Kinetics of the Reaction between Nitroxide and Thiyl Radicals: Nitroxides as Antioxidants in the Presence of Thiols

Sara Goldstein,*,† Amram Samuni,§ and Gabor Merenyi#

Institute of Chemistry and the Accelerator Laboratory, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; Department of Molecular Biology, Hebrew University—Hadassah Medical School, P.O. Box 12000, Jerusalem 91120, Israel; and Department of Chemistry, Nuclear Chemistry, The Royal Institute of Technology, S-10044 Stockholm 70, Sweden

Received: May 29, 2008; Revised Manuscript Received: July 3, 2008

Cyclic nitroxides effectively protect cells, tissues, isolated organs, and laboratory animals from radical-induced damage. The present study focuses on the kinetics and mechanisms of the reactions of piperidine and pyrrolidine nitroxides with thiyl radicals, which are involved in free radical "repair" equilibria, but being strong oxidants can also produce cell damage. Thiyl radicals derived from glutathione, cysteine, and penicillamine were generated in water by pulse radiolysis, and the rate constants of their reactions with 2,2,6,6-tetramethylpiperidine-1-oxyl (TPO), 4-OH-TPO, and 3-carbamoyl-proxyl were determined to be $(5-7) \times 10^8$ M^{-1} s^{-1} at pH 5−7, independent of the structure of the nitroxide and the thiyl radical. It is suggested that the reaction of nitroxide (>NO$^\bullet$) with thiyl radical (RS$^\bullet$) yields an unstable adduct (>NOSR). The deprotonated form of this adduct decomposes via heterolysis of the N−O bond, yielding the respective amine (>NH) and sulfinic acid (RS(O)OH). The protonated form of the adduct decomposes via homolysis of the N−O bond, forming the aminium radical (>NH$^{\bullet+}$) and sulfinyl radical (RSO$^\bullet$), which by subsequent reactions involving thiol and nitroxide produce the respective amine and sulfonic acid ($RS(O)_2OH$). Nitroxides that are oxidized to the respective oxoammonium cations (>N$^+$=O) are recovered in the presence of NADH but not in the presence of thiols. This suggests that the reaction of >N$^+$=O with thiols yields the respective amine. Two alternative mechanisms are suggested, where >N$^+$=O reacts with thiolate (RS$^-$) directly generating the adduct >NOSR or indirectly forming >NO$^\bullet$ and RS$^\bullet$, which subsequently together yield the adduct >NOSR. Under physiological conditions the adduct is mainly deprotonated, and therefore nitroxides can detoxify thiyl radicals. The proposed mechanism can account for the protective effect of nitroxides against reactive oxygen- and nitrogen-derived species in the presence of thiols.

Introduction

Cyclic nitroxides (>NO$^\bullet$) are cell-permeable stable radicals, which effectively protect cells, tissues, isolated organs, and laboratory animals from radical-induced damage.[1−10] Their protective effects have, in part, been attributed to their ability to catalyze the dismutation of superoxide radicals[11,12] and scavenge a large variety of deleterious species such as carbon-centered radicals,[13] $^\bullet$OH,[14,15] peroxyl radicals ($RO_2^\bullet$),[16] $^\bullet NO_2$,[17,18] $CO_3^{\bullet-}$, [18,19] and thiyl radicals.[20−22] However, efficient scavenging of $^\bullet$OH radicals within the cell is practically impossible as a result of their rapid reaction with cellular constituents. Instead, the protective activity of nitroxides may result from their reactions with less reactive species, such as $^\bullet NO_2$ or secondary intermediates including peroxyl and thiyl radicals.

Glutathione (GSH) is considered to be the major thiol−disulfide redox buffer of the cell, the concentration of which in the cytosol is 1−11 mM.[23] Numerous proteins contain sulfhydryl groups due to their cysteine content. In fact, their concentration in cells and tissues is much greater than that of GSH.[23] The oxidation of thiols by various damaging radicals, for example,$^\bullet$OH, $^\bullet NO_2$, and $CO_3^{\bullet-}$, [24−29] forms the respective thiyl radicals (RS$^\bullet$), which are involved in free radical "repair" equilibria.[30] However, thiyl radicals are strong oxidants and can induce cell damage by reacting with protein thiols as well as with unsaturated acyl chains of phospholipids in the membrane.[31]

