The role of glutathione in the transport and catabolism of nitric oxide

Neil Hogg, Ravinder J. Singh, B. Kalyanaraman*

Biophysics Research Institute, Medical College of Wisconsin, Milwaukee, WI 53226, USA

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Abstract Nitric oxide acts as a neuronal and vascular messenger implying diffusion through intracellular environments containing 5–10 mM glutathione. Nitric oxide reacts with glutathione under aerobic conditions generating *S*-nitrosoglutathione (GSNO). GSNO reacts with glutathione ($k = 8.3\times10^{-3}$ $M^{-1}\cdot s^{-1}$) to generate nitrous oxide and glutathione disulfide (GSSG). Anaerobically, glutathione reacts with nitric oxide generating nitrous oxide and GSSG ($k = 4.8\times10^{-4}$ s^{-1} at 5 mM GSH). In both aerobic and anaerobic situations the nitroxyl anion may be an intermediate in the synthesis of nitrous oxide and, under aerobic conditions, nitroxyl anion may generate peroxynitrite. We present a hypothesis for the intracellular interaction between nitric oxide and glutathione.

Key words: Nitric oxide; Glutathione; Nitrous oxide; Peroxynitrite; Nitrosothiol

1. Introduction

Central to the concept of nitric oxide as a messenger molecule is the assumption that nitric oxide is able to diffuse, or be transported, through cells and tissues to its site of action. A large body of experimental evidence bears out this assumption. For example, endothelial nitric oxide can be detected extracellularly by either electrochemical means [1] or by using a reporter cell system [2]. It has been suggested repeatedly that nitric oxide requires a carrier system in order to reach its pharmacological destination because of its inherently high reactivity with oxygen [3]. The rate constant for the reaction of nitric oxide with oxygen is about 6×10^6 $M^{-2}\cdot s^{-1}$ [4]. Nitric oxide is generated continuously at a particular locus and diffuses to its site of action in the presence of an oxygen concentration gradient. If we assume that physiological concentrations of nitric oxide are ≤ 0.1 μM [1] and average intracellular concentrations of oxygen are 20–50 μM [5] the rate of the reaction between nitric oxide and oxygen will be 1–3 pM/s. In the first minute of the reaction, less than 0.4% of the nitric oxide will have been consumed. As most physiological actions of nitric oxide occur on the time scale of seconds, the reaction with oxygen will affect minimally the diffusion of nitric oxide within and between cells.

It has been suggested that thiols may be intrinsically involved in the metabolism and mobilization of nitric oxide. The two most widely disseminated mechanisms for this are: (i) a ferrous iron-thiol complex binds nitric oxide and transports it to the site of action [6], and (ii) nitrosothiols, formed from the reaction between nitric oxide, thiols and oxygen, release nitric oxide after intercellular diffusion and uptake into target cells [7]. It has been suggested that endothelial derived relaxing factor is more closely akin to a nitrosothiol than to nitric oxide [8] although this has been disputed [9]. Protein nitrosothiols and low molecular weight nitrosothiols formed from endogenous and exogenous nitric oxide have been detected in plasma [10] and in pulmonary lavage [11], respectively.

A misconception that is prevalent in the literature is that nitric oxide reacts directly with thiols to form nitrosothiols in a reversible process [7]. Recently it has been demonstrated that the formation of *S*-nitrosoglutathione (GNSO) from glutathione (GSH) and nitric oxide is an oxygen dependent process [12]. However nitric oxide has been shown to oxidize thiols, to the corresponding disulfide, under anaerobic conditions [13].

In this paper we show that nitric oxide reacts with GSH under aerobic and anaerobic conditions and present the hypothesis that GSH is important in the mobilization and, under some circumstances, perhaps the catabolism of nitric oxide. The perspective here, however, is not that nitric oxide requires a carrier or transporter to reach its pharmacological target but that nitric oxide, to reach such a destination, has to diffuse through cells in the presence of 5–10 mM GSH.

2. Materials and methods

2.1. Materials

GSH, sodium dithionite and diethylenetriaminepentaacetic acid (DTPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Spermine NONOate was purchased from Cayman Chemical Co. (Ann Arbor, MI). Nitrous oxide was purchased from Airco Gases (Murrey Hill, NJ), nitric oxide was purchased from Matheson Gas (Joliet, IL) and *S*-nitrosoglutathione (GSNO) was synthesized as previously described [14].

