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Glutathiyl Radical

by

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Abbreviations:

DMPO: 5.5-dimethylpyrroline-N-oxide
$GS^{\bullet}$: Glutathiyl radical
$GSO^{\bullet}$: GS-sulfinyl radical
GSO_2OOH: GS-sulfonyl peroxide
GSOH: GS-sulfenic acid
GSSG: Glutathione disulfide
H_2O_2: Hydrogen peroxide
HRP: Horseradish peroxidase
$Ox^{\bullet}$: One-electron oxidants
PBN: α-Phenyl-tert-burylnitrone
ESR: Electron Paramagnetic Resonance
GSH:Glutathione
$GSO_2^{\bullet}$: Thiol peroxyl radical isomer
GSO_3H: GS-sulfonic acid
$GSOO^{\bullet}$: Thiol peroxyl radical
$GSSG^{\bullet-}$: Glutathione disulfide radical anion
$HO^{\bullet}$: Hydroxyl radical
NADH: Nicotamide adenine dinucleotide
$^1O_2$: singlet oxygen

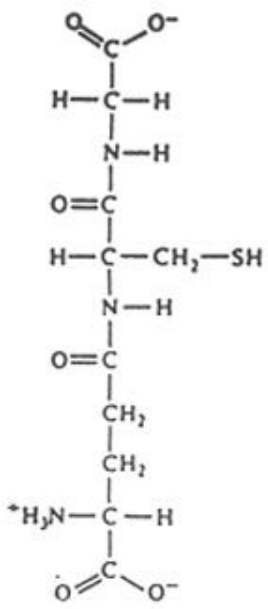
Outline

Abstract

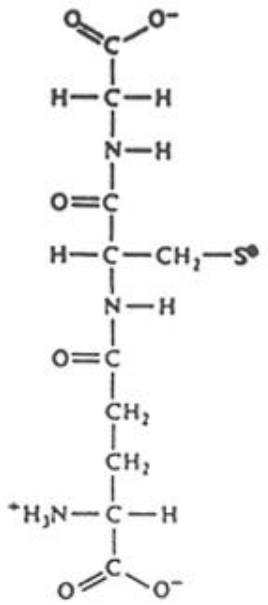
Thiols, acting as radical scavengers, are important in the protection of cells against ionizing radiation, as well as against reactive free radicals formed in normal metabolism. During the scavenging process, typically, thiyl radicals are formed. This paper will focus on the formation, reaction, detection and biological effects of glutathiyl radical, produced by the one-electron oxidation of glutathione.

Introduction

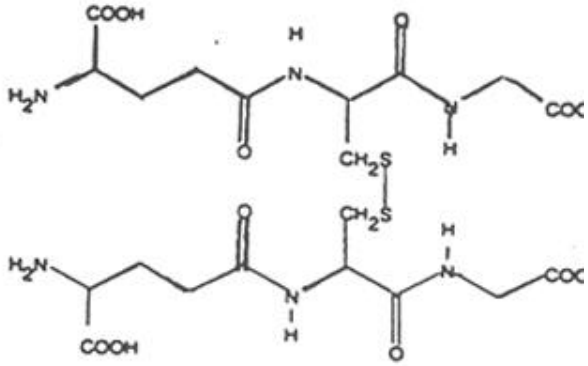
Glutathione (GSH) has two basic forms: the reduced form (GSH) and the disulfide form of glutathione (GSSG). In most human tissues, the GSH/GSSG ratio is more than 10/1 [1]. Following is the structure of glutathione:



Glutathione



Glutathiyl Radical



Glutathione Disulfide

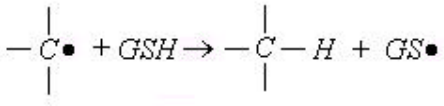
Glutathione plays an important role in nomal cell funtion as well as in toxicology, carcinogenesis, radiotherapy, chemotherapy and diverse other areas. It acts as a scavenger to remove reactive species, such as hydroxyl radical ($^{\bullet}OH$), peroxynitrite ($NO_2^{\bullet}$), $RO^{\bullet}$, $RO_2^{\bullet}$, carbon-centered radicals and singlet oxygen ($^1O_2$) [1]. When it reacts with free radicals, it will generate glutathiyl radicals ($GS^{\bullet}$) that control the balance between either oxidative or reductive free radical chemistry of glutathione [2].