The reaction between glutathionyl radical (GS$^\bullet$) and nitroxides has been suggested to protect cells from GS$^\bullet$ toxicity and also as a potential pathway of formation of secondary amines, which are known as major metabolites of nitroxides in cells.[21,22] We have previously demonstrated that the reaction of nitroxides with $HO_2^\bullet$, $RO_2^\bullet$, $^\bullet NO_2$, and $CO_3^{\bullet-}$ radicals yields the corresponding oxoammonium cations, which could be toxic as oxidizing species. Also, the reactivity of nitroxides toward $HO_2^\bullet$ and $RO_2^\bullet$, although not toward $^\bullet NO_2$ and $CO_3^{\bullet-}$, depends on the nature of the ring side chain and particularly on the ring size.[12,16,17] The rate constant for the reaction of GS$^\bullet$ in aqueous solutions with 4-((9-acridinecarbonyl)amino)-2,2,6,6-tetramethylpiperidine-1-oxyl (Ac-TPO) has been estimated to be 10^8-10^9 M^{-1} s^{-1} and that of TPO in acetonitrile with arylsulfenyl and arylsulfinyl radicals to be $\sim 10^9$ M^{-1} s^{-1}.[20] The main product of the nitroxide reaction with thiyl radicals was identified as the corresponding amine.[21,22,32,33] In the present study, the reactions of piperidine and pyrrolidine nitroxides with thiyl radicals in water have been investigated to gain better understanding of the antioxidative activity of nitroxides.

Experimental Section

Water for solution preparation was purified using a Milli-Q system. All chemicals were of the highest available grade and

* Corresponding author (fax +972-2-6586925; e-mail sarag@vms.huji.ac.il).
† The Hebrew University of Jerusalem.
§ Hebrew University—Hadassah Medical School.
# The Royal Institute of Technology.

10.1021/jp804743g CCC: $40.75
Published on Web 08/27/2008

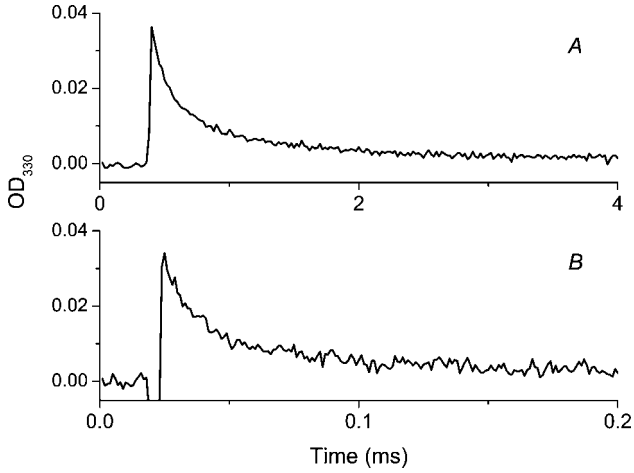


Figure 1. Effect of 3-CP on the decay rate of GS• monitored at 330 nm. All solutions were N_2O-saturated and contained 1 mM GSH and 2.8 mM phosphate buffer (pH 6.1) without 3-CP (A) or in the presence of 100 μM 3-CP (B).

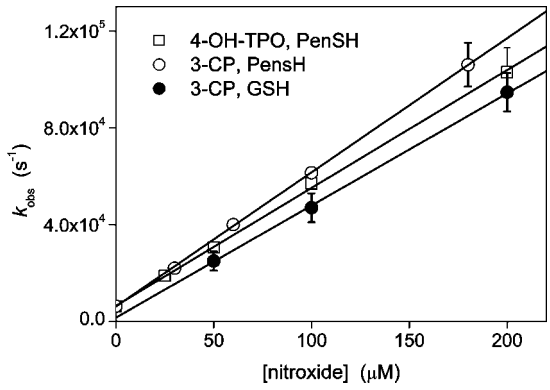


Figure 2. Effects of [nitroxide] on the observed rate constant of RS• decay rate measured at 330 nm. All solutions were N_2O-saturated and contained 2 mM RSH and 4 mM phosphate buffer at pH 5.6 (GSH) or 5.9 (PenSH) using a dose of 8.3 Gy (~5 μM RS•).

were used as received: L-glutathione oxidized, GSSG (Fluka); L-glutathione reduced, GSH (Sigma); D-penicillamine, PenSH (Sigma); L-cysteine, CysSH (Fluka); 2,2,6,6-tetramethylpiperidinoxyl, TPO (Aldrich); 4-OH-TPO (Aldrich); and 3-carbamoylproxyl, 3-CP (Aldrich). Reduced β-nicotinamide adenine dinucleotide (NADH) grade III from yeast was obtained from Sigma. The concentration of NADH was determined spectrophotometrically using $\varepsilon_{340} = 6200\ M^{-1}\ cm^{-1}$. The oxoammonium cations were prepared in aerated solutions via electrooxidation of the respective nitroxides using an electrochemical reactor, similar to that previously described.[12] The concentration of the oxoammonium cation was determined by adding an excess of ferrocyanide and measuring the yield of ferricyanide ($\varepsilon_{420} = 1000\ M^{-1}\ cm^{-1}$). The oxoammonium cations derived from TPO and 3-CP were stable for at least 2−3 h at room temperature.