2.2. Spectrophotometric methods

UV-visible spectra were obtained using an HP8452 diode array spectrophotometer (Hewlett Packard Co., Palo Alto, CA). Samples were prepared in quartz cuvettes that were sealed with a Suba-seal (Aldrich Chemical Co., Milwaukee, WI). Anaerobic samples were prepared from de-aerated buffer that had equilibrated with a pure nitrogen atmosphere for at least 24 h. All operations were performed within a nitrogen atmosphere using a glove-box. After sealing, the cuvette was transferred to the spectrophotometer. GSNO concentration was calculated using an extinction coefficient of 767 $M^{-1}\cdot cm^{-1}$ at 330 nm [15].

2.3. Nitrous oxide measurements

Samples (5 ml total volume) were prepared either aerobically or anaerobically and sealed inside 8 ml glass vials. Head space gas was removed at various intervals for nitrous oxide determination by gas chromatography [16]. Nitrous oxide concentration was calculated as previously described [17].

The rate constant for the reaction between nitric oxide and GSH was determined by incubating GSH with an aqueous, anaerobic solution of nitric oxide (4 ml total volume) in an 8 ml glass vial and nitrous oxide formation was monitored as a function of time.

2.4. Oxygen consumption

Oxygen consumption was measured using an oxygen electrode (Yel-

*Corresponding author. Fax: (1) (414) 266 8515;
E-mail: balarama@its.mcw.edu

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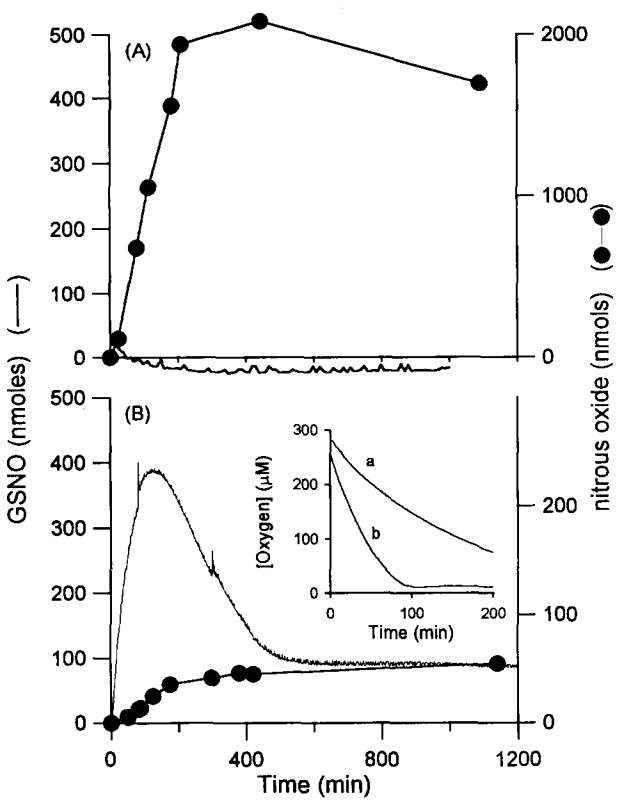


Fig. 1. The reaction between GSH and nitric oxide. GSH (1 mM) was incubated with spermine NONOate (1 mM) in PBS containing DTPA (100 μM) at 37°C either anaerobically (A) or aerobically (B). GSNO (—) was monitored continuously at 330 nm. Nitrous oxide was monitored by gas chromatography (●). Inset: Spermine NONOate (1 mM) was incubated either alone (a) or in the presence of GSH (1 mM) (b) in PBS containing DTPA (100 μM) at 37°C and oxygen consumption was monitored continuously using an oxygen electrode.

low Springs Instrument Co., Yellow Springs, OH) maintained at 37°C by a circulating water bath. The electrode was calibrated at 240 μM oxygen (water at 37°C) and 0 μM oxygen (sodium dithionitrite-treated water) and assumed to be linear within this range.

2.5. Measurement of nitric oxide

Nitric oxide release from spermine NONOate was monitored by elecron spin resonance using nitronyl nitroxide as previously described [18].

