This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Glutathiyl radicals: a mini review

by

Ling Li

Free Radical and Radiation Biology Program
B180 Medical Laboratories
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2001

8 February , 2001

Abbreviations:

ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate)
DMPO, 5, 5-dimethylphrroline-N-oxide
ESR, Electron Spin Resonance
EPR, Electron Paramagnetic Resonance
GS$^{\bullet}$, glutathiyl radical
GSH, glutathione
GSSG$^{\bullet-}$, glutathione disulfide radical anion
GSO$^{\bullet}$, GS-sulfinyl radical
$GSO_2^{\bullet}$, thiol peroxyl radical isomer
$GSO_2OO^{\bullet}$,glutathione sulfinyl peroxyl radical
GSO_2OOH, GS-sulfonyl peroxide
GSO_3H, GS-sulfonic acid
GSOH, GS-sulfenic acid
GSSO$^{\bullet}$, thiol peroxyl radical
GSSO$^{\bullet}$, thiol peroxyl radical
GSSG, glutathione disulfide
H_2O_2, hydrogen peroxide
HO$^{\bullet}$, hydroxyl radical
HRP, horseradish peroxidase
TMPD, *N*,*N*,*N'*,*N'*-tetramethyl-1,4-phenylenediamine

Outline

Abstract

Glutathione (GSH) is a ubiquitous constituent of living organisms. It can act as a radical scavenger, radioprotector and antioxidant. Reactions of glutathione with many oxidizing free radicals as well as nonradicals yield the glutathiyl radical ($GS^{\bullet}$). Techniques used to generate glutathiyl radicals are radiolysis and laser flash photolysis. This radical can further react with oxygen to form thiol peroxyl radical ($GSOO^{\bullet}$). These reactions will initiate a cyclic process that can generate various free radicals. The main techniques in detecting the glutathiyl radicals are Electron Spin Resonance (ESR) and absorption spectrophotometry.

Introduction

Thiols are widely distributed in nature and known to act as radical scavengers, a property that makes them both radioprotectors and antioxidants [1,2]. Typically, thiyl free radicals ($RS^{\bullet}$) are produced when a thiol functions as a radical scavenger. $RS^{\bullet}$ are also formed in certain metabolic pathways. Glutathione, one of the most important thiols, is involved in many biological reactions, for example, detoxification of hydrogen peroxide (H_2O_2) and other xenobiotics, amino acid transport, leukotriene synthesis and scavenging of free radicals [3]. Owing to their reducing properties, thiols participate in many cellular redox processes according to the general reaction (1) [4]:

$$RSH \; ? \quad RS^{\bullet} + e? + H^{+} \qquad (1)$$

Free radical "repair" by glutathione is frequently typified by reactions of the form [4]:

$$\begin{array}{c} | \\ -C^{\bullet} \\ | \end{array} + GSH \; ? \quad \begin{array}{c} | \\ -CH \\ | \end{array} + GS^{\bullet} \qquad (2)$$

Glutathiyl radicals are the result of the one-electron oxidation of GSH.

Formation of $GS^{\bullet}$

1. Reactions that form $GS^{\bullet}$

The thiyl radical, $GS^{\bullet}$ can be formed mainly *via* several different ways:

(1) Hydrogen donation to a free radical

The hydrogen donation in reaction (2) is energetically favorable because the C-H bond strength is higher than that of the S-H bond. These reactions can be very rapid. Table 1 presents values of the rate constants for some representative carbon-centered radicals (or mixtures of radicals).

Table 1. Rate constants for production of $GS^{\bullet}$ by carbon-centered radicals*

Radical source (major radical)	k /(10^{-7} $M^{-1}s^{-1}$)
Methanol ($^{\bullet}CH_2OH$)	4.0
Ethanol ($CH_3{}^{\bullet}CHOH$)	5.9
	11.0
2-Propanol ($(CH_3)_2{}^{\bullet}COH$)	18.0
Glucose	0.7
Deoxyribose	3.5

*Adapted from [4].

