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Reaction of hydroxyl radicals with *S*-nitrosothiols: determination of rate constants and end product analysis

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The reaction of the hydroxyl radical ($^{\bullet}OH$) with *S*-nitroso derivatives of cysteine, acetylcysteine and glutathione was studied at neutral and acidic pH. The second-order rate constants were determined by a competition kinetic method using a deoxyribose–thiobarbituric acid assay. The rate constants were diffusion controlled and were 2.27, 1.94 and 1.46×10^{10} dm^3 mol^{-1} s^{-1}, for *S*-nitrosocysteine, *S*-nitrosoacetylcysteine and *S*-nitrosoglutathione respectively, at neutral pH. The major products of the degradation induced by $^{\bullet}OH$ were found to be the corresponding disulfide (–S–S–) and nitrite (NO_2^-) at neutral pH as well as at pH 3. Simultaneous proton formation has also been observed. A plausible mechanism based on the formation of an intermediate thiol radical ($RS^{\bullet}$), as a result of electron transfer from the *S*-nitrosothiols (RSNOs) to $^{\bullet}OH$, is proposed for the formation of disulfide and nitrite at both pHs. The high rate constant values and the degradation of these compounds demonstrate the potential role of $^{\bullet}OH$ in RSNO metabolism under physiological conditions.

Introduction

S-Nitrosothiols (RSNOs) are an important class of compounds that are believed to play a major role *in vivo*, in connection with the storage and transport of nitric oxide ($^{\bullet}NO$) within the body.[1,2] The mechanism of formation of RSNOs from the reaction of $^{\bullet}NO$ (produced from L-arginine) with protein thiols, in the presence of oxygen, is reasonably well understood.[3–5] In a recent study, we reported the most probable reaction mechanism of RSNO formation *in vivo* using a kinetic model.[6] Among the various biological functions of RSNOs it is reported that the cytotoxicity depends on the decomposition time of RSNO. The compounds that release NO rapidly are less cytotoxic than the compounds that release NO slowly.[7] The involvement of RSNOs in the storage and transport of $^{\bullet}NO$ within the body makes them potential candidates for medical applications. For example *S*-nitrosoglutathione (GSNO) is currently used to inhibit platelet aggregation during some operations.[8,9] In this context, the kinetics and mechanism of the release of $^{\bullet}NO$ by RSNO is very important. Reports on the kinetics and mechanism of the degradation of RSNO, leading to the release of $^{\bullet}NO$ by metal ions and some nucleophiles, are available. Metal ions such as Cu^+ and Hg^{2+} accelerate the degradation of RSNO.[10–12] The reaction of these metal ions causes liberation of $^{\bullet}NO$ and nitrite (NO_2^-) respectively from RSNO. The presence of ascorbate at high concentrations also enhances the degradation of RSNO leading to the formation of $^{\bullet}NO$ and the thiol (from which RSNO is formed), in the absence of any metal ions, while low concentrations of ascorbate generate $^{\bullet}NO$ and disulfide.[13] Based on the ability of ascorbic acid to decompose GSNO, it is also proposed that it might act as a modulator for RSNO metabolism.[14] Reactions of *S*-nitrosocysteine with hydrogen peroxide yields the peroxynitrite anion.[15] It is also reported that GSNO reacts with superoxide radicals ($O_2^{\bullet -}$) generating glutathione disulfide (GSSG) and equimolar quantities of nitrite and nitrate. Here too, $O_2^{\bullet -}$ could act as a physiological modulator of *S*-nitrosation reactions.[16,17] However, a recent study demonstrated that the reaction of $O_2^{\bullet -}$ with GSNO is unlikely to be biologically important based on the rate constant, which is only of the order of 300 dm^3 mol^{-1} s^{-1}.[18] This rate constant is much lower than the rate constant of the reaction between $O_2^{\bullet -}$ and $^{\bullet}NO$, hence the former reaction may not compete with the latter.