3. Results and discussion

3.1. The reaction of nitric oxide with GSH under anaerobic conditions

Nitric oxide was generated from the spontaneous decomposition of spermine NONOate ($t_{1/2}$ = 40 min) [19]. The reaction between nitric oxide and GSH under anaerobic conditions resulted in the almost stoichiometric conversion of nitric oxide to nitrous oxide (Fig. 1A). After 400 min 70% of the added nitric oxide was detected as nitrous oxide (assuming a stoichiometry of 2:1 nitric oxide/nitrous oxide). During the reaction a stoichiometric conversion of GSH to GSSG was observed and no GSNO was detected (Fig. 1A). This was more clearly seen in Fig. 2A where incubation of GSH with nitric oxide did not result in the appearance of an absorbance band centered at 340 nm, characteristic of GSNO. These results may be explained by Eq. 1–3 as was postulated previously for the reaction between nitric oxide and thiols [13]. GSH reacts with nitric oxide to give an intermediate radical adduct (Eq. 1). This adduct then

$$GSH + {}^{\bullet}NO \rightleftharpoons GS{-}\dot{N}{-}OH \quad (1)$$

$$2GS{-}\dot{N}{-}OH \rightarrow GSSG + HONNOH \quad (2)$$

$$HONNOH \rightarrow N_2O + H_2O \quad (3)$$

$$GS{-}\dot{N}{-}OH + {}^{\bullet}NO \rightarrow GSOH + N_2O \quad (4)$$

$$GSOH + GSH \rightarrow GSSG + H_2O \quad (5)$$

decays by a second order process to give GSSG and hyponitrous acid (Eq. 2). Decomposition of hyponitrous acid (Eq. 3) leads to the formation of nitrous oxide and water. However the presence of nitrite and acidic pH can lead to the formation of nitrate, molecular nitrogen and nitric oxide [20]. Alternatively, by analogy with the reaction between nitric oxide and protein cysteinyl residues [21], GSṄOH may react with nitric oxide to give glutathione sulfenic acid (GSOH) and nitrous oxide (Eq. 4). GSOH will then react rapidly with GSH to give GSSG as shown in Eq. 5.

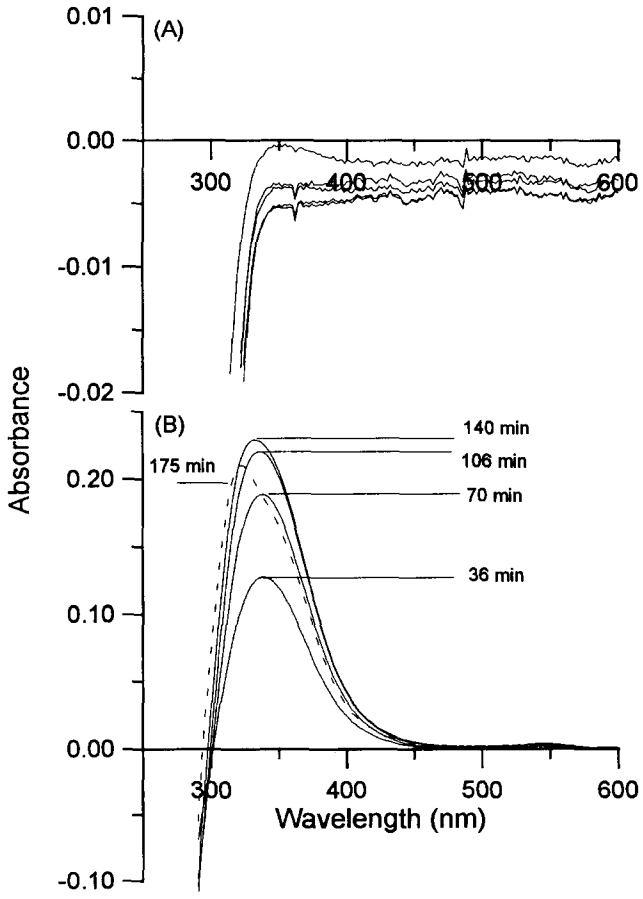


Fig. 2. Spectrophotometric changes during the reaction between GSH and nitric oxide. GSH (1 mM) was incubated with spermine NONOate (1 mM) in PBS containing DTPA (100 μM) at 37°C under anaerobic (A) or aerobic (B) conditions. Spectrophotometric changes were monitored between 250 and 600 nm at zero time and at the indicated times and the zero time point spectrum was subtracted from all subsequent measurements.