Pulse radiolysis experiments were carried out using a 5-MeV Varian 7715 linear accelerator (0.05−0.5 μs electron pulses, 200 mA current). A 200 W Xe lamp provided the analyzing light. Appropriate cutoff filters were used to minimize photochemistry. All measurements were made at room temperature using a 4 cm Spectrosil cell and applying three light passes (apparent optical path length = 12.1 cm). The dose per pulse, which was determined in N_2O-saturated solutions containing 5 mM KSCN,[34] varied between 1.0 and 8.3 Gy. γ-Irradiation was carried out at room temperature using a ^{60}Co source, and the dose rate was determined by Fricke dosimetry. Electron paramagnetic resonance (EPR) spectra were recorded on a Varian spectrometer working at X band with center field set at 3370 G, 100 kHz modulation frequency, 1 G modulation amplitude, and 10 mW incident microwave power. The nitroxide concentration was calculated from the EPR signal intensity using standard solutions of the nitroxide.

Results

Most experiments involved the formation of thiyl radicals upon pulse irradiation of N_2O-saturated solutions containing 0.5−4 mM thiol via reactions 1−5.[24−27]

$$H_2O \xrightarrow{\gamma}$$

$$e^-_{aq}\,(2.6),\ {}^\bullet OH\,(2.7), H^\bullet\,(0.6), H_3O^+\,(2.6), H_2O_2\,(0.72) \quad (1)$$

The numbers in parentheses are the radiation−chemical yields of the species (in 10^{-7} M Gy^{-1}) and are somewhat higher in the presence of high solute concentrations.

$$e^-_{aq} + N_2O \rightarrow N_2 + OH^- + {}^\bullet OH \quad (2)$$

$${}^\bullet OH + RSH \rightarrow RS^\bullet + H_2O \quad (3)$$

$$H^\bullet + RSH \rightarrow RS^\bullet + H_2 \ \text{ and } \ R^\bullet + H_2S \quad (4)$$

$$R^\bullet + RSH \rightarrow RS^\bullet + RH \quad (5)$$

A few experiments involved the generation of GS• in deaerated solutions containing 1 mM GSSG and 0.2 M *tert*-butanol. Under these conditions •OH is scavenged by *tert*-butanol, forming the respective relatively unreactive $^\bullet CH_2C(OH)(CH_3)_2$ radical, and GS• is formed via reactions 6−9.[24,26,35]

$$e^-_{aq} + GSSG \rightarrow (GSSG)^{\bullet -} \quad (6)$$

$$H^\bullet + GSSG \rightarrow GSSGH^\bullet \quad (7)$$

$$GSSGH^\bullet \rightleftharpoons (GSSG)^{\bullet -} + H^+ \quad (8)$$

$$(GSSG)^{\bullet -} \rightleftharpoons GS^\bullet + GS^- \quad (9)$$

The formation and decay of ~5 μM RS• was monitored directly at 330 nm. Under all experimental conditions the thiyl radicals studied decayed via second-order kinetics, the second-order rate constant being independent of [RSH]. Because $k_{11}[RS^-] + k_{-11} \gg k_{10} + k_{12}$,[24−27] this indicates that under the present conditions [$(RSSR)^{\bullet -}$] is negligible compared to [RS•].

$$RS^\bullet + RS^\bullet \rightarrow RSSR \quad (10)$$

$$RS^\bullet + RS^- \rightleftharpoons (RSSR)^{\bullet -} \quad (11)$$

$$(RSSR)^{\bullet -} + RS^\bullet \rightarrow RSSR + RS^- \quad (12)$$

Upon the addition of nitroxide, the decay of RS• ($\varepsilon_{330} = 390 \pm 50$, 650 ± 60, and $1120 \pm 90\ M^{-1}\ cm^{-1}$ for CysS•, GS•, and PenS•, respectively) turned from second- to first-order kinetics due to reaction 13.

$$RS^\bullet + {>}NO^\bullet \rightarrow \text{products} \quad (13)$$

Typical kinetic plots are given in Figure 1 displaying the effect of 3-CP on the decay rate of GS•. Figure 2 shows that the observed first-order rate constant, k_{obs}, increased linearly with increasing [>NO•]. From the slope of the lines we calculated k_{13}, and the values are listed in Table 1. The observed rate constant was independent of [RSH] at 1−4 mM at pH 5.9−6.1

