{red}(ONOO^-)$, as well as lipophilicities (distribution between n-octanol and water, log P_{OW}) is given in Table 1.

Table 1. *Physicochemical properties of redox-active Mn porphyrin-based SOD mimics MnTE-2-PyP^{5+} and MnTnBuOE-2-PyP^{5+}. Data are taken from references [3-5,11-16] ; [a] at 25 °C; [b] at 37 °C.*

	Ar	 Ethyl	 n-Butoxyethyl
	$E_{1/2}$ (mV *vs*. NHE)	+ 228	+ 277
	log k_{cat} ($O_2^{\bullet -}$) [a]	7.76	7.83
	log k_{red} ($ONOO^-$) [b]	7.53	7.54
	log P_{OW}	-7.79	-4.10

Comprehensive studies on bioavailability of these compounds have been performed recently as well [17-19], in order to gain further insight into the biodistribution of MnPs, but also provide guidelines for proper dosing regimens. After assessing preliminary efficacy and toxicity data, the porphyrins were administered to mice intraperitoneally (ip) or intravenously (iv) at following doses: 10 mg/kg of MnTE-2-PyP^{5+} (ip) and 2 mg/kg of MnTnBuOE-2-PyP^{5+} (iv). Drug levels in plasma and different organs were followed during at least 24 hours after a single dose, thus allowing the calculation of relevant pharmacokinetic (PK) parameters *via* non-compartmental analysis of PK curves.

These studies showed the highest MnP bioavailability in liver (maximum concentration, C_{max}, values of 8.1 and 13.1 µmol per L of tissue homogenate for MnTE-2-PyP^{5+} and MnTnBuOE-2-PyP^{5+}, respectively) and kidney, followed by spleen, lung and heart, whereas lower levels were found in plasma and lowest in the brain. Remarkably, MnTnBuOE-2-PyP^{5+} has 8-fold higher brain bioavailability (expressed as the area under PK curve, AUC, divided by dose) than the less lipophilic MnTE-2-PyP^{5+} (Figure 1), which may be highly significant for its potential application as a brain tumor radio- and chemo-therapeutic [20-21].

Additionally, it has been shown that $k_{cat}(O_2^{\bullet -})$ parallels the ability of MnPs to reduce $ONOO^-$ [4-5,14], due to the same thermodynamic and kinetic factors facilitating the reaction of electron-deficient cationic MnPs with electron-donating anionic species, such as $O_2^{\bullet -}$ and $ONOO^-$ [3-5,22]. Specifically, the removal of $ONOO^-$ can occur either through its binding to the Mn(III) site followed by its one-electron reduction to $NO_2^{\bullet}$ radical (coupled to the oxidation of Mn^{III}P to Mn^{IV}P), or the two-electron reduction of $ONOO^-$ by the Mn^{II}P species (coupled to the oxidation of Mn^{II}P to Mn^{IV}P). The latter reaction produces less toxic nitrite, NO_2^-, and might in fact be dominant *in vivo* due to (i) the abundance of cellular reductants keeping MnPs in Mn(II) state [1,13,23-25] and (ii) a higher driving force making it thermodynamically favorable [26].