Hydroxyl radicals ($^{\bullet}OH$) are the main DNA damaging agent which can be produced *in vivo* during oxidative stress and on exposure to ionizing radiation.[19] $^{\bullet}OH$ is known to react with many organic molecules including GSH at a diffusion controlled rate.[20] Understanding of the reaction between $^{\bullet}OH$ and RSNO is, therefore, a matter of the utmost importance from a biological perspective. It is probable that $^{\bullet}OH$ could act as a physiological modulator of the *S*-nitrosation reaction compared to $O_2^{\bullet -}$ as it is expected that the rate constants of the reactions of $^{\bullet}OH$ with RSNOs are several orders of magnitude greater than that of $O_2^{\bullet -}$. However, the exact rate constants for the reactions of $^{\bullet}OH$ with RSNOs are not yet reported. Therefore, we have determined the second-order rate constants in the case of the *S*-nitroso derivatives of cysteine (CYSH), acetylcysteine (ACYSH) and glutathione (GSH) at neutral pH with the help of a competition kinetic method using a deoxyribose–TBA assay. This study also presents a detailed product analysis at different pH values of the three RSNOs along with a probable reaction mechanism. Cysteine is the basic reagent unit for the formation of a –S–NO bond in any reaction of $^{\bullet}NO$ with free thiols (of proteins or glutathione) and therefore, it would be ideal to investigate its degradation by $^{\bullet}OH$. Glutathione (GSH) is the most abundant sulfur-containing intracellular entity (cellular concentration ~5 mM) and therefore, the endothelial nitric oxide has to diffuse through the cells in the presence of GSH. This leads to the assumption that, *in vivo*, the most likely *S*-nitrosation product could be GSNO.[21] In a preliminary report, we demonstrated that $^{\bullet}OH$ reacts with GSNO leading to the formation of glutathione disulfide and nitrite at neutral pH.[22] One of the major difficulties involved in the study of $^{\bullet}OH$ reactions with RSNOs is that the components of most of the $^{\bullet}OH$ generating systems such as H_2O_2 photolysis, Fenton reaction *etc.* can themselves induce degradation of RSNOs. In this context, radiation chemical method is an ideal choice, where ionizing radiation such as γ-rays can radiolyze water and produce both oxidising and reducing radicals. Therefore, in the present work we have used radiation chemical techniques to produce $^{\bullet}OH$ (eqns. (1) and (2)).

$$H_2O \rightsquigarrow e_{aq}^-, H^{\bullet}, {}^{\bullet}OH, H_2, H_2O_2, H_3O^+ \quad (1)$$

$$N_2O + e_{aq}^- \longrightarrow {}^{\bullet}OH + OH^- + N_2 \quad (2)$$

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Results

$^{\bullet}$OH reacts with cysteine and glutathione at a diffusion controlled rate.[20,32] Such high rate constants are generally determined either by direct monitoring of the formation of the resulting intermediates using pulse radiolysis or by competition kinetic methods with an $^{\bullet}$OH scavenger, using pulse or steady state radiolysis technique. In an earlier set of experiments, we attempted to monitor the possible intermediates from the reaction of $^{\bullet}$OH with GSNO at pH 7 using pulse radiolysis connected to transient absorption spectroscopy in the range, 300–700 nm. No intermediates were observed in this wavelength range indicating that no intermediates with optical absorption properties in the region 300–700 nm, were formed from the reaction of $^{\bullet}$OH with GSNO. Therefore, it was practically difficult to monitor the build-up of intermediates for the rate constant measurements. On the other hand, competition kinetic methods using pulse radiolysis require an absorbing transient intermediate such as dimethyl sulfoxide (DMSO) as the $^{\bullet}$OH scavenger. The RSNOs were found to be unstable in solutions containing DMSO. Therefore, a competition kinetic method, using 2′-deoxy-D-ribose as the $^{\bullet}$OH scavenger followed by a thiobarbituric acid (TBA) assay using a steady state radiolysis technique, was found to be the most suitable method for the determination of rate constants.