TABLE 1: Rate Constants for the Reaction of RS• with Nitroxide (10^8 M^{-1} s^{-1})

	GS•	CysS•	PenS•
3-CP	4.6 ± 0.6^a 5.2 ± 0.3^b 5.9 ± 0.4^d	6.6 ± 0.2^b	5.5 ± 0.2^a 5.8 ± 0.1^c
4-OH-TPO	5.0 ± 0.2^b 5.2 ± 0.4^d	5.6 ± 0.2^b	4.9 ± 0.2^a
TPO	7.5 ± 0.2^b 6.7 ± 0.5^d		

[a] Determined directly by following the decay of RS• produced in N_2O-saturated solutions containing 1−4 mM RSH at pH 5.6−6.1. [b] Determined by competition kinetics using eq 15 for RS• between nitroxide and $ABTS^{2-}$ in N_2O-saturated solutions containing 1 mM RSH at pH 6.0 or 7.1. [c] Determined by competition kinetics using eq 16 for RS• between nitroxide and $ABTS^{2-}$ in N_2O-saturated solutions containing 2 mM RSH at pH 5.9. [d] Determined by competition kinetics for GS• between nitroxide and $ABTS^{2-}$ in deaerated solutions containing 1 mM GSSG at pH 6.4−6.6.

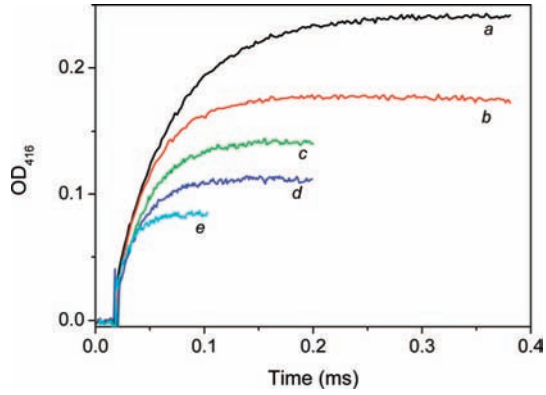


Figure 3. Effect of [TPO] on the rates of formation and yields of $ABTS^{\bullet-}$. All solutions were N_2O-saturated and contained 1 mM GSH and 2.8 mM phosphate buffer (pH 7.1) with various concentrations of TPO: 0 (a) 10 μM (b), 15 μM (c), 25 μM (d), and 45 μM (e).

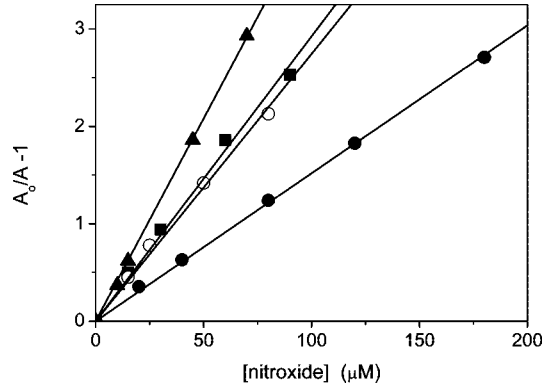


Figure 4. Effect of [nitroxide] on the yield of $ABTS^{\bullet-}$ analyzed according to eq 15. Solutions (N_2O-saturated) contained (■) 3-CP, 2 mM GSH, 100 μM $ABTS^{2-}$, 4 mM phosphate buffer, pH 5.6; (●) 3-CP or (○) 4-OH-TPO or (▲) TPO and 1 mM GSH, 50 μM $ABTS^{2-}$, 2.8 mM phosphate buffer, pH 7.1. The dose was 1 Gy/pulse.

and independent of pH as measured in the presence of 2 mM PenSH at pH 5.9 and 7.4.

The reaction of GS• with 4-OH-TPO around pH 6 could not be studied accurately due to the formation of a transient species absorbing at 330 nm. The absorption of CysS• is relatively weak ($\varepsilon_{336} = 390 \pm 50$ M^{-1} cm^{-1}), and therefore its reaction with >NO• could not be studied accurately. Hence, the reactions of

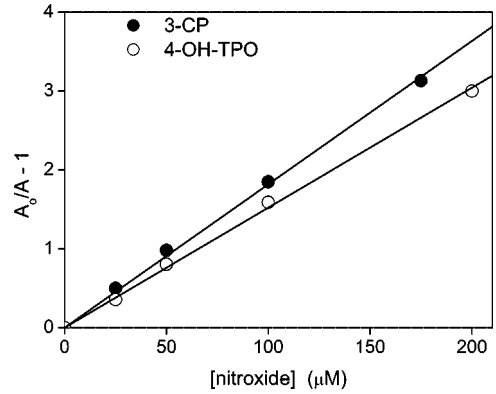


Figure 5. Effect of [nitroxide] on the yield of $ABTS^{\bullet-}$ analyzed according to eq 15. Solutions (N_2O-saturated) contained 2 mM CysSH, 50 μM $ABTS^{2-}$, 4 mM phosphate buffer, pH 6.0, and were irradiated with a dose of 1 Gy/pulse.