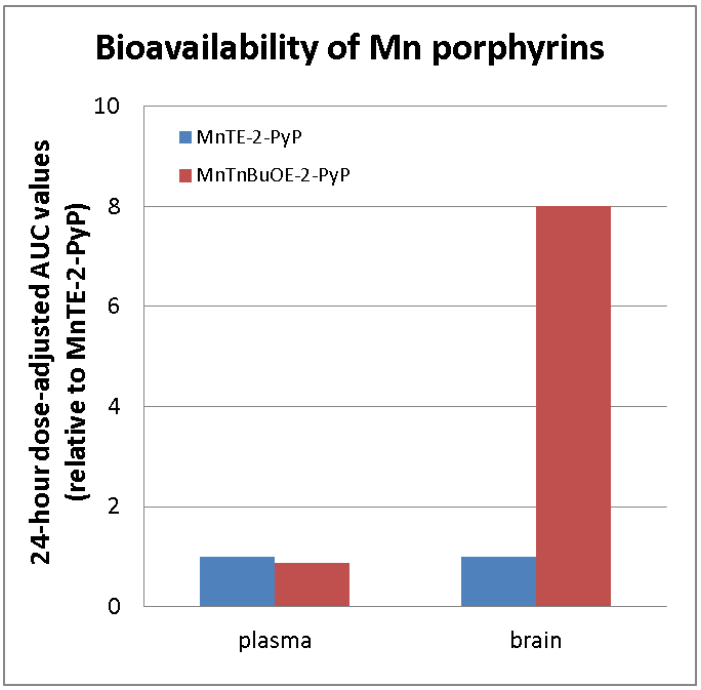


Figure 1. *Bioavailability of redox-active Mn porphyrin-based SOD mimics MnTE-2-PyP^{5+} and MnTnBuOE-2-PyP^{5+}, demonstrated as 24-hour dose-adjusted AUC values: raw AUC values have been divided by dose (10 mg/kg for MnTE-2-PyP^{5+} and 2 mg/kg for MnTnBuOE-2-PyP^{5+}). The values for MnTE-2-PyP^{5+} determined via iv route are taken as 100 % availability (plasma bioavailability via ip route was found to be approximately 84% of bioavailability via iv route for MnTE-2-PyP^{5+}) [18]. MnPs' charges have been omitted from the plot for simplicity.*

MnTnBuOE-2-PyP^{5+} represents the latest generation of redox-active compounds and is currently undergoing preclinical exploration. This work is intended to complement the already extensive research of its chemical and biological properties by a simple electrochemical study.

Experimental

The investigated metalloporphyrin, Mn^{III}TnBuOE-2-PyPCl$_5$ was synthesized according to the published procedure [11]. Stock solution was prepared by dissolution of purified and characterized solid (known molar absorbance coefficient, ε_{456} (MnTnBuOE-2-PyP^{5+}) = 1.78 × 10^5 M^{-1} cm^{-1}) [11]. Cyclic voltammetry of buffered and deaerated solutions was performed as reported in a previous study [26]. All potentials are reported *vs.* the normal hydrogen electrode (NHE), using the known potential of MnTE-2-PyP^{5+}, $E_{1/2}$ = + 228 mV *vs.* NHE at pH 7.8, as a reference [11,27-28].

Results and Discussion

Cyclic voltammograms of the aqueous solutions of Mn^{III}Ps can show two distinct electron transfers: only one current peak pair attributed to the Mn^{III}P/Mn^{II}P redox couple is observed in a neutral or mildly basic medium (pH = 7.8, Figure 2), whereas in the more basic medium an additional current peak pair attributed to the Mn^{IV}P/Mn^{III}P redox couple appears at more positive potentials (pH = 11, Figure 2). The cathodic and anodic current peak potentials, E_{pc} and E_{pa}, of both redox couples shift toward more negative values upon the increased pH of the solution. The cathodic-anodic peak separation, $\Delta E_p = |E_{pc} - E_{pa}|$ for both redox processes is larger than 59 mV even at the scan rate of 0.02 V s^{-1}, indicating quasi-reversible electron transfer processes.