N_2O-saturated aqueous solutions containing RSNOs (CYSNO, ACYSNO or GSNO) and EDTA at pH 7 were irradiated with a ^{60}Co-γ-source and the dose dependent decay of RSNOs was initially confirmed using a UV/VIS spectrophotometer. The chemical changes brought about by radiation can be due to either a *direct effect* or an *indirect effect*. The direct effect is due to the direct deposition of radiation energy and the indirect effect due to the reaction of water-derived free radicals with the solute molecules. In the case of low concentrations (mM) of solute molecules in an aqueous medium, the indirect effect will be the dominant effect.[19] Therefore, the most dominant reaction in the radiolysis of the N_2O-saturated aqueous solutions is with $^{\bullet}$OH, and the dose dependent decay of RSNOs is a clear indication of this reaction.

2′-Deoxy-D-ribose (DR) is a well known $^{\bullet}$OH scavenger and this results in the formation of malondialdehyde (MDA) which produces a pink coloured chromogen with TBA (TBA–Chr) with λ_{max} at 532 nm and an absorption coefficient of 153,000 dm^3 mol^{-1} cm^{-1}.[33] A number of trial experiments were initially carried out to confirm the stability of RSNOs in the presence of DR and it was found that there is no interaction between these compounds which would affect the measurements to any significant extent.

A competition between the reaction of $^{\bullet}$OH with DR and of $^{\bullet}$OH with RSNO will depend on the rate constants of these two reactions. Consider equations 3 and 4 where k_{DR} and k_R are the second-order rate constants for the reaction of $^{\bullet}$OH with DR and RSNO, respectively:

$$DR + {}^{\bullet}OH \xrightarrow{k_{DR}} MDA \rightarrow \text{Products with TBA} \quad (3)$$

$$RSNO + {}^{\bullet}OH \xrightarrow{k_R} \text{Products} \quad (4)$$

Therefore, the rate equation can be written as in eqn. 5.

$$\frac{\frac{d[{}^{\bullet}OH]}{dt}}{\frac{d[TBA-Chr]}{dt}} = 1 + \frac{k_R[R]}{k_{DR}[DR]} \quad (5)$$

In the absence of RSNO, the rate of the reaction of $^{\bullet}$OH with DR is equal to the rate of $^{\bullet}$OH production, *i.e.*, $\frac{d[{}^{\bullet}OH]}{dt}$ can be taken as A^0. The rate of reaction in the presence of RSNO is equal to $-\frac{d[TBA\text{-}Chr]}{dt}$ which can be taken as A^*. Then, eqn. 5 can be rewritten as:

$$\frac{1}{A^*} = \frac{1}{A^0} + \frac{k_R[R]}{k_{DR}[DR]A^0} \quad (6)$$

A plot of $\frac{1}{A^*}$ *versus* [RSNO] must give a straight line of slope $\frac{k_R}{k_{DR}[DR]A^0}$ with an intercept on the Y-axis of $\frac{1}{A^0}$. From the slope, the second-order rate constant, k_R, can be determined. We used a value of 3.1×10^9 dm^3 mol^{-1} s^{-1} for k_{DR}.[34,35]

Using this concept, the absorbance of TBA–Chr was monitored at different concentrations of RSNO for a fixed time of irradiation and $\frac{1}{A^*}$ *versus* [RSNO] was plotted. Straight line plots with good correlation coefficients were obtained ($R^2 \geq 0.98$) (Fig. 1). The second-order rate constants were thus calculated from the slope and are summarised in Table 1.