RS• with >NO• were also studied using competition kinetics with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{2-}$). The values of k_{14} at pH >5.5 have been recently redetermined for GS•, PenS•, and CysS• to be $(3.8 \pm 0.2) \times 10^8$, $(4.4 \pm 0.1) \times 10^8$, and $(4.8 \pm 0.1) \times 10^8$ M^{-1} s^{-1}, respectively.[36]

$$RS^{\bullet} + ABTS^{2-} \rightarrow RS^{-} + ABTS^{\bullet-} \qquad (14)$$

The formation of $ABTS^{\bullet-}$ was monitored at 416 nm ($\varepsilon = 3.6 \times 10^4$ M^{-1} cm^{-1}). Under all experimental conditions the reactions of $ABTS^{\bullet-}$ with RSH/RS^- and >NO• were sufficiently slow as not to affect the kinetics of formation or the yield of $ABTS^{\bullet-}$. Thiyl radicals in the presence of excess $ABTS^{2-}$ generated $ABTS^{\bullet-}$ as monitored by a first-order buildup of its absorbance, A_0 ($k^0_{obs} = k_{14}[ABTS^{2-}]_0$). Upon formation of RS• in solutions containing both $ABTS^{2-}$ and >NO•, where reactions 13 and 14 alone are taking place, the yield of $ABTS^{\bullet-}$ (A) should decrease and the first-order rate constant of the formation of $ABTS^{\bullet-}$ (k_{obs}) should increase as [>NO•] increases. Typical kinetic plots are given in Figure 3.

At constant [$ABTS^{2-}$], the dependencies of $ABTS^{\bullet-}$ yield and k_{obs} on [>NO•] are described by relationships 15 and 16, respectively.

$$A_0/A = 1 + k_{13}[>NO^{\bullet}]/k^0_{obs} \qquad (15)$$

$$k_{obs} = k^0_{obs} + k_{13}[>NO^{\bullet}] \qquad (16)$$

Figures 4 and 5 show the results according to expression 15 in the case of GS• and CysS• reaction with 3-CP, TPO, or 4-OH-TPO, whereas Figure 16 shows the results obtained from expression 16 for PenS• reaction with 3-CP or 4-OH-TPO. The values of k_{13} derived from Figures 4−6 are listed in Table 1. They seem to agree, within experimental accuracy, with the directly determined values.

In some experiments GS• was produced through the reduction of GSSG by e^-_{aq} according to reactions 6−9. The results are shown in Figure 7, and the derived k_{13} values are listed in Table 1, showing that they are indistinguishable from those obtained by generating GS• via oxidation of GSH by •OH/H•.

At pH >7, the formation of $ABTS^{\bullet-}$ in the presence of RSH and >NO• followed single-exponential kinetics, which was attributed to the competition between RS• and >NO• for $ABTS^{2-}$. Upon lowering the pH, a subsequent first-order formation of $ABTS^{\bullet-}$ was observed. Typical kinetic traces are shown in Figure 8.

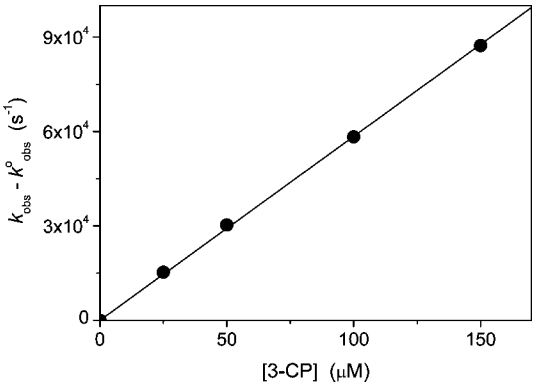


Figure 6. Effect of [3-CP] on the rate of $ABTS^{\bullet-}$ formation analyzed according to eq 16. Solutions (N_2O-saturated) contained 2 mM PenSH, 50 μM $ABTS^{2-}$, 4 mM phosphate buffer, pH 5.9, and were irradiated with a dose of 1 Gy/pulse.