Formation of Glutathiyl Radicals

Glutathiyl radical can be formed through several ways:

1. GSH donates a hydrogen to an oxidizing radical:

   Acting as a free radical scavenger, glutathione can “repair” free radicals, *e.g.*



(1)

This hydrogen atom donation reaction happens very rapidly. For example, methanol radical can receive hydrogen from GSH, at room temperature and in aqueous solution, k=5.9 x10^7 $M^{-1}s^{-1}$ [3]. Hydroxyl radicals are much more reactive than carbon-centered radicals with glutathione, but producing other radicals besides $GS^{\bullet}$.

2. Electron Donation to a One-electron Oxidant

One-electron oxidants ($Ox^{\bullet}$), such as azide ($N_3^{\bullet}$) and sulphate radicals ($SO_4^{\bullet-}$), are useful courses of thiyl radicals *via* the following reaction:

$$Ox^{\bullet} + GS^{-} \rightarrow Ox^{-} + GS^{\bullet} \qquad (2)$$

The simplest one-electron oxidants are halogens atom losing one electron ($X^{\bullet}$). Usually they are in the form of $X_2^{\bullet-}$, because

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

For example:

$$Br_2^{\bullet-} + GSH \rightarrow 2Br^{\bullet-} + GS^{\bullet} + H^{+} \qquad (4)$$

However, for this reaction, k is pH dependent. Oxidants typically react much faster with GS^- at high pH than with GSH with low pH where GSH is undissociated

$$GSH \Leftrightarrow GS^{-} + H^{+} \quad \text{pKa} \approx 9.2 \text{ [1]} \qquad (5)$$

3. Oxidation Glutathione by Oxidases

The production of glutathione thiyl radical can be seen during the oxidation of glutathione by hame peroxidase and other hame proteins. In the presence of H_2O_2, horseradish peroxidase (HRP) and other non-specific peorxidases can oxidize thiols into thiyl radicals [4]

$$H_2O_2 + 2GSH \xrightarrow{HRP} 2H_2O + 2GS^{\bullet} \qquad (6)$$

However, glutathione peroxidase does not oxidize H_2O_2 to form $GS^{\bullet}$, instead

$$H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSSG \text{ [2]} \qquad (7)$$

Then why GPx catalyzes the reaction of GSH and H_2O_2 to form GSSG instead of $GS^{\bullet}$? The reasons are: first, this reaction involves a two-electron oxidation process; second, "glutathione peroxidase does not support oxygen consumption in the presence of H_2O_2" [5].

4. Metals Catalyze

Thiyl radicals can be generated when thiols react with transition metals ions, for example:

$$GS^- + Cu^{2+} \rightarrow GS^{\bullet} + Cu^+ \qquad (8)$$

$$Fe^{3+} + GS^- \rightarrow Fe^{2+} + GS^{\bullet} + H^+ \qquad (9)$$

In 1978, Willson [6] proposed that in the presence of thiols, free ferrous irons can form iron/thiol complexes, which is more reductive than either GS^- or Fe^{2+}:

$$Fe^{11} + 2GS^- \Leftrightarrow Fe^{11}(GS)_2 \qquad (10)$$

$$Fe^{11}(GS)_2 + O_2 \Leftrightarrow [Fe^{111}(GS)_2 - O_2^{\bullet -}] \qquad (11)$$

$$[Fe^{111}(GS)_2 - O_2^{\bullet -}] + GS^- \rightarrow Fe^{11}(GS)_2 + O_2^{\bullet -} + GS^{\bullet} \qquad (12)$$

5. Dissociation of Disulfide Radical Anions

$GSSG^{\bullet -}$ can generate GS through hemolytic fission:

$$GSSG^{\bullet -} \Leftrightarrow GS^{\bullet} + GS^- \qquad (13)$$

However, since E (GSSG/$GSSG^{\bullet -}$) ≈ -1700mV [7], only very powerful reductants can reduce GSSG to $GSSG^{\bullet -}$, such as the hydrated electron (e_{aq}^-), $CO_2^{\bullet -}$ radical anion and alcohol radicals ($(CH_3)_2CO^{\bullet -}$) [2].

Reactions of Glutathiyl Radicals

Once $GS^{\bullet}$ is formed, it can react rapidly with various organic species and in particular molecular oxygen.