Table 1 The second-order rate constant obtained for the reaction of $^{\bullet}$OH with RSNOs using a deoxyribose–TBA assay at neutral pH

RSNO	Rate/10^{10} dm^3 mol^{-1} s^{-1}
CYSNO	2.27
ACYSNO	1.94
GSNO	1.46

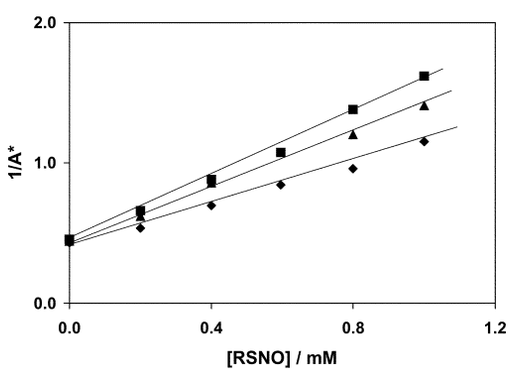

Fig. 1 Scavenging of hydroxyl radicals by varying concentrations of CYSNO (■), ACYSNO (▲) and GSNO (◆) in the presence of 2′-deoxy-D-ribose (3×10^{-3} mol dm^{-3}).

It can be seen from the table that all of the three rate constants are diffusion controlled. In the recent study by Ford *et al.*,[18] a second-order rate constant in the case of GSNO was estimated from a FACSIMILE simulation as 1.3×10^{10} dm^3 mol^{-1} s^{-1} and the experimentally determined value in the present case (1.46×10^{10} dm^3 mol^{-1} s^{-1}) matches very well with the former value.

N_2O-saturated aqueous solutions containing 1 mM RSNO (CYSNO, ACYSNO or GSNO) and 0.1 mM EDTA at pH 7 and 3 were irradiated at different doses in a ^{60}Co-γ-source and the decay of RSNO was monitored by a UV/VIS spectrophotometer. The UV/VIS spectra recorded after different irradiation times (and hence different doses) with all of these compounds showed a dose dependent decay in the wavelength region 200–600 nm at pH 3 as well as at pH 7. A typical spectral decay obtained with CYSNO is shown in Fig. 2.

As can be seen from the figure, the CYSNO characterised by its absorption maximum at 334 nm (and at 550 nm) was substantially degraded after about 30 minutes of irradiation (dose

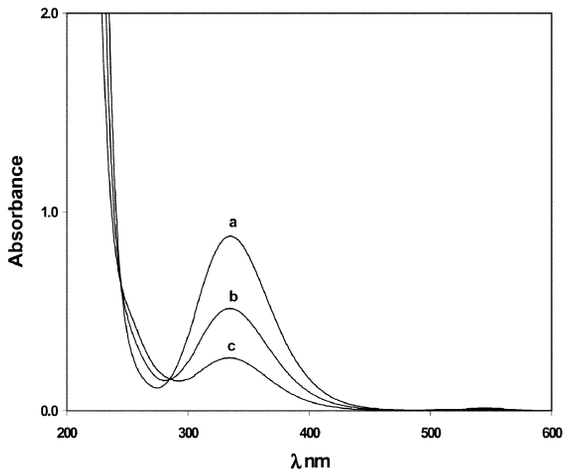

Fig. 2 Decay profile of N_2O-saturated CYSNO (1 mM), after gamma irradiation at doses a) 0 Gy, b) 515 Gy and c) 2060 Gy, using UV/VIS spectrophotometry.

absorbed = 2060 Gy). The decayed spectrum has shown a red-shift in its absorbance in the 240–270 nm region (Fig. 2). Experiments were also carried out to obtain a similar decay pattern at pH > 9, however, such measurements were hampered by the self decay of RSNO. RSNOs are known to undergo hydrolysis at basic pH.[36]

The decay patterns were also monitored using HPLC. The chromatograms obtained at pH 3 and 7 have shown a dose dependent decay of all of these compounds. A typical decay profile obtained with CYSNO is shown in Fig. 3. Subsequent HPLC analysis has further revealed that nitrite (NO_2^-) and disulfide (–S–S–) are the major products formed from the degradation of the respective *S*-nitrosothiols at pH 7 as well as at pH 3. The possibility of the formation of nitrate is excluded as the reaction is carried out in N_2O-saturated solutions where the presence of oxygen is negligible. The unirradiated solutions of RSNOs were found to be stable for many hours when protected from light. The dose dependent formation of the corresponding disulfide and the nitrite at pH 7 in the case of CYSNO is shown in Fig. 3. Similar product patterns were obtained in the case of ACYSNO and GSNO.