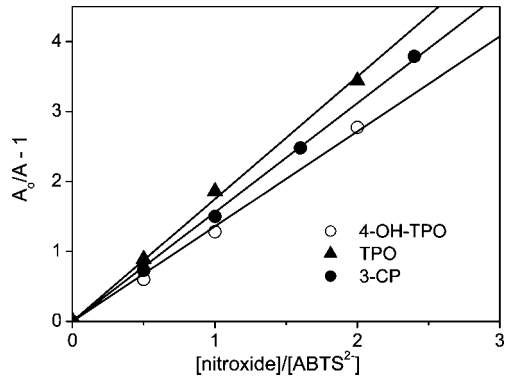


Figure 7. Effect of added nitroxide on the yield of $ABTS^{\bullet-}$ analyzed according to eq 15. Deaerated solutions (Ar-saturated) contained 1 mM GSSG, 0.2 M *tert*-butanol, 50 or 100 μM $ABTS^{2-}$, 2.4 mM phosphate buffer, pH 6.4−6.6, and a dose of 1.7 Gy/pulse.

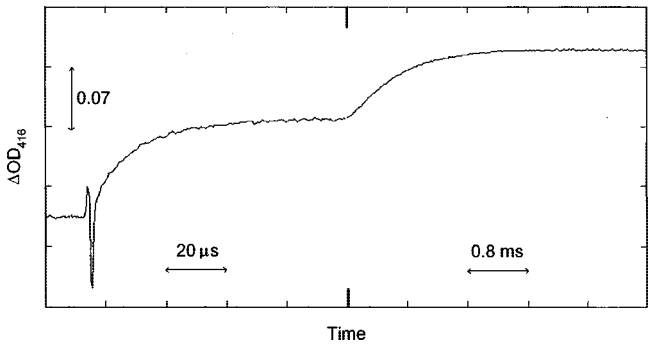


Figure 8. Formation of $ABTS^{\bullet-}$ upon pulse irradiation of N_2O-saturated solution containing 2 mM GSH, 60 μM 3-CP, 50 μM $ABTS^{2-}$, and 4 mM phosphate buffer at pH 5.8.

The stability of 3-CP in the presence of GSH in acidic solutions allowed a more detailed study of the mechanism of the reaction in the presence of this nitroxide and GSH. The rate of the slow process was independent of [3-CP] and almost independent of [GSH] and [$ABTS^{2-}$]. This finding reveals that the rate-determining step in the slow process is the decomposition of an intermediate adduct to yield a species capable of rapidly oxidizing $ABTS^{2-}$. On closer inspection, however, the rate is found to slightly increase with [$ABTS^{2-}$], indicating that adduct decomposition is not infinitely slower than the subsequent oxidation of $ABTS^{2-}$. In addition, the rate slightly increases with [GSH] as well. The reason for this is not sufficiently clear. It could reflect some side reaction between the adduct and GSH or, as will be discussed later, it could imply a competition

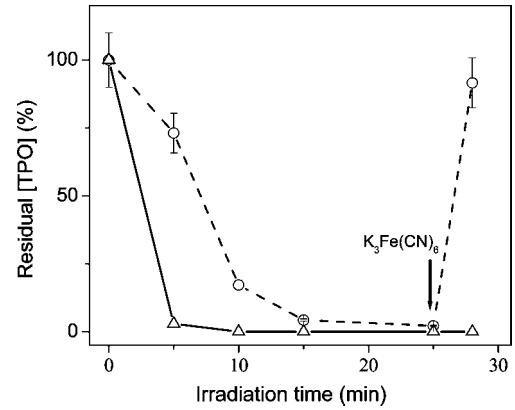


Figure 9. Oxidation of TPO by superoxide in the presence of NADH or GSH. Oxygenated solutions containing 200 μM TPO, 0.1 M formate, and 1 mM NADH (circles, dashed line) or GSH (triangles, solid line) in 0.2 M PB (pH 7.4) were γ-irradiated. The residual EPR signal of TPO was monitored at various points of time. After 25 min, 1 mM $K_3Fe(CN)_6$ was added and the EPR signal was monitored again.

between 3-CP and GSH for an oxidizing species that is formed from the adduct. These complications could probably be avoided, if $GS^{\bullet}$ is generated by reduction of GSSG in the presence of adequate amounts of $ABTS^{2-}$ while the [$>NO^{\bullet}$]/[$ABTS^{2-}$] ratio is maintained sufficiently large. The rate of the slow formation of $ABTS^{\bullet-}$ increases as the pH decreases until the two processes, that is, the fast and the slow one, merge. Under no experimental conditions did the yield of $ABTS^{\bullet-}$ exceed that of $GS^{\bullet}$ produced radiolytically, and sometimes the former was significantly lower than the latter. At pH <4 a single-exponential buildup was observed, where [$ABTS^{\bullet-}$] ~ [$GS^{\bullet}$]$_0$ and k_{obs} increased with increasing [$ABTS^{2-}$] and was independent of [GSH]. Similar results were obtained in the case of PenSH, but the appearance of the slow buildup was observed only at pH below 6.