1. The conjugation of $GS^{\bullet}$ with GS^{-}

In cells, the concentration of GSH is in the millimolar range. At human physiological pH, about 1-2% of GSH will be dissociated:

$$GSH \Leftrightarrow GS^{-} + H^{+} \quad \text{pKa} = 9.2 \qquad (5)$$

While GS^{-} will react with $GS^{\bullet}$ rapidly [1]:

$$GS^{-} + GS^{\bullet} \rightarrow GSSG^{\bullet -} \qquad k = 8 \times 10^{8}\ M^{-1}s^{-1} \quad (14)$$

This reaction depends not only on the concentration of glutathione, but also on pH. $GSSG^{\bullet -}$, as a powerful reductant, can then reduce metal ions and O_2 to form O_2 [1]:

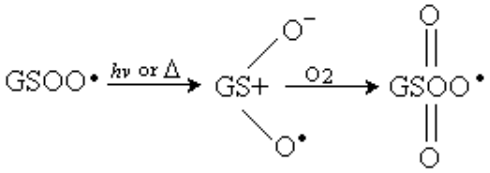
$$GSSG^{\bullet -} + O_2 \rightarrow GSSG + O_2^{\bullet -} \qquad k = 5 \times 10^{8}\ M^{-1}s^{-1} \qquad (15)$$

2. The conjugation of $GS^{\bullet}$ with oxyge n

Reacting with O_2, thiyl radicals can generate peroxyl radicals:

$$GS^{\bullet} + O_2 \Leftrightarrow GSOO^{\bullet} \qquad k_f = 2.0 \times 10^{9}\ M^{-1}s^{-1};\ k_b = 6.2 \times 10^{5}\ M^{-1}s^{-1}\ [2] \qquad (16)$$

However, as Buettner [9] pointed out, this glutathiyl peroxyl radical is not stable and will undergo isomerization at low temperature to GS-sulphonyl radical, the latter will further react with O_2 to produce GS-sulphonyl peroxyl radical [9]:



(17)

GS-sulphonyl radical and GS-sulphonyl peroxyl radical can in turn reduce agents [9]:

$$GSO_2OO^{\bullet} \xrightarrow{\text{reducing agent}} GSO_2OOH \xrightarrow{\text{reducing agent}} GSO_3H + H_2O \quad (18)$$

$$GSO^{\bullet} \xrightarrow{\text{reducing agent}} GSOH \xrightarrow{GSH} GSSG + H_2O \qquad (19)$$

In addition, $GSOO^{\bullet}$ can react with more GSH as [9]:

$$GSOO^{\bullet} \xrightarrow{\Delta, GSH} GSO^{\bullet} + GSOH \qquad (20)$$

In summary, "end products of GSH oxidation by oxygen radicals under aerobic conditions include GSSG, sulfenic acid (GSOH) and sulfonic acid (GSO_3H)." [1]

3. Electron Transfer reactions

Forni [10] pointed out that $GS^{\bullet}$ can "more readily enters into an electron rather that hydrogen transfer reactions".

As a good electron donor, ascorbate ($AscH^-$) can "repair" $GS^{\bullet}$ as follow [10]:

$$AscH^- + GS^{\bullet} \rightarrow GSH + Asc^{\bullet -} + H^+ \qquad k = 6 \times 10^8\ M^{-1}s^{-1} \text{ at pH } 6.5 \qquad (21)$$

$GS^{\bullet}$ can also oxidize nicotamide adenine dinucleotide (NADH) as [2]

$$NADH + GS^{\bullet} \rightarrow GS^- + NAD^{\bullet} + H^+ \qquad k = 2.3 \times 10^8\ M^{-1}s^{-1} \qquad (22)$$

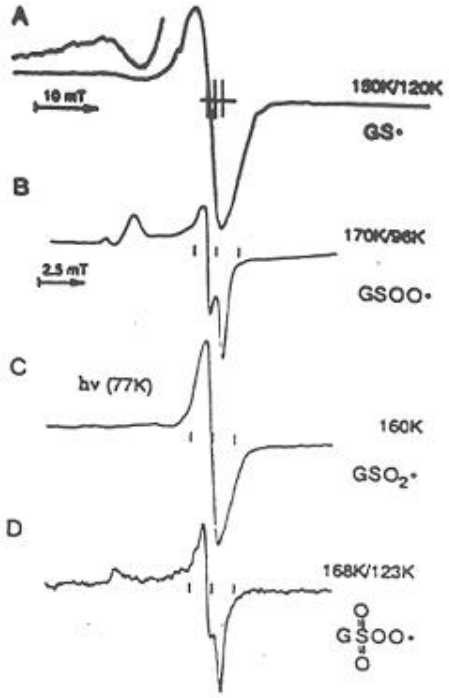
<u>Detection of Glutathiyl Radicals</u>

Electron spin resonance (ESR) is the only technique to directly detect free radicals. Since ESR is not sensitive enough to measure the fast-reacting $GS^{\bullet}$ in room temperature, aqueous solution, a spin trap is used. Both 5,5-Dimethylpyrroline-N-oxide (DMPO) and α-Phenyl-*tert*-butylnitrone (PBN) can trap thiyl radicals. However, the spectra of PBN-trapping are less characteristic than that of DMPO. Figure 1 [11] shows the direct ESR spectra of the DMPO-thiyl radicals of glutathione after γ-irradiation.