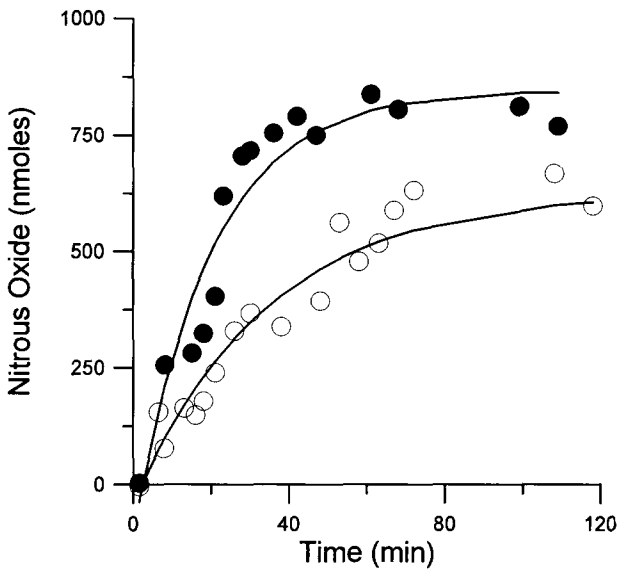


Fig. 3. The kinetics of formation of nitrous oxide. Nitric oxide (500 μM) was incubated in the presence of either 50 mM (●) or 5 mM (○) GSH in phosphate buffer (400 mM, pH 7.4) containing DTPA (1 mM) at 37°C under anaerobic conditions. Head space gas (100 μl) was removed and the nitrous oxide content was measured by gas chromatogrpahy. The data were fitted to a single exponential and the pseudo-first order rate constants were determined to be 4.8×10^{-4} s^{-1} (5 mM GSH) and 8.3×10^{-4} s^{-1} (50 mM GSH).

The rate constant for the reaction between nitric oxide and GSH was estimated by following the rate of formation of nitrous oxide. The reaction of nitric oxide with 10- and 100-fold excess of glutathione resulted in a time-dependent formation of nitrous oxide that could be approximated to a single exponential as shown in Fig. 3. This indicates a first order dependence on nitric oxide concentration. Because of the limitations of the present experimental protocol we were unable to accurately determine the order of the reaction with respect to GSH, however, the pseudo-first order rate constant in the presence of a physiological concentration of GSH (5 mM) was 4.8×10^{-4} s^{-1} ($t_{1/2}$ = 24 min).

3.2. The aerobic reaction between nitric oxide and GSH

In oxygen- (or air-) saturated solutions, bolus addition of nitric oxide to GSH results in the formation of GSNO. GSNO has also been detected during the slow release of nitric oxide from nitric oxide donors into an aerobic solution of GSH [12]. Fig. 1B shows the formation of GSNO from the reaction between spermine NONOate and GSH. GSNO formation was monitored by observing the change in absorbance at 330. In contrast to Fig. 2A, spectra taken between 250 and 600 nm revealed the formation of absorbance maxima at 340 nm and 550 nm indicating the production of GSNO (Fig. 2B). Eq. 6–8 indicate a plausible mechanism for the formation of GSNO under these conditions. It is also possible that other oxides of nitrogen can directly nitrosylate GSH

$$2^{\bullet}NO + O_2 \rightarrow 2^{\bullet}NO_2 \quad (6)$$

$$^{\bullet}NO + {}^{\bullet}NO_2 \rightarrow N_2O_3 \quad (7)$$

$$GSH + N_2O_3 \rightarrow GSNO + HNO_2 \quad (8)$$

[4]. The one electron oxidation of GSH by nitrogen dioxide is facile and results in the formation of glutathionyl radical. However, the possibility that glutathionyl radical is an intermediate in the formation of GSNO was discounted as 5, 5′-dimethyl-1-pyrroline-*N*-oxide (DMPO, 100 mM), an efficient thiyl radical trap, had no effect on the yield of GSNO. The consumption of oxygen during the decomposition of spermine NONOate (Fig. 1B, inset) is consistent with Eq. 6. However the addition of GSH caused a large increase in the rate of oxygen consumption. GSH alone did not result in oxyen consumption, nor did it affect the rate of spermine NONOate decay, as measured by UV-spectrophotometry and by nitric oxide release as measured by electron spin resonance. This implies that an intermediate in either the formation or the decay of GSNO reacts with oxygen.