As previously reported,[37] the oxidation of nitroxide by superoxide radical in the presence of NADH yields eventually the respective hydroxylamine, which can be oxidized to the parent nitroxide by one-electron oxidants such as ferricyanide. Conversely, upon oxidation of nitroxide in the presence of thiol, such as cysteine or GSH, the nitroxide is not recoverable by ferricyanide. This is demonstrated in Figure 9 showing the progressive loss of TPO upon exposure to radiolytically formed superoxide in oxygenated buffered solutions (pH 7.4) containing formate and NADH or GSH. Under such conditions all the water-derived radicals (reaction 1) are converted into superoxide radicals (reactions 17 − 20), which react predominantly with TPO because NADH and GSH react relatively slowly with supeoxide.[38]

$$e^-_{aq} + O_2 \rightarrow O_2^{\bullet-} \quad (17)$$

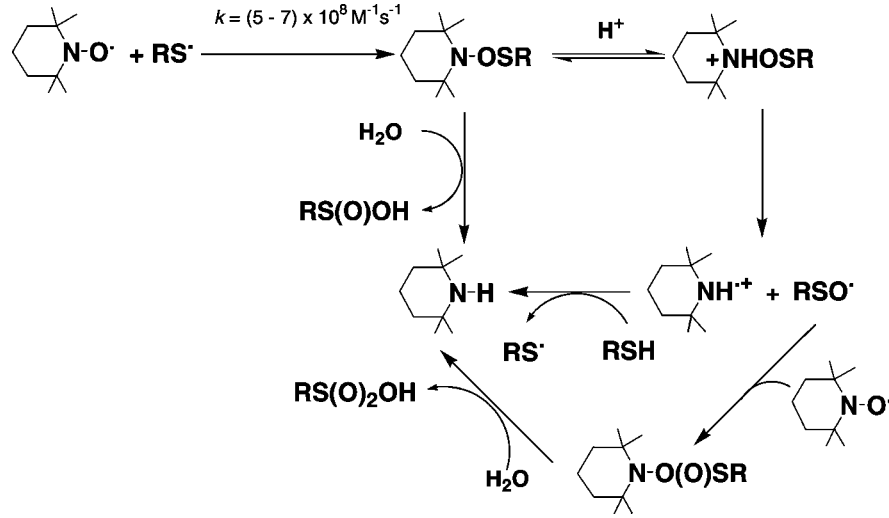
$$^{\bullet}OH/H^{\bullet} + HCO_2^- \rightarrow CO_2^{\bullet-} + H_2O/H_2 \quad (18)$$

$$CO_2^{\bullet-} + O_2 \rightarrow CO_2 + O_2^{\bullet-} \quad (19)$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \quad (20)$$

The oxidation of TPO by superoxide yields the respective oxoammonium cation, which is reduced by NADH via a two-electron transfer reaction to the respective hydroxylamine.[12] In contrast, the reaction of the oxoammonium cation with GSH could form the respective amine.[39] To check this assumption, we studied the reactions of the oxoammomium cations derived from TPO and 3-CP with NADH, GSH, or CysSH. When the

SCHEME 1



oxoammonium cation reacted with an excess of 1 mM NADH, a full recovery of the EPR signal was observed after the addition of 1 mM $K_3Fe(CN)_6$, supporting our previously suggested two-electron transfer mechanism.[12] In the case of GSH or CysSH no EPR signal was detected after the addition of ferricyanide.

Discussion

The rate constant for the reaction of nitroxides with thiyl radicals is independent of the structure of the nitroxide and thiyl radicals and is similar to that determined previously for the reactions of nitroxides with $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ radicals, $k = (5-7) \times 10^8$ M^{-1} s^{-1}.[17,19] In contrast to $^{\bullet}NO_2$, $CO_3^{\bullet-}$ and peroxyl radicals, the reaction of nitroxides with thiyl radicals does not form the respective oxoammonium cation, which rapidly oxidizes $ABTS^{2-}$.[12,17,18,40]

At pH <7, the reaction of the nitroxide with thiyl radicals produces a species that oxidizes $ABTS^{2-}$ to $ABTS^{\bullet-}$. As was mentioned before, this oxidizing species arises during the rate-determining decomposition of an adduct, although the rate of this process increases slightly as [$ABTS^{2-}$] or [GSH] increases. This might imply that the formation of $ABTS^{\bullet-}$ takes place through the reaction of this oxidizing species with $ABTS^{2-}$ in competition with its oxidation of GSH to $GS^{\bullet}$, which subsequently oxidizes $ABTS^{2-}$. This conclusion is further supported by the slight decrease of the yield of $ABTS^{\bullet-}$ as [GSH] increases, given that the nitroxide competes with $ABTS^{2-}$ for the $GS^{\bullet}$ radicals. Our proposed mechanism is given in more detail in Scheme 1.