(2) Electron donation to a one-electron oxidant

The simplest one-electron oxidants are radicals derived by electron loss (oxidation) from halogens (X^-) or pseudohalogens (SCN^-), which are largely in the form $X_2^{\bullet-}$ at reasonable concentrations of X^- because of the equilibrium:

$$X^{\bullet} + X^- \Leftrightarrow X_2^{\bullet-} \quad (3)$$

These have more selective oxidizing characteristics than hydroxyl radicals but still oxidize glutathione very rapidly:

$$Br_2^{\bullet-} + GSH \;?\; 2Br^- + GS^{\bullet} + H^+ \quad (4)$$

Formation of $GS^{\bullet}$ will be essentially complete *ca* 10 μs after pulse radiolysis of a solution containing, *e.g.* 0.1 mol dm^{-3} KBr and 1 mmol dm^{-3} GSH [4].

Azide ($N_3^{\bullet}$) and sulphate ($SO_4^{\bullet-}$) radicals are some other useful one-electron oxidants ($Ox^{\bullet}$) as alternative, convenient sources of thiyl radicals *via* reactions of the general form [4]:

$$Ox^{\bullet} + GS^- \;?\; Ox^- + GS^{\bullet} \quad (5)$$

(3) Oxidation of glutathione *via* oxidases

The production of thiyl radicals has been detected by electron spin resonance spin trapping techniques in the oxidation of glutathione by hydrogen peroxide, catalysed by oxidases but without additional substrates such as the phenol and amines [4]:

$$H_2O_2 + 2GSH \xrightarrow{\text{peroxidases}} 2H_2O + 2GS^{\bullet} \quad (6)$$

Glutathione can act as a reductant of prostaglandin hydroperoxides, with catalysis by prostaglandin H synthase.

(4) Oxidation by the superoxide radical

Thiyl radicals are produced when thiols are autoxidized, especially when enzymes capable of producing superoxide are present:

$$O_2^{\bullet-} + GSH + H^+ \rightleftharpoons H_2O_2 + GS^{\bullet} \quad (7)$$

Another route to thiyl radicals not involving one-electron oxidation of thiols by $HO_2^{\bullet}$/ $O_2^{\bullet-}$ is the generation of hydroxyl radicals by Fenton chemistry (the "iron-catalysed Haber-Weiss" system) [5]:

$$Fe^{2+} + O_2 \rightleftharpoons Fe^{3+} + O_2^{\bullet-} \quad (8)$$

$$2\ O_2^{\bullet-} + 2H^+ \rightleftharpoons H_2O_2 + O_2 \quad (9)$$

$$Fe^{2+} + H_2O_2 \rightleftharpoons Fe^{3+} + {}^{\bullet}OH + OH^- \quad (10)$$

The hydroxyl radicals then abstract hydrogen to yield thiyl radicals:

$$^{\bullet}OH + GSH \rightleftharpoons H_2O + GS^{\bullet} \quad (11)$$

2. Techniques used to produce $GS^{\bullet}$

(1) Pulse radiolysis [6]

Reactive water radiolysis products are formed at known yields, G-values (μM/Gy), and in homogeneous distribution within about 10^{-8} s after ionization:

$$H_2O \rightleftharpoons {}^{\bullet}OH\ (0.28) + H^{\bullet}\ (0.06) + e_{aq}^-\ (0.28) \quad (12)$$

Solutions were deaerated by gentle bubbling (> 30 min) with N_2 or N_2O. In both environments $GS^{\bullet}$ is generated at a yield of up to 0.62 (μM/Gy) *via* reactions (11) to (16):

$$^{\bullet}OH + GSH \rightarrow H_2O + GS^{\bullet} \quad \text{(and other products)} \qquad (11)$$

$$e_{aq}^{-} + GSH \rightarrow SH^{-} + G^{\bullet} \text{ and } S^{-} + H^{\bullet} \qquad (13)$$