3.3. The reaction of GSNO with GSH

It was observed that the GSNO formed from the reaction between nitric oxide and GSH under aerobic conditions was unstable in the presence of excess GSH (Fig. 1B). This indicates a direct reaction between GSNO and GSH. Fig. 4 shows the decrease in GSNO and the formation of nitrous oxide from the reaction between GSNO and GSH in the presence of DTPA. Under anaerobic conditions (Fig. 4A) GSNO completely decayed within about 400 min and approximately 75% of the nitrosyl functional group appeared as nitrous oxide. Under aerobic conditions (Fig. 4B), the rate of GSNO decay was identical to the anaerobic system, however the yield of nitrous oxide was dramatically reduced. Under these condi-

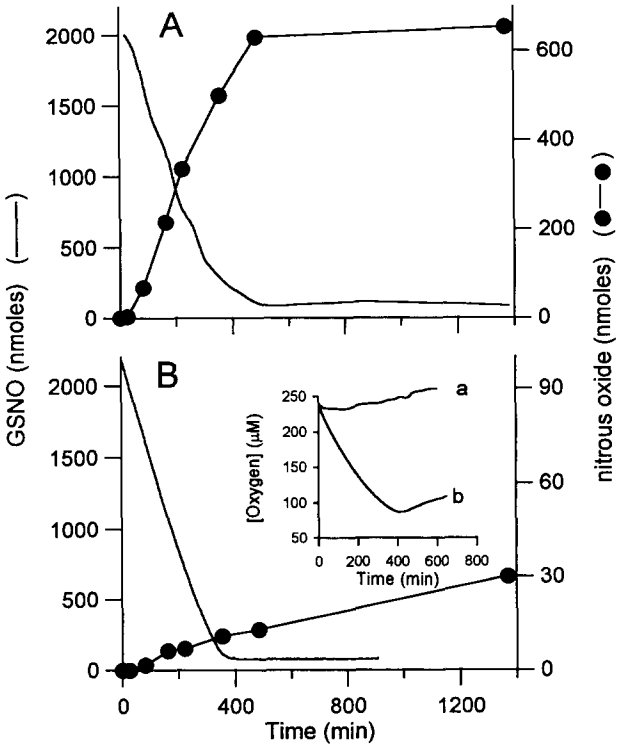


Fig. 4. The reaction between GSH and GSNO. GSH (1 mM) was incubated with GSNO (1 mM) in PBS containing DTPA (100 μM) at 37°C under anaerobic (A) or aerobic (B) conditions. GSNO (—) was monitored continuously at 330 nm. Nitrous oxide was monitored by gas chromatography (●). Inset: GSNO (1 mM) was incubated either alone (a) or in the presence of GSH (1 mM) (b) in PBS containing DTPA (100 μM) at 37°C and oxygen consumption was monitored continously using an oxygen electrode.

