ChemComm

Hydroxyl radical induced decomposition of *S*-nitrosoglutathione

Veleeparambil M. Manoj and Charuvila T. Aravindakumar*

School of Chemical Sciences, Mahatma Gandhi University, Kottayam 686 560, Kerala, India.
E-mail: mgu@md2.vsnl.net.in

Received (in Cambridge, UK) 13th June 2000, Accepted 16th October 2000
First published as an Advance Article on the web

_S_-Nitrosoglutathione (GSNO) undergoes decomposition induced by hydroxyl radicals (·OH) in aqueous medium at neutral pH forming nitrite (NO_2^-) and glutathione disulfide (GSSG) and therefore it is proposed that ·OH could interfere in the GSNO metabolism.

S-Nitrosothiols (RSNO) are important class of compounds which are now believed to play a major role *in vivo* in connection with the storage and transport of nitric oxide (·NO) within the body.[1,2] The mechanism of the formation of RSNO from the reaction of ·NO with protein thiols in the presence and in the absence of oxygen is reasonably well understood.[3–6] The involvement of RSNOs in the storage and transport of ·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.[7,8] Therefore, the kinetics and mechanism of the release of ·NO by RSNO is very important. Excellent reports on the kinetics and mechanism of the degradation of RSNO leading to the release of ·NO by metal ions and some nucleophiles are now available.[9,10] Reaction of *S*-nitrosocysteine (SNCys) with hydrogen peroxide yields peroxynitrite anion.[11] It is also reported that GSNO reacts with superoxide radicals ($O_2^{\cdot -}$) generating glutathione disulfide (GSSG) and equimolar quantities of nitrite and nitrate.[12]

Glutathione (GSH) is the most abundant sulfur-containing intracellular entity (cellular concentration *ca.* 5 mM) and therefore the endothelial nitric oxide has to diffuse through the cells in presence of GSH. This leads to the assumption that *in vivo* conditions, the most likely *S*-nitrosation product could be the GSNO.[13] Hydroxyl radicals (·OH) are the main DNA damaging agent which can be produced *in vivo* during oxidative stress and on exposure to ionizing radiations.[14] Understanding of the reaction between ·OH and GSNO is, therefore, a matter of utmost importance in a biological perspective. The present communication describes a novel reaction pathway for the decomposition of GSNO in presence of ·OH at neutral pH. To our knowledge, this is the first report on the reaction of ·OH with a possible reservoir for ·NO in biological systems.

One of the major difficulties involved in the ·OH reaction with RSNOs is that the components of most of the ·OH generating systems such as H_2O_2 photolysis, Fenton reaction, *etc*., themselves can induce degradation of RSNOs. In this context, radiation chemical method is an ideal choice where ionizing radiations such as γ-rays can radiolyze water and produce both oxidising and reducing radicals. Therefore, in the present work we have used radiation chemical technique to produce ·OH as shown in reactions (1) and (2).

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

$$N_2O + e_{aq}^- + H_2O \rightarrow {}^{\cdot}OH + OH^- + N_2 \quad (2)$$

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, in SI units, the yields are, $G(^{\cdot}OH) \approx G(e_{aq}^-) \approx G(H_3O^+) = 0.28$, $G(^{\cdot}H) = 0.062$, $G(H_2O_2) = 0.072$ and $G(H_2) = 0.047$ μmol J^{-1}.[15] In the presence of N_2O the $G(^{\cdot}OH) = 0.56$ and $G(OH^-) = 0.28$ μmol J^{-1} as per reaction (2).

GSNO was synthesised from $NaNO_2$ in presence of HCl.[16] N_2O saturated solutions containing GSNO (10^{-3} M) and EDTA (10^{-4} M) at pH 7.3 were irradiated at different doses in a ^{60}Co-γ-source and the decay of GSNO was monitored by both UV–VIS spectrophotometry and HPLC. The unirradiated solution of GSNO was found to be stable for many hours when protected from light. The $G(-GSNO)$ values obtained in both cases were 0.53 and 0.54 μmol J^{-1} respectively. GSSG and nitrite were found to be the major products of radiolysis from the HPLC analysis. The decay of GSNO and the corresponding products build up are shown in Fig. 1. The calculated G(GSSG) and $G(NO_2^-)$ are 0.13 and 0.41 μmol J^{-1}, respectively. The pH of the solutions were determined before and after irradiation and a dose dependent reduction in pH was observed which is tabulated in Table 1. A blank solution without GSNO under similar conditions was also irradiated and obtained no major pH changes.