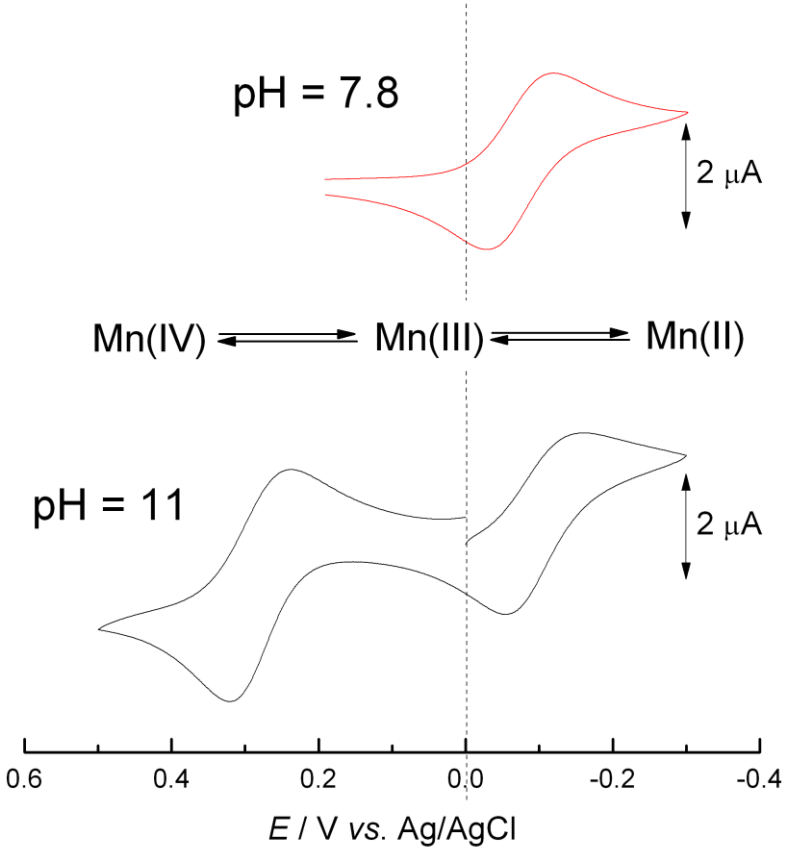


Figure 2. *Comparison of cyclic voltammograms of 0.5 mM aqueous solutions of MnTnBuOE-2-PyP^{5+} at pH=7.8 and pH = 11, v = 0.02 V s^{-1}, [NaCl] = 0.1 M, θ = 25 °C. Similar behavior has been reported previously for methyl, ethyl and butyl analogues [15,26,29]. Redox couples shown relate to the Mn^{III}P/Mn^{II}P (right cycle) and the Mn^{IV}P/Mn^{III}P couples (left cycle).*

These results are in agreement with the previously established scheme of behavior of MnPs in aqueous solutions [26,30]. At pH = 7.8 only a single-electron reduction of Mn^{III}P can be observed, shown by eq. (1), whereas at pH = 11 the single-electron reduction of Mn^{IV}P is accompanied by a simultaneous dissociation of two protons, according to eq. (2). The latter electron transfer cannot be observed in a neutral medium due to a formal potential more positive than that of water ($E^{0'}$(O$_2$(g),H$^+$(aq.)/H$_2$O(l)) = 816 mV *vs*. SHE at pH = 7) [30-31].

$$(H_2O)_2\,Mn^{III}TnBuOE-2-PyP^{5+} + e^- \rightleftarrows (H_2O)Mn^{II}TnBuOE-2-PyP^{4+} + H_2O \quad (1)$$

$$(O)(H_2O)Mn^{IV}TnBuOE-2-PyP^{4+} + 2H^+ + e^- \rightleftarrows (H_2O)_2\,Mn^{III}TnBuOE-2-PyP^{5+} \quad (2)$$

Table 2. *Calculated reduction potentials for the Mn^{III}P/Mn^{II}P, Mn^{IV}P/Mn^{III}P and Mn^{IV}P/Mn^{II}P redox couples of MnTnBuOE-2-PyP^{5+}. Values for MnTE-2-PyP^{5+} are taken from reference [26] and adjusted to the value $E_{1/2}$ = + 228 mV vs. NHE for its Mn^{III}P/Mn^{II}P redox couple at pH=7.8. The reduction potential of the Mn^{IV}P/Mn^{II}P couple is calculated as: $E_{1/2}$(Mn^{IV}P/Mn^{II}P) = [$E_{1/2}$(Mn^{III}P/Mn^{II}P) + $E_{1/2}$(Mn^{IV}P/Mn^{III}P)]/2.*

MnP	$E_{1/2}$ (mV *vs*. NHE)			
	pH = 7.8	pH = 11		
	Mn^{III}P/Mn^{II}P	Mn^{III}P/Mn^{II}P	Mn^{IV}P/Mn^{III}P	Mn^{IV}P/Mn^{II}P
MnTnBuOE-2-PyP^{5+}	+ 277	+ 235	+ 624	+ 430
MnTE-2-PyP^{5+}	+ 228	+ 208	+ 592	+ 400