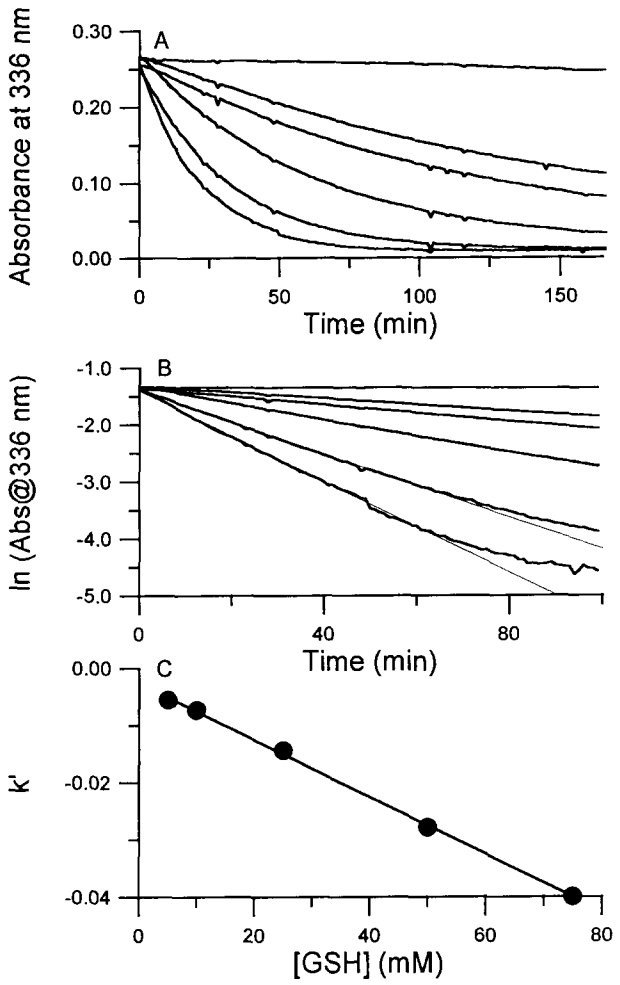


Fig. 5. Determination of the rate constant for the reaction between GSH and GSNO. (A) GSNO (350 μM) was incubated with GSH (0, 5, 10, 25, 50 and 75 mM) in phosphate buffer (200 mM, pH 7.4) containing DTPA (100 μM) at 37°C and the absorbance was monitored at 336 nm. (B) Data from (A) replotted using the natural log of the absorbance (heavy lines) and a linear regression fit to this data (light lines). (C) The gradient of the data in (B), representing the pseudo-first order rate constants (k') plotted as a function of GSH concentration giving a second order rate constant of 8.3×10^{-3} $M^{-1}\cdot s^{-1}$.

tions oxygen consumption was also observed and had synchronous kinetics to GSNO decay (Fig. 4, inset). The decay of GSNO in the presence of GSH exhibited second order behavior and, under pseudo first order conditions, GSNO decay was a single exponential (Fig. 5A). Thus plotting the natural log of the absorbance data gave linear curves the slope of which represents the pseudo first order rate constant, k' (Fig. 5B). A second order rate constant of $k_9 = 8.3\times10^{-3}$ $M^{-1}\cdot s^{-1}$ was calculated from k' as a function of GSH concentration (Fig. 5C). These results are consistent with the following mechanism (Eq. 9, 10, 11). Nucleophilic attack

$$GS^- + GSNO \rightarrow GSSG + NO^- \quad (9)$$

$$NO^- + NO^- + 2H^+ \rightarrow HONNOH \quad (10)$$

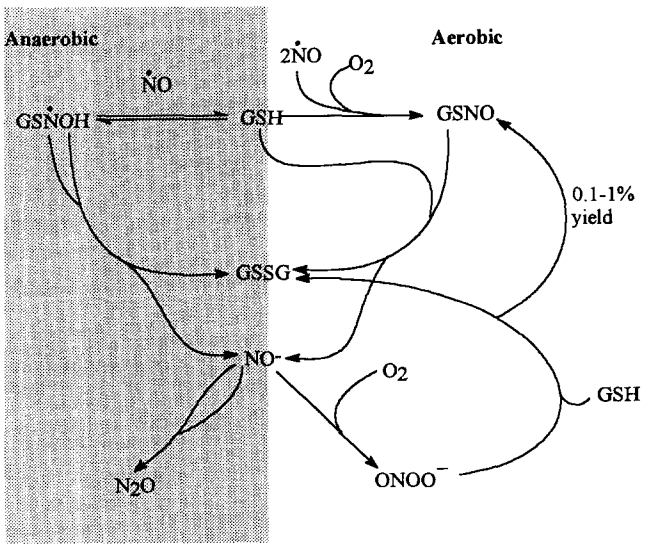
$$NO^- + O_2 \rightarrow ONOO^- \quad (11)$$

of GS^- on GSNO to give GSSG and NO^- (Eq. 9) has been previously described in organic solvents and may be used for the synthesis of mixed disulfides [22]. Under anaerobic conditions, NO^- dimerizes to eventually give nitrous oxide (Eqs. 10 and 3). Alternatively NO^- may react with GSH to give GSSG and hydroxylamine [23]. This reaction may account for the less than 100% yield of nitrous oxide. The formation of nitrous oxide and hydroxylamine has been previously reported during the oxidation of dithiols by nitric oxide [24]. Under aerobic conditions, however, NO^- reacts with oxygen (Eq. 11) to give peroxynitrite, consequently decreasing nitrous oxide production [25,26]. Peroxynitrite has previously been shown to oxidize GSH to give GSSG and low yields of GSNO and glutathionyl radical [27–29].