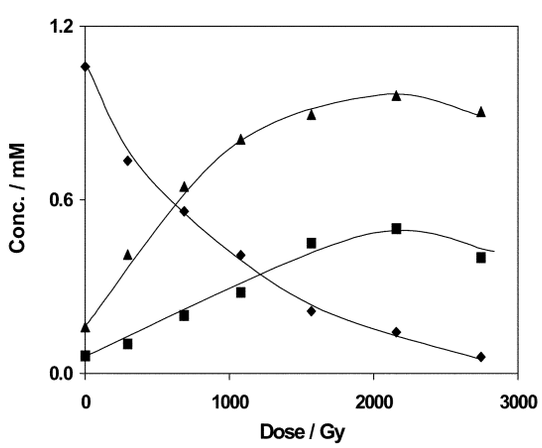

Fig. 3 Dose dependent decay of CYSNO (◆) at pH 7 and the corresponding formation of NO_2^- (▲) and CYSSCY (■) determined by using HPLC; mixture of disodium phosphate (1 mM) and sodium sulfate (10 mM) in water (pH 6) was used as eluent with a flow rate of 0.8 ml min^{-1}.

The red-shift of the decayed spectra of RSNO (see Fig. 2) is an additional indication of the formation of disulfide as this has an absorption around 250 nm region. The *G*(-RSNO)s were calculated from the slopes of the initial decay of RSNOs at pH 3 and 7 and are tabulated in Table 2. The *G*(-RSNO) values

Table 2 The *G* values determined after gamma radiolysis of N_2O-saturated RSNO solutions (1 mM) containing EDTA (0.1 mM)

	$G \times 10^7$ mol J^{-1}					
	−RSNO[a]		Disulfide		Nitrite	
RSNO	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
CYSNO	0.52	0.53	0.13	0.14	0.38	0.40
ACYSNO	0.54	0.53	0.09	0.12	0.41	0.39
GSNO	0.53	0.54	0.12	0.13	0.42	0.41

[a] Negative sign represents the degradation yield.

obtained at both pHs are close to the $G(^{\bullet}OH)$ value (~0.56 μmol J^{-1}) and therefore, it can be understood that the decay is nearly quantitative. Similarly the *G* values of the products, nitrite and disulfide, were also determined at pH 3 and 7 (Table 2). It is thus clear from Table 2 that both the decay and product build-up with CYSNO, ACYSNO and GSNO are similar. It was further observed that the pHs of the irradiated RSNO solutions were considerably lower than the unirradiated solutions. The pH of the solutions was, therefore, measured before and after different irradiation times at pH 7 and a clear dose dependent decrease was observed. The dose dependent pH changes obtained with CYSNO, ACYSNO and GSNO are shown in Fig. 4. A blank solution without RSNO under similar conditions was also irradiated and no major pH changes were observed.

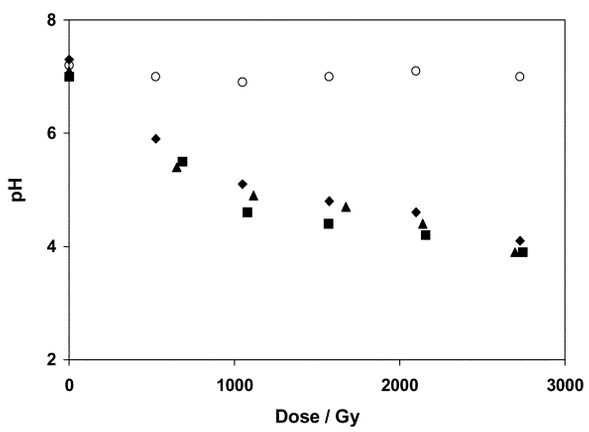

Fig. 4 pH changes in a gamma irradiated, N_2O-saturated solution containing RSNO (1 mM) and EDTA (0.1 mM) at different dose values. CYSNO (■), ACYSNO (▲), GSNO (◆) and without RSNO (○).