As shown previously [11], the $E_{1/2}$ of $Mn^{III}P/Mn^{II}P$ redox couple of MnTnBuOE-2-PyP^{5+} is close to the value of the Mn SOD enzyme itself, accounting for its high reactivity towards $O_2^{\bullet-}$ (even higher than that of MnTE-2-PyP^{5+}, Table 1). Thermodynamic parameters related to the $Mn^{IV}P$ species of MnTnBuOE-2-PyP^{5+} determined in this work (Table 2) support its observed reactivity as an efficient scavenger of peroxynitrite. Estimating the shift of the $E_{1/2}$ ($Mn^{IV}P/Mn^{III}P$) value for +118 mV per unit decrease of pH from pH = 11, yields the values of $E_{1/2}$ ($Mn^{IV}P/Mn^{III}P$) = + 1049 mV *vs*. NHE at the physiological pH, according to eq. (2). The $E_{1/2}$ ($Mn^{IV}P/Mn^{II}P$) at the physiological pH can then be calculated as $E_{1/2}(Mn^{IV}P/Mn^{II}P) = [E_{1/2}(Mn^{III}P/Mn^{II}P) + E_{1/2}(Mn^{IV}P/Mn^{III}P)]/2$ = + 663 mV *vs*. NHE. Finally, the driving forces for the single-electron and two-electron reduction of $ONOO^-$, *EMF* ($1e^-$) = 551 mV and *EMF* ($2e^-$) = 637 mV, can then be calculated as $EMF = E_c - E_a$, where the values of E_c are the formal potentials $E^{0'}(ONOO^-/NO_2^{\bullet})$ = 1.6 V and $E^{0'}(ONOO^-/NO_2^-)$ = 1.3 V [32], respectively, whereas the values of E_a are the formal potentials $E_{1/2}(Mn^{IV}P/Mn^{III}P)$ and $E_{1/2}(Mn^{IV}P/Mn^{II}P)$, respectively.

Conclusions

Considerable interest in the impact of oxidative stress on cellular function prompted an intensive search for natural and synthetic antioxidants. High bioavailability of MnTnBuOE-2-PyP^{5+}, coupled with its exceptional physicochemical properties as a redox-active ROS/RNS scavenger, makes it a promising candidate in the ongoing cancer and radioprotection preclinical studies.

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References

[1] I. Batinic-Haberle, A. Tovmasyan, E. R. Roberts, Z. Vujaskovic, K. W. Leong and I. Spasojevic *Antioxidants & Redox Signaling* **20** (2014) 2372-415.

[2] I. Batinic-Haberle, I. Spasojevic, H. M. Tse, A. Tovmasyan, Z. Rajic, D. K. St Clair, Z. Vujaskovic, M. W. Dewhirst and J. D. Piganelli *Amino Acids* **42** (2012) 95-113.

[3] I. Batinic-Haberle, J. S. Reboucas, L. Benov and I. Spasojevic In *Handbook of Porphyrin Science, Vols.11-15*; K.M. Kadish, K.M. Smith, R. Guillard, Eds.; World Scientific: Singapore, 2011; pp 291-393.

[4] I. Batinic-Haberle, Z. Rajic, A. Tovmasyan, J. S. Reboucas, X. Ye, K. W. Leong, M. W. Dewhirst, Z. Vujaskovic, L. Benov and I. Spasojevic *Free Radical Biology and Medicine* **51** (2011) 1035-53.

[5] I. Batinić-Haberle, J. S. Rebouças and I. Spasojević *Antioxidants & Redox Signaling* **13** (2010) 877-918.

[6] P. M. Wood *Biochemical Journal* **253** (1988) 287-289.

[7] D. T. Sawyer and J. S. Valentine *Accounts of Chemical Research* **14** (1981), 393-400.

[8] I. Batinić-Haberle, I. Spasojević, R. D. Stevens, P. Hambright and I. Fridovich *Dalton Transactions* (2002) 2689-2696.

[9] I. Spasojevic, S. Miriyala, A. Tovmasyan, D. Salvemini, P. Fan, Z. Vujaskovic, I. Batinic-Haberle and D. K. S. Clair *Free Radical Biology and Medicine* **51** (2011) S98-S99.

[10] I. Spasojevic, T. Weitner, A. Tovmasyan, H. Sheng, S. Miriyala, D. Leu, Z. Rajic, D. S. Warner, D. S. Clair, T.-T. Huang and I. Batinic-Haberle *Free Radical Biology and Medicine* **65** (2013) S132.

[11] Z. Rajic, A. Tovmasyan, I. Spasojevic, H. Sheng, M. Lu, A. M. Li, E. B. Gralla, D. S. Warner, L. Benov and I. Batinic-Haberle *Free Radical Biology and Medicine* **52** (2012) 1828-1834.

[12] S. Carballal, V. Valez, I. Batinic-Haberle, G. Ferrer-Sueta and R. Radi *Free Radical Biology and Medicine* **65** (2013) S121-S122.