A good material balance can be observed from the G(GSSG) and $G(NO_2^-)$ values as $G(-GSNO) \approx G(GSSG) + G(NO_2^-)$. A minor contribution of NO_2^- is anticipated from the decay of peroxynitrite which could be formed as a result of the reaction of GSNO with H_2O_2 formed during radiolysis, as reported earlier.[11] However, this contribution is expected to be ⩽ 0.072 μmol J^{-1} [this value corresponds to $G(H_2O_2) = 0.072$ μmol J^{-1}]. Therefore, the major reaction is definitely between ·OH and GSNO. ·OH generally reacts with thiols including GSH by

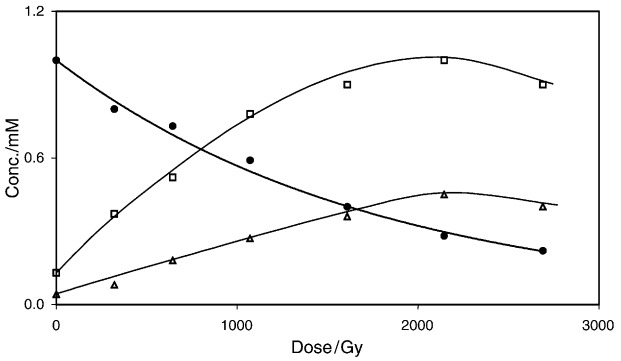

Fig. 1 Dose dependent decay of GSNO (●) at pH 7.3 and the corresponding formation of NO_2^- (□) and GSSG (Δ) determined by using HPLC (Column: 25 cm, Nucleosil, 5C-18; eluent: mixture of sodium phosphate (10^{-3} M) and sodium sulfate (10^{-2} M) in water; flow rate: 1 ml min^{-1}; λ = 210 nm).

Table 1 The observed pH changes in a γ-irradiated N_2O saturated solution containing GSNO (10^{-3} M) and EDTA (10^{-4} M) at different dose values

Dose/Gy	pH
0	7.3
525	5.9
1049	5.1
1574	4.8
2098	4.6
2728	4.1

DOI: 10.1039/b004706f

H-abstraction (from –SH) forming thiyl radicals ($RS^{\cdot}$) as the main intermediate as reported earlier.[17,18] Although the sulfur is bonded to NO in GSNO, the most potential site for $^{\cdot}OH$ attack is expected at the sulfur centre. On the other hand, the H-abstraction reaction which is reported in the case of GSH will not be possible in the present case. Therefore, based on the above observations we propose a reaction mechanism involving the attack of $^{\cdot}OH$ at the electron rich sulfur centre of GSNO [reactions (3)–(6)]. The initial attack of $^{\cdot}OH$ in GSNO (electron transfer) would produce a highly unstable cationic species as shown in reaction (3), which may lead to the breakage of the S–N bond and result in the formation of $GS^{\cdot}$ and NO^{+}. However, such a cationic intermediate ($GS^{+}NO$) is expected to be very short lived and no experimental evidence for its exact identity as well as its transnitrosation reaction [reaction (4)] is available at this moment. The subsequent reactions of NO^{+} with OH^{-} which is formed as shown in reaction (3), can lead to nitrite and H^{+} formation. The combination of two sulfur centered radicals ($RS^{\cdot}$) and the corresponding formation of disulfide (RSSR) is a well known reaction reported in the case of low molecular weight thiols.[19] Therefore, a similar radical–radical reaction of $GS^{\cdot}$ is proposed for the formation of GSSG.