Discussion

Determination of the second-order rate constant for the reaction of $^{\bullet}OH$ with RSNOs is important in predicting any kind of biological significance of this reaction. This really is a challenge as many of the commonly used methodologies fail in this case because either the intermediate has no optical absorption properties (*e.g.* as in the case of pulse radiolysis), or the RSNOs are highly unstable in the presence of most of the $^{\bullet}OH$ generating reagents. The most suitable method for this purpose is therefore proposed to be the radiolytic production of $^{\bullet}OH$ and competition, using 2′-deoxy-D-ribose, followed by TBA assay. It is expected that this can be used as a general method for the determination of the second-order rate constant for the reaction of $^{\bullet}OH$ with any RSNO. The high rate constants obtained in the present case give an indication that this reaction is of biological relevance. Although the rate constant values are comparable, one can see a noticeable decrease in the rate constant value on going from CYSNO to GSNO as $k(^{\bullet}OH$ + CYSNO) > $k(^{\bullet}OH$ + ACYSNO) > $k(^{\bullet}OH$ + GSNO). This

decrease can be best attributed to the contribution of steric factors.

It is important to note that a good material balance is obtained from the G(RSSR) and $G(NO_2^-)$ values as G(-RSNO) ≈ G(RSSR) + $G(NO_2^-)$ (see Table 2). This implies that there is a quantitative production of RSSR and NO_2^- from the decay of all of the selected RSNOs. In one of the earlier studies,[12] production of peroxynitrite is reported from the reaction of H_2O_2 with GSNO. The decay of peroxynitrite would also yield NO_2^-. Therefore, in the present case too, it is probable that peroxynitrite – a yield equal to the yield of H_2O_2 ($G(H_2O_2)$ = 0.072 μmol J^{-1}) – is formed during radiolysis (see eqn. 1). As the yield of NO_2^- obtained in all three cases is very high compared to the yield of H_2O_2 ($G(NO_2^-)$ ≈ 0.4 μmol J^{-1}), it is undoubtedly clear that NO_2^- results mainly from the reaction of $^{\bullet}OH$ with RSNO.

From the G values of both –S–S– and NO_2^-, it is obvious that these two are the major products and that the elucidation of a reaction mechanism based on the formation of only these two products would be highly logical. Based on the more or less similar G values of these two products with all the RSNOs, it is assumed that the reaction of $^{\bullet}OH$ proceeds with the same mechanism in all these cases. It is also obvious from the dose dependent decrease of pH (see Fig. 4) that a proton is simultaneously released. $^{\bullet}OH$ generally reacts with thiols including GSH by H-abstraction (from –SH), forming thiyl radicals ($RS^{\bullet}$) as the main intermediate, as reported earlier.[17] As the sulfur is bonded to NO in the present case, the reaction mechanism would be different. On the other hand, it is expected that the most likely potential site for $^{\bullet}OH$ is still at the sulfur which is the most electron rich centre. Therefore, an electron transfer mechanism is proposed in the present case between the electrophilic $^{\bullet}OH$ and the electron rich sulfur. The immediate product of such an electron transfer reaction may lead to the production of a RSNO cation (RS^+NO). Such a cationic species is expected to be highly unstable and could result in the breakage of the S–NO bond leading to a thiyl radical ($RS^{\bullet}$) and NO^+ as shown in eqn. 9. In the reaction of $^{\bullet}OH$ with RSH, the intermediate radicals, observed using pulse radiolysis in earlier studies,[37] were identified as disulfide radical anions ($RSSR^{\bullet -}$). This species is formed by the reaction of the thiyl radical with the thiolate ion in an equilibrium reaction.