The reaction between GSH (or GS^-) and GSNO (Eq. 9) must be considered in experimental systems where *S*-nitrosothiols are used as nitric oxide donor compounds in the presence of large quantities of thiol. In such systems the potential for formation of nitrous oxide and peroxynitrite exists and it is far from certain that the predominant reaction of GSNO is homolytic cleavage to give nitric oxide.

3.4. Hypothetical mechanism for GSH-dependent nitric oxide transport in cells

The diffusion of nitric oxide through intracellular environments directly implies that nitric oxide is able to pass, relatively unscathed, through solutions of 5–10 mM GSH. However, the data presented here indicate that nitric oxide is able to react with GSH under both anaerobic and aerobic conditions (refer to Scheme 1). During these reactions, GSH is oxidized to GSSG. Under anaerobic conditions GSSG production occurs directly and generates nitrous oxide. Under aerobic conditions the reaction proceeds, at least partially, through the intermediate formation of GSNO that decays by reaction with GSH. The latter reaction also leads to the generation of nitrous oxide. As discussed above, the reaction of nitric oxide with oxygen is probably insignificant in the intracellular environment (however, see below). This minimizes the contribution of reactions 6–8 and indicates that GSNO formation by this mechanism is kinetically unlikely.



Scheme 1. Possible mechanism for the reactions between nitric oxide and GSH in both anaerobic and aerobic conditions.

This however, does not preclude the formation of *S*-nitrosothiols, such as serum albumin–NO, in plasma [30].

We hypothesize that the relevant reaction determining the fate of nitric oxide in the presence of high concentrations of GSH is the reversible association of nitric oxide with GSH to form GSṄOH (Eq. 1). It is envisaged that nitric oxide will diffuse through a cell in equilibrium with GSṄOH. At the plasma membrane nitric oxide will diffuse approximately equally in three dimensions whereas the diffusion of GSH will be restricted by the plasma membrane. The net result of this will be the release of nitric oxide from the cell, pulling the equilibrium of reaction 1 to the left near the plasma membrane. This allows a mechanism for the release of nitric oxide into the extracellular milieu or for diffusion between adjacent cells. A second order reaction between either two molecules of GSṄOH or nitric oxide and GSṄOH leads to the formation of GSSG and N_2O (Eq. 2, 3, 4, 5), possibly through the intermediate formation of NO^-. Alternative fates of NO^- include the reaction with GSH to give hydroxylamine [24] and formation of peroxynitrite from the reaction with oxygen (Eq. 11). NO^-, like molecular oxygen, has a ground-state biradical triplet multiplicity and an excited state singlet multiplicity. Thus there is no spin restriction for the reaction between the triplet ground-states of oxygen and NO^- [31]. At low rates of nitric oxide generation the steady state concentration of GSṄOH will be low and consequently decomposition to form N_2O will be slow. However at higher rates of nitric oxide synthesis, as may be derived from inducible nitric oxide synthase, formation and decomposition of GSṄOH may be at least partially responsible for nitric oxide catabolism.