$$GSNO + {}^{\cdot}OH \rightarrow [GS^{+}NO] + OH^{-} \quad (3)$$

$$[GS^{+}NO] \rightarrow GS^{\cdot} + NO^{+} \quad (4)$$

$$OH^{-} + NO^{+} \rightarrow H^{+} + NO_2^{-} \quad (5)$$

$$GS^{\cdot} \rightarrow \tfrac{1}{2}\,GSSG \quad (6)$$

Therefore, the overall reaction mechanism can be written as

$$GSNO + {}^{\cdot}OH \rightarrow \tfrac{1}{2}\,GSSG + NO_2^{-} + H^{+} \quad (7)$$

The dose dependent reduction in the pH values (Table 1) provides clear support to the above mechanism. Further, we exclude the possibility of the formation of nitrate as the reaction is carried out in N_2O saturated solutions where the presence of oxygen is insignificant.

In conclusion, the mechanistic aspects of the reaction of $^{\cdot}OH$ with GSNO, one among the biologically important *S*-nitrosothiols, have been proposed for the first time. The fast decay of GSNO in the presence of $^{\cdot}OH$ and the corresponding formation of nitrite and glutathione disulfide provide evidence for the possible interference of $^{\cdot}OH$ in the GSNO metabolism. One more question to be asked from these findings is, does GSNO have any sacrificial role like glutathione in terms of its protective role against oxidative stress? However, such a role can be established only after a clear understanding of the concentration of GSNO *in vivo* and of the exact rate constant of $^{\cdot}OH$ with GSNO, which are yet to be investigated. Our work is currently being concentrated in these directions.

We thank the Rubber Research Institute of India, Kottayam, for the γ-irradiation experiments. V. M. M. thanks the Nuclear Science Centre, New Delhi, for a fellowship. Part of the financial support for this work is from the Board of Research in Nuclear Sciences (BRNS), Govt. of India.

Notes and references

1 J. S. Stamler, O. Jaraki, J. Osborne, D. I. Simon, J. Keaney, J. Vita, D. Singel, C. R. Valeri and J. Loscalzo, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 76746.
2 B. Gaston, *Biochim. Biophys. Acta*, 1999, **1411**, 323.
3 V. G. Kharitonov, A. R. Sundquist and V. S. Sharma, *J. Biol. Chem.*, 1995, **270**, 28 158.
4 A. J. Gow, D. G. Buerk and H. Ischiropoulos, *J. Biol. Chem.*, 1997, **272**, 2841.
5 C. T. Aravindakumar, J. Ceulemans and M. De Ley, *Biochem. J.*, 1999, **344**, 253.
6 C. T. Aravindakumar, J. Ceulemans and M. De Ley, *Biophys. Chem.*, 2000, **85**, 1.
7 E. J. Langford, A. S. Brown, R. J. Wainwright, A. J. de Belder, M. R. Thomas, R. E. A. Smith, M. W. Radomski, J. F. Martin and S. Moncada, *Lancet*, 1994, **344**, 1458.
8 A. de Belder, C. Lees, J. Martin, S. Moncada and S. Campbell, *Lancet*, 1995, **345**, 124.
9 H. R. Swift and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1933.
10 A. P. Munro and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1999, 1989.
11 P. J. Coupe and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1999, 1057.
12 J. David, T. M. Christie, F. L. Stephen, A. W. David and B. G. Matthew, *Biochem. Biophys. Res. Commun.*, 1998, **244**, 525.
13 J. S. Stamler, *Curr. Top. Microbiol. Immunol.*, 1995, **196**, 19.
14 C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor & Francis, London, 1987.
15 J. W. Spinks and R. S. Wood, *An Introduction to Radiation Chemistry*, John Wiley & Sons, Inc., New York, 3rd edn., 1990.
16 T. W. Hart, *Tetrahedron Lett.*, 1985, **26**, 2013.
17 W. A. Armstrong and W. G. Humphries, *Can. J. Chem.*, 1967, **45**, 2589.
18 A. B. Ross, W. G. Mallard, W. P. Helman, G.V. Buxton, R. E. Hui and P. Neta, NDRL-NIST Solution Kinetics Database: ver.2.0, National Institute of Standards and Technology, Gaithersburg, MD, 1994.
19 W. A. Pryor, D. F. Church, C. K. Govindan and G. Gank, *J. Org. Chem.*, 1982, **47**, 159.