$$RS^{\bullet} + RS^- \rightleftharpoons RSSR^{\bullet -} \quad (7)$$

$RSSR^{\bullet -}$ has an absorption maximum of around 450 nm. On the other hand, the thiyl radical ($RS^{\bullet}$) has no measurable absorbance in the wave length range >300 nm.[38] It may be for this reason that we did not observe the build-up of any absorbing species using pulse radiolysis. In this case, the presence of RSH or RS^- is negligible. Therefore, the probable fate of $RS^{\bullet}$ is bimolecular interaction. The subsequent reaction of NO^+ with OH^-, which is formed as shown in eqn. 8, can lead to nitrite and H^+ formation. The mutual coupling of $RS^{\bullet}$ would eventually lead to the formation of disulfide. The combination of two sulfur-centered radicals and the corresponding formation of disulfide (RSSR) is a well known reaction reported in the case of low molecular weight thiols.[38] Therefore, a similar radical–radical reaction of $RS^{\bullet}$ is proposed for the formation of RSSR with all these compounds. The proposed mechanism for the formation of RSSR is displayed in reactions 8–11.

$$RSNO + {}^{\bullet}OH \longrightarrow [RS^+NO] + OH^- \quad (8)$$

$$[RS^+NO] \longrightarrow RS^{\bullet} + NO^+ \quad (9)$$

$$OH^- + NO^+ \longrightarrow HNO_2\ (H^+ + NO_2^-) \quad (10)$$

$$RS^{\bullet} \longrightarrow \tfrac{1}{2}\ RSSR \quad (11)$$

Therefore, the overall reaction mechanism can be written as:

$$RSNO + {}^{\bullet}OH \longrightarrow \tfrac{1}{2}\ RSSR + HNO_2\ (H^+ + NO_2^-) \quad (12)$$

In conclusion, evidence for the degradation of RSNOs induced by $^{\bullet}OH$ is presented. The high rate constants (≈10^{10} dm^3 mol^{-1} s^{-1}) obtained for this reaction indicate the probable involvement of $^{\bullet}OH$ in the metabolism of GSNO and CYSNO. Based on the rate constant for the reaction of $O_2^{\bullet -}$ with GSNO reported earlier[18] and that of $^{\bullet}OH$ with RSNOs in the present study, it is proposed that $^{\bullet}OH$ could possibly act as a physiological modulator of RSNOs compared to $O_2^{\bullet -}$. However, the role of $^{\bullet}OH$ in a cellular medium cannot be over-emphasised as GSH is known to have a sacrificial role in scavenging $^{\bullet}OH$. It reacts with $^{\bullet}OH$ with a high rate constant (1.3×10^{10} dm^3 mol^{-1} s^{-1}) and the concentration of GSH in cells is approximately 5 mM. As the concentration of GSNO under similar conditions is expected to be only around 10^{-6}–10^{-7} mol dm^{-3} (assuming the quantitative formation of GSNO from NO),[6] the availability of $^{\bullet}OH$ for the degradation reaction of GSNO may not be significant. Nonetheless, the high reactivity of $^{\bullet}OH$ with RSNOs is an obvious reason why this reaction must be considered carefully.

Materials and methods

Materials

Commercially available high purity glutathione (GSH), cysteine (CYSH), *N*-acetyl-L-cysteine (ACYSH) (from Aldrich), sodium nitrite ($NaNO_2$) and ethylenediaminetetraacetic acid (EDTA) (from Merck) were used without further purification.