Another approach to detect glutathiyl radical is UV or visible light absorption spectrophotometry. Typically $RS^{\bullet}$ display an optical absorption spectrum between 300~330 nm region. Figure 2 [12] illustrates the transient absorption spectra of the more relevant radicals derived from the pulse radiolysis of GSH at different experimental conditions, including $GS^{\bullet}$, $GSOO^{\bullet}$ and $GSSG^{\bullet -}$.

Figure 1 [11]. ESR spectra of a γ-irradiated frozen solution of glutathion.

In N_2 saturated solution, the spectrum of $GS^{\bullet}$ found after reaction of $Cl_2^{\bullet}$ with GSH In O_2 saturated solution, $GSOO^{\bullet}$ formation Photobleaching $GSOO^{\bullet}$ at 77K results in an isomer radical, $GSO_2^{\bullet}$ Annealing of C results in mobilization of O_2 and the generation of $GSO_2OO^{\bullet}$



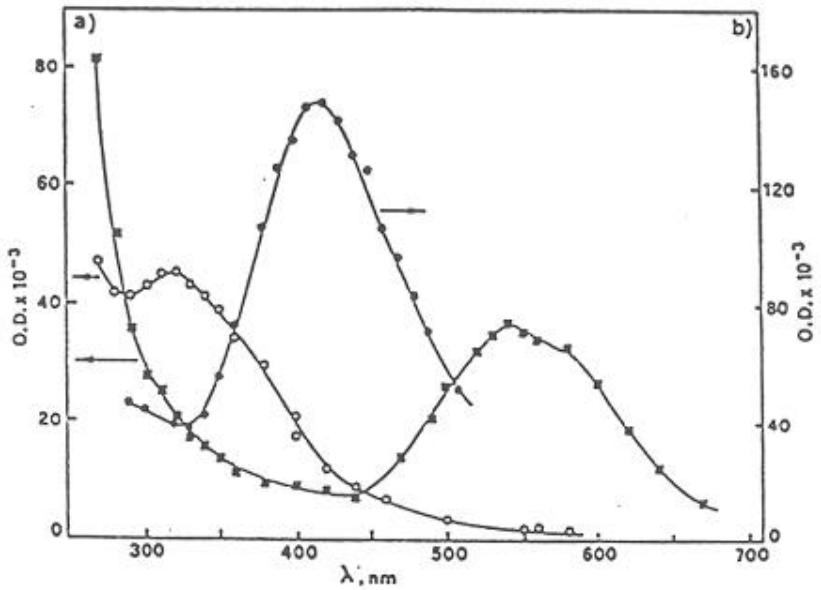


Figure 2 [12] a) Transient spectra of 1 mM GSH at PH 5.5: $GS^{\bullet}$ (?), N_2O saturated solutions, 4 μs after the pulse; $GSOO^{\bullet}$ (¦) N_2O/O_2 (60:40v/v) saturated solutions, 7μs after the pulse. Irradiation dose = 25Gy. b) $GSSG^{\bullet-}$ (?) N_2O saturated solutions of 4.6 mM GSH at pH 8.4, 6 μs after the pulse. Irradiation dose = 11 Gy. Cell pathlength = 5 cm.

Biological Effect of glutathiyl Radicals

In living organisms, glutathione is very important. Extensive studies have shown that it is involved in various biological reactions, such as the detoxification of hydrogen peroxide,

amino acid transport, leukotriene synthesis and scavenging of free radicals. Having a thiol group, glutathione is a very good radioprotector. In the process of “repairing” cellular radicals, glutathiyl radicals will be generated. Glutathiyl radicals, as a detoxifier, scavenge reactive oxygen species. However, it can still cause some biological problems. Glatt [13] noted that at physiological concentration, glutathione have the effect of mutagenesis, which is due to the production of glutathiyl radicals.

Schoneich [14] compared the reactivity of thiol radicals to that of the oxygen-centered radicals against polyunsaturated fatty acid:

$(CH_3)_3CO^{\bullet} > RS^{\bullet} > CCl_3OO^{\bullet} > (CH_3)_2C(OH)OO^{\bullet} > HO_2^{\bullet} >$ (fatty acid)-$OO^{\bullet}$

Summary

As an antioxidant, glutathion protect cells against the damage of free radicals. However, glutathiyl radical sometimes is generated. Whether thiyl radical is an antioxidant or pro-oxidant is depending on the circumstances.

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