As seen in Scheme 1, the reaction of $>NO^{\bullet}$ with $RS^{\bullet}$ forms an unstable adduct (>NOSR) via a radical−radical recombination with $k = (5-7) \times 10^8$ M^{-1} s^{-1}. The deprotonated form of the adduct decomposes in water via heterolysis of the N−O bond, forming the respective amine (>NH) and sulfinic acid (RS(O)OH). The protonated form of the adduct homolyzes along the N−O bond, forming the respective aminium radical ($>NH^{\bullet+}$) and sulfenyl radical ($RSO^{\bullet}$). This can be rationalized by the significantly higher pK_a of the aminium radical, for example, $pK_a = 7.5-8$ for the 2,2,6,6-tetramethylpiperidinium radical,[41] as compared to that of the adduct, which should be well below 4. Because $RSO^{\bullet}$ is a reducing species, it does not react with $ABTS^{2-}$, and we suggest that it decomposes via reaction with the nitroxide, yielding the respective sulfonic acid ($RS(O)_2OH$) and amine (Scheme 1). The aminium radical can oxidize thiols, for example, $k \sim (2-6) \times 10^6$ M^{-1} s^{-1} for the reaction of the pyridinium radical with $CysS^{\bullet}$ and $PenS^{\bullet}$,[42] and if a similar rate constant also applies in our case, then the rate constant for the reaction of $>NH^{\bullet+}$ with $ABTS^{2-}$ should be about an order of magnitude higher. This estimation is based on the competition of $ABTS^{2-}$ and RSH for $>NH^{\bullet+}$, where the concentration of RSH is 1−2 orders of magnitude higher than that of $ABTS^{2-}$. As demonstrated in Scheme 1, the final products of the reaction of nitroxide with thiyl radicals are the respective amine, sulfinic, and sulfonic acids. Our proposed mechanism is in accordance with previously reported findings, where the main products of the reaction of $GS^{\bullet}$ with 4-((9 acridinecarbonyl)-amino)-TPO in aerated solutions were identified as the respective amine, sulfonic acid, sulfenic acid, and glutathione disulfide.[21]

Nitroxides are recovered after being oxidized to the respective oxoammonium cations ($>N^+{=}O$) in the presence of NADH but not in the presence of thiols. This suggests that the reaction of $>N^+{=}O$ with thiols yields the respective amines. Two alternative mechanisms are suggested whereby $>N^+{=}O$ reacts with thiolate (RS^-) directly generating >NOSR (reaction 21) or indirectly forming $>NO^{\bullet}$ and $RS^{\bullet}$ (reaction 22), which subsequently yields >NOSR (see Scheme 1).

$$>N^+{=}O + RS^- \rightarrow >NOSR \quad (21)$$

$$>N^+{=}O + RS^- \rightarrow >NO^{\bullet} + RS^{\bullet} \quad (22)$$

Under physiological conditions the unstable adduct >NOSR decomposes mainly to the respective amine via heterolysis of the unstable adduct >NOSR. Therefore, nitroxides can detoxify thiyl radicals without producing any toxic intermediates. This mechanism can also contribute to the protective effect of nitroxides against $^{\bullet}NO_2$, $CO_3^{\bullet-}$, and peroxyl radicals in the presence of thiols because their reaction with nitroxides yields the respective oxoammonium cations.

Conclusions

The rate constant for the reaction of cyclic nitroxides with thiyl radicals has been determined to be $(5-7) \times 10^8$ M^{-1} s^{-1} at pH 5−7, independent of the structure of the nitroxide and the thiyl radical. It is proposed that this reaction yields an unstable adduct, which decomposes via a complex mechanism to yield the respective amine, sulfinic, and sulfonic acids. Under physiological conditions the adduct is deprotonated and decomposes via heterolysis of the N−O bond, yielding the respective

amine and sulfinic acid. This mechanism accounts for the protective effect of nitroxides against thiyl radicals and also against reactive oxygen- and nitrogen-derived species in the presence of thiols.

Acknowledgment. This work was supported by the Israel Science Foundation of the Israel Academy of Sciences. The preparation of the manuscript was aided by support from the European Science Foundation COST Programme, Action CM0603 (Free Radicals in Biology).

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JP804743G