[13] G. Ferrer-Sueta, D. Vitturi, I. Batinic-Haberle, I. Fridovich, S. Goldstein, G. Czapski and R. Radi *Journal of Biological Chemistry* **278** (2003) 27432-27438.

[14] G. Ferrer-Sueta, C. Quijano, B. Alvarez and R. Radi *Methods in Enzymology* **349** (2002) 23-37.

[15] G. Ferrer-Sueta, I. Batinic-Haberle, I. Spasojevic, I. Fridovich and R. Radi *Chemical Research in Toxicology* **12** (1999) 442-449.

[16] S. Carballal and R. Radi, unpublished.

[17] A. Tovmasyan, T. Weitner, H. Sheng, X. Chen, K. Ashcraft, P. Fan, M. W. Dewhirst, D. S. Warner, Z. Vujaskovic, I. Batinic-Haberle and I. Spasojevic, (2014) in preparation.

[18] T. Weitner, I. Kos, H. Sheng, A. Tovmasyan, J. S. Reboucas, P. Fan, D. S. Warner, Z. Vujaskovic, I. Batinic-Haberle and I. Spasojevic *Free Radical Biology and Medicine* **58** (2013) 73-80.

[19] I. Spasojevic, Y. Chen, T. J. Noel, P. Fan, L. Zhang, J. S. Rebouças, D. K. St. Clair and I. Batinic-Haberle *Free Radical Biology and Medicine* **45** (2008) 943-949.

[20] D. H. Weitzel, A. Tovmasyan, K. A. Ashcraft, Z. Rajic, T. Weitner, C. Liu, W. Li, A. F. Buckley, M. R. Prasad, K. H. Young, R. M. Rodriguiz, W. C. Wetsel, K. B. Peters, I. Spasojevic, J. I. Herndon II, I. Batinic-Haberle and M. W. Dewhirst *Molecular Cancer Therapeutics*, (2014) in revision.

[21] D. Leu, Y. Zou, T. Weitner, A. Tovmasyan, I. Spasojevic, I. Batinic-Haberle and T.-T. Huang In *60th Annual Meeting of the Radiation Research Society*: Las Vegas, Nevada, USA, 2014.

[22] J. S. Rebouças, I. Spasojević, D. H. Tjahjono, A. Richaud, F. Méndez, L. Benov and I. Batinić-Haberle *Dalton Transactions* (2008) 1233-1242.

[23] G. Ferrer-Sueta, L. Hannibal, I. Batinic-Haberle and R. Radi *Free Radical Biology and Medicine* **41** (2006) 503-512.

[24] V. Valez, A. Cassina, I. Batinic-Haberle, B. Kalyanaraman, G. Ferrer-Sueta and R. Radi *Archives of Biochemistry and Biophysics* **529** (2013) 45-54.

[25] M. C. Jaramillo, M. M. Briehl, J. D. Crapo, I. Batinic-Haberle and M. E. Tome *Free Radical Biology and Medicine* **52** (2012) 1272-1284.

[26] T. Weitner, I. Kos, Z. Mandic, I. Batinic-Haberle and M. Birus *Dalton Transactions* **42** (2013) 14757-14765.

[27] A. G. Tovmasyan, Z. Rajic, I. Spasojevic, J. S. Reboucas, X. Chen, D. Salvemini, H. Sheng, D. S. Warner, L. Benov and I. Batinic-Haberle *Dalton Transactions* **40** (2011) 4111-4121.

[28] I. Kos, J. S. Rebouças, G. DeFreitas-Silva, D. Salvemini, Z. Vujaskovic, M. W. Dewhirst, I. Spasojevic and I. Batinic-Haberle *Free Radical Biology and Medicine* **47** (2009) 72-78.

[29] A. Budimir, T. Šmuc, T. Weitner, I. Batinic-Haberle and M. Biruš *Journal of Coordination Chemistry* **63** (2010) 2750-2765.

[30] T. Weitner, A. Budimir, I. Kos, I. Batinic-Haberle and M. Birus *Dalton Transactions* **39** (2010) 11568-11576.

[31] P. W. Atkins *Physical Chemistry*; 6th ed.; Oxford University Press: Oxford, 1998, 936.

[32] W. H. Koppenol *Free Radical Biology and Medicine* **25** (1998) 385-391.