$$H^{\bullet} + GSH \rightarrow GH + GS^{\bullet} \qquad (14)$$

$$G^{\bullet} + GSH \rightarrow GH + GS^{\bullet} \qquad (15)$$

$$e_{aq}^{-} + N_2O + H^{+} \rightarrow N_2 + {}^{\bullet}OH \qquad (16)$$

In N_2O-saturated solution (23 mM N_2O) the reaction sequence (13) to (15) is replaced by the reactions (16) and (11). Reactions were also initiated by $CO_2^{\bullet-}$ radicals, generated at a yield of 0.62 (μM/Gy) by radiolysis of N_2O-saturated solutions of $HCOO^{-}$:

$$^{\bullet}OH, H^{\bullet} + HCOO^{-} \rightarrow H_2O, H_2 + CO_2^{\bullet-} \qquad (17)$$

$$CO_2^{\bullet-} + GSH^{-} \rightarrow HCOO^{-} + GS^{\bullet} \qquad (18)$$

or by $Br_2^{\bullet-}$, the product of $^{\bullet}OH$ – induced oxidation of Br^{-}. In some experiments GSH solutions were saturated with N_2O/O_2 mixtures at molar ratios of 4:1 or 20:1.

(2) Laser flash photolysis [7]

Glutathiyl radicals can also be generated by photolysis. One method is to produce carbon-centered radicals (~1×10^{-6} M) by the visible (490 nm) laser flash photolysis of aqueous solutions containing R'Co ([14]aneN_4)$(H_2O)^{2+}$ (typically 1×10^{-4} M), as in equation (19). With a water-soluble thiol–GSH present, the reaction is employed to generate glutathiyl radicals. Because thiyl radicals are not highly colored, two kinetic probes can be used. They can react with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) ion ($ABTS^{2-}$) in a known reaction (21) to yield the highly colored radical anion $ABTS^{\bullet-}$. Another probe that can be used is *N,N,N',N'*–tetramethyl-1,4-phenylenediamine (TMPD), which is easily oxidized to the highly colored radical cation $TMPD^{\bullet+}$. Thiyl radicals are observed to

oxidize TMPD in neutral solution but not in acidic solution, as the protonated amine is much less easily oxidized.

$$R'Co([14]aneN_4)^{2+} + h\nu \rightarrow R'^{\bullet} + Co([14]aneN_4)^{2+} \quad (19)$$

$$R'^{\bullet} + GSH \rightarrow R'H + GS^{\bullet} \quad (20)$$

$$RS^{\bullet} + ABTS^{2-} \rightarrow RS^{\bullet} + ABTS^{\bullet-} \quad (21)$$

Reactions and properties of $GS^{\bullet}$

Glutathione can react with various highly oxidizing species ($^{\bullet}OH$, $RO^{\bullet}$, $ROO^{\bullet}$) to form $GS^{\bullet}$. Glutathiyl radicals, which is less oxidizing can react with another GSH, most efficiently *via* GS^- [8]:

$$GS^{\bullet} + GS^- \rightarrow GSSG^{\bullet-} \qquad K_{22} = 3.3\times10^3\ M^{-1} \quad (22)$$

$GSSG^{\bullet-}$ is a strongly reducing species. The very negative potential of the GSSG/$GSSG^{\bullet-}$ couple ($E^{O'}$ ~ -1500 mV) makes $GSSG^{\bullet-}$ probably the most reducing species that can arise in a biological setting. This species can reduce metals as well as produce $O_2^{\bullet-}$ ($E^{O'}$ ~ +1200 mV).

$$GSSG^{\bullet-} + O_2 \rightarrow O_2^{\bullet-} + GSSG \qquad k_{23}=1.6\times10^8\ M^{-1}s^{-1} \quad (23)$$

$GS^{\bullet}$ can react with molecular oxygen causing a chain reaction. This reaction forms a colored species [9]:

$$GS^{\bullet} + O_2 \rightarrow GSOO^{\bullet} \qquad K_{24}=3.2\times10^3\ M^{-1} \quad (24)$$