Synthesis of RSNOs. The *S*-nitroso derivatives of GSH, CYSH and ACYSH were synthesized by a procedure reported earlier.[23] Equimolar quantities of $NaNO_2$ were added to ice-cold solutions of GSH, CYSH, and ACYSH in water containing 2 M HCl. The mixtures of GSH and $NaNO_2$ were kept for 40 min, and that of CYSH and ACYSH were kept for 15 min at 5 °C. The pale red solutions of CYSNO and ACYSNO were used as stock solutions. Conditions were adjusted to get a complete conversion of *S*-nitroso derivatives. The GSH–$NaNO_2$ mixture was then treated with acetone and stirred for about 10 min. The pale red precipitate (GSNO) was filtered, washed and dried. The concentrations of *S*-nitroso derivatives were calculated spectrophotometrically using the molar absorptivities at 336 nm as, GSNO = 770, CYSNO = 670 and ACYSNO = 870 dm^3 mol^{-1} cm^{-1}.[24] The purity of the RSNOs was checked by HPLC.

Preparation of RSNO solutions. Millimolar solutions of GSNO, CYSNO and ACYSNO were prepared in high purity water which contained 0.1 mM EDTA. The role of EDTA is to scavenge any traces of metal ions such as Cu^{2+} which can induce decomposition of RSNO.[11] Experiments were carried out at two typical pHs (pH 3 and 7) and the pH was adjusted by HCl and NaOH. All of the solutions were covered with black paper throughout the experiments to protect from light as RSNOs are known to be light sensitive.[7]

Pulse radiolysis. A few pulse radiolysis experiments were carried out to look at the transient intermediates from the reaction of $^{\bullet}OH$ with GSNO. This set-up consists of a linear accelerator delivering 7 MeV electron pulses of 50 ns duration and is connected to an optical detection set-up. This apparatus allows the detection of short-lived intermediates with life times from several nanoseconds to seconds with optical absorption properties in the 250–700 nm region. Details of the set-up have been published elsewhere.[25,26]

γ-Radiolysis and the production of $^{\bullet}$OH. γ-Irradiations were carried out with a ^{60}Co-γ-source. The dose rate was determined by cerric sulfate dosimetry[27] and was about 100 Gy min^{-1}.

The yields of various radicals and molecular products are normally expressed as G values which are defined as the number of molecules formed or destroyed per 100 eV absorption of radiation energy and in SI units, the yields are: $G(^{\bullet}OH) \approx G(e_{aq}^{-}) \approx G(H_3O^{+}) = 0.28$, $G(^{\bullet}H) = 0.062$, $G(H_2O_2) = 0.072$ and $G(H_2) = 0.047$ μmol J^{-1}.[28] In the presence of N_2O, $G(^{\bullet}OH) = 0.56$ μmol J^{-1} due to the conversion of e_{aq}^{-} into $^{\bullet}$OH according to eqn. 2, though a variation in this value can be observed depending on the concentration of the reactive substrate and the reaction rate constant.[29]

UV/VIS and HPLC analyses. The decay of the RSNOs was monitored by recording their absorption spectra from 200–600 nm using a UV/VIS spectrophotometer (Shimadzu UV160A). The decay as well as the decay products from the RSNOs were also analysed by HPLC (Shimadzu LC-10 AS) with a UV/VIS detector (Shimadzu SPD10A). A mixture of disodium phosphate (1 mM) and sodium sulfate (10 mM) in water (pH 6) was used as eluent with a flow rate of 1 ml min^{-1} for GSNO and with a flow rate of 0.8 ml min^{-1} for CYSNO and ACYSNO, using a 25 cm, Nucleosil, 5C-18 column.

Deoxyribose–TBA assay. This assay has been carried out based on a reported procedure.[30,31] Equal volumes of irradiated sample, which contained 2′-deoxy-D-ribose (DR), and 1% thiobarbituric acid (TBA) in the presence of 2.8% HCl were heated on a water bath for 20 min at 100 °C. The mixture was then cooled to room temperature and absorbance at 532 nm was recorded and the concentration of TBA–Chr was determined using an absorption coefficient of 153,000 dm^3 mol^{-1} cm^{-1}.[30,31] An unirradiated solution under the same conditions was used as a reference and no significant amount of TBA–Chr was formed in this reference solution. The concentration of DR was fixed at 3×10^{-3} mol dm^{-3}.

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