The thiol peroxyl radical ($GSOO^{\bullet}$) is very unstable. Electron spin resonance (ESR) at low temperature showed that visible light would bring about the isomerization of $GSOO^{\bullet}$:

$$GSOO^{\bullet} \xrightarrow{\lambda\nu} GSO_2^{\bullet} \quad (25)$$

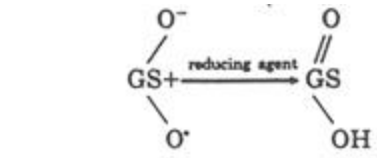
Evidence also showed that $GSSO^{\bullet}$ could isomerize through another pathway besides photolytic isomerization. Thermal isomerization can alter the structure at temperatures nearing 300 K producing a sulfonyl radical, and it is more thermodynamically stable than $GSOO^{\bullet}$ [8]. The isomer radical can react with O_2 further:

$$GSO_2^{\bullet} + O_2 \; ? \quad GSO_2OO^{\bullet} \qquad (26)$$

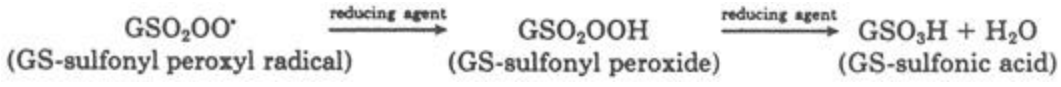
Glutathione sulfonyl peroxyl radical is not affected by light. However, the cycle is still not completed. It can continue by adding GSH (27) until oxygen is depleted (24):

$$GSO_2OO^{\bullet} + GSH \; ? \quad GS^{\bullet} + GSO_2OOH \qquad (27)$$

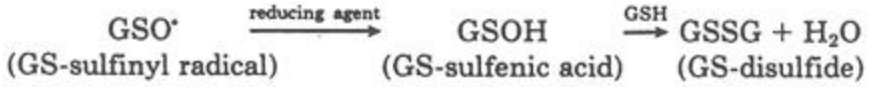
Some of the final stable end products are GS-suflenic acid, GS-disulfide and GS-sulfonic acid (28, 29, 30) [8].



(GS-sulfonyl radical) (GS-sulfinic acid) (28)



(GS-sulfonyl peroxyl radical) (GS-sulfonyl peroxide) (GS-sulfonic acid) (29)



(GS-sulfinyl radical) (GS-sulfenic acid) (GS-disulfide) (30)

Detection of GS•

1. Electron Spin Resonance (ESR)

Glutathiyl radicals can be detected by ESR spectroscopy using a spin trap. In aqueous solutions at room temperature GS• is not directly detectable by ESR. Therefore, it is usually detected by using a spin trap. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) is a commonly used spin trap for the detection of the thiyl radical. The ESR spectrum of the DMPO-glutathionyl radicals formed in a system of GSH is shown in Figure 1 [10].

2. Detection by Absorption Spectrophotometry

The glutathiyl radical can also be detected by absorption spectrophotometry. When free radicals are formed, the reaction is followed directly by an overall change in

absorption taking place at different wavelengths due to the loss of reactant or the formation of product [11]. Figure 2 shows the transient spectrum of $GS^{\bullet}$ obtained by pulse radiolysis of 0.5 mM GSSG solution (pH 7.0) saturated with N_2O, molar absorption coefficient ε^{330} ca. 500 M^{-1} cm^{-1} [12].

ca. 500 M^{-1} cm^{-1} [12].

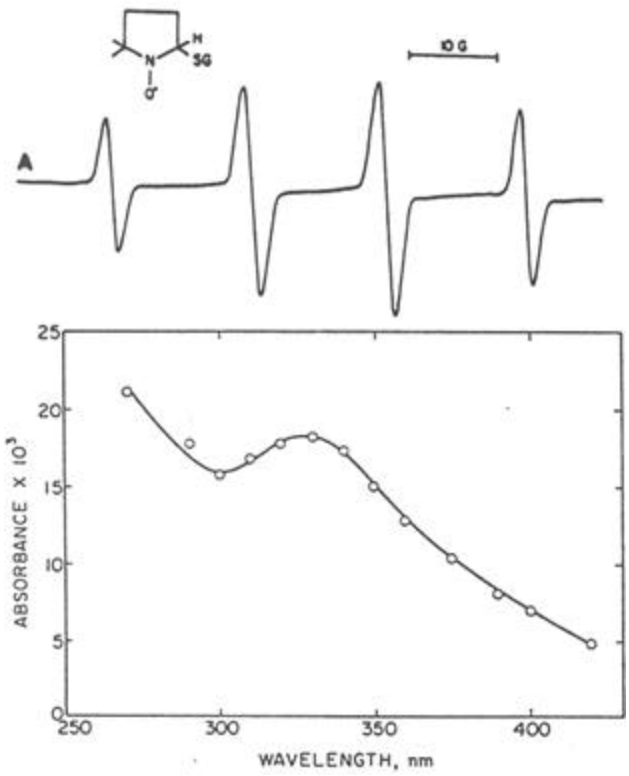


Figure 1. ESR spectra of the DMPO-glutathione thiyl radical adduct produced in a system of HRP and H_2O_2 under aerobic conditions. a^N = 15.4G, a^H = 16.2G.

Figure 2**. Transient spectrum of $GS^{\bullet}$**

Summary

Reaction of GSH with oxidizing free radicals yield the $GS^{\bullet}$, which can further react with GSH to form $GSSG^{\bullet-}$. This species can reduce to produce $O_2^{\bullet-}$ (reaction 23). The possibility of this reaction has led to the proposal that superoxide dismutase and GSH in combination are an integral component of the cellular antioxidant defense [13, 14].

Reference:

1. Henriksen T, Melo TB, Saxebol G. (1986) Free radical formation in proteins and protection from radiation damage. In: Pryor WA. ed. *Free Radicals in Biology*, Vol. II. New York: Academic Press; pp213-256.

2. von Sonntag, C. (1987) *The Chemical Basis of Radiation Biology*. New York: Taylor & Francis.

3. Huxtable AJ. (1986) *Biochemistry of Sulfur.* New York London: Plenum Press.

4. Wardman P. (1988) Conjugation and oxidation of glutathione via thiyl free radicals. H, Ketterer B (eds), *Glutathione Conjugation*. New York: Academic Press.

5. Winterbourn CC. (1993) Superoxide as an intracellular radical sink. *Free Radic Biol Med.* **14**: 85-90.

6. Prütz WA, Butler J, Land EJ. (1994) The glutathione free radical equilibrium, $GS^{\bullet} + GS^{-}$? $GSS^{\bullet-}G$, mediation electron transfer to Fe(III) – cytochrome *c*. *Biophy Chem.* **49**: 101-111.

7. Huston P, Espenson JH, Bakac A. (1992). Reactions of thiyl radicals with transition-metal complexes. *J AM Chem Soc*. **114**: 9510-9516.

8. Buettner GR. (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, a-tocopherol, and ascorbate. *Arch Biochm Biophy*. **300**: 535-543.

9. Sevilla MD, Becker D, Yan M. (1990) The formation and structure of the sulfoxyl radicals $RSO^{\bullet}$, $RSO_2^{\bullet}$ and $RSO_2OO^{\bullet}$ from the reaction of cysteine, glutathione and penicillamine thiyl radicals with molecular oxygen. *Int J Radiat Biol.* **57**:65-81.

10. Harmon LS, Carver DK, Schreiber J, Mason RP. (1986) One- and two-electron oxidation of reduced glutathione by peroxidases. *J Biol Chem.* **261**: 1642-1648.

11. Dunster C, Willson RL. (1990) Thiyl free radicals: Electron transfer, addition or hydrogen abstraction reactions in chemistry and biology, and the catalytic role of sulfur compounds. In: Chatgilialoglu C, Amus KD. ed. *Sulfur Centered Reactive Intermediates in Biology and Chemistry*. New York: Plenum Press. pp.377-387.

12. Quintliani M, Badiello R, Tamba M, Esfandi A, Gorin G. (1977) Radiolysis of glutathione in oxygen-containing solutions of pH 7. *Int J Radiat Biol.* **32**: 195-202.

13. Munday R, Winterbourn CC. (1989) Reduced glutathione in combination with superoxide dismutase as an important biological antioxidant defense mechanism. *Biochem Pharm.* **38**: 4349-4352.

14. Winterbourn CC. (1993) Superoxide as an intracellular radical sink. *Free Radi Biol Med.* **14**:85-90.