This hypothesis assumes that GSṄOH does not react with other biological species, however, this may not be the case. The reaction of nitric oxide with GSH can be compared with the reaction of nitric oxide with oxygen. The rate constants for the reaction of nitric oxide with oxygen (6×10^6 $M^{-2}\cdot s^{-1}$ [4]) and GSH (4.8×10^{-4} s^{-1} at 5 mM GSH) indicate that at physiological concentrations (oxygen, 20 μM; GSH, 5 mM; nitric oxide, 0.1 μM) the reaction of nitric oxide with GSH will proceed about 40 times faster than the reaction of ˙NO with oxygen (48 pM/s vs. 1.2 pM/s, respectively). However, both reactions appear to proceed through intermediary radicals (ONOO˙ in the case of oxygen and GSṄOH in the case of GSH). It has been suggested that if ONOO˙ undergoes reactions with biological molecules then the reaction of nitric oxide with oxygen cannot be discounted on kinetic grounds [32]. This is also true for GSṄOH and the observation that there is at least 100-fold greater concentration of GSH than oxygen in the intracellular environment may make this possibility more likely. This conjecture awaits a full kinetic understanding of these two reaction mechanisms. It is clear that the reaction of nitric oxide with GSH is too slow to explain the rapid biological half life of nitric oxide of about 5 s. However, rapid consumption of GSṄOH by reactions other than those described here may contribute to the observed rate of nitric oxide inactivation. There is no evidence as yet for the reaction of either GSṄOH or ONOO˙ in biological systems.

4. Conclusion

The reaction between nitric oxide and GSH has the potential to generate GSNO, nitrous oxide and peroxynitrite. If the reaction of nitric oxide with oxygen is a major determinant of the extent of nitric oxide diffusion, in vivo, then the formation of GSNO may occur. If, as is likely, the low oxygen tension within the cell minimizes the importance of nitric oxide autoxidation then the formation of GSNO will not occur by this mechanism. In this scenario nitric oxide may diffuse through cells in transient association with GSH allowing for diffusion and subsequent release of nitric oxide into extracellular environments.

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References

[1] Malinski, T. and Taha, Z. (1992) Nature 358, 676–678.
[2] Ishii, K., Sheng, H., Warner, T.D., Forstermann, U. and Murad, F. (1991) Am J. Physiol. 261, H598–H603.
[3] Pietraforte, D., Mallozzi, C., Scorza, G. and Minetti, M. (1995) Biochemistry 34, 7177–7185.
[4] Wink, D.A., Darbyshire, J.F., Nims, R.W., Saavedra, J.E. and Ford, P.C. (1993) Chem. Res. Toxicol. 6, 23–27.
[5] Takehara, Y., Kanno, T., Yoshioka, T., Inoue, M. and Utsumi, K. (1995) Arch. Biochem. Biophys. 323, 27–32.
[6] Mulsch, A., Mordvincev, P.I., Vanin, A.F. and Busse, R. (1993) Biochem. Biophys. Res. Commun. 196, 1303–1308.
[7] Girard, P. and Potier, P. (1993) FEBS Lett. 320, 7–8.
[8] Myers, P.R., Minor Jr., R.L., Guerra Jr., R., Bates, J.N. and Harrison, D.G. (1990) Nature 345, 161–163.
[9] Feelisch, M., te Poel, M., Zamora, R., Deussen, A. and Moncada, D. (1994) Nature 368, 62–65.
[10] Stamler, J.S., Jaraki, O., Osborne, J., Simon, D.I., Keaney, J., Vita, J., Singel, D., Valeri, C.R. and Loscalzo, J. (1992) Proc. Natl. Acad. Sci. USA 89, 7674–7677.
[11] Gaston, B., Reilly, J., Drazen, J.M., Fackler, J., Ramdev, P., Arnelle, D., Mullins, M.E., Sugarbaker, D.J., Chee, C., Singel, D.J., Loscalzo, J. and Stamler, J.S. (1993) Proc. Natl. Acad. Sci. USA 90, 10957–10961.
[12] Wink, D.A., Nims, R.W., Darbyshire, J.F., Christodoulou, D., Hanbauer, I., Cox, G.W., Laval, F., Laval, J., Cook, J.A., Krishna, M.C., DeGraff, W.G. and Mitchell, J.B. (1994) Chem. Res. Toxicol. 7, 519–525.
[13] Pryor, W.A., Church, D.F., Govindan, C.K. and Crank, G. (1982) J. Org. Chem. 47, 159–161.
[14] Field, L., Dilts, R.V., Ravichandran, R., Lenhert, P.G. and Carnahan, G.F. (1978) J. Chem. Soc. Chem. Commun. 249–250.
[15] Mathews, W.R. and Kerr, S.W. (1993) J. Pharmacol. Exp. Ther. 267, 1529–1537.
[16] Fukuto, J.M., Wallace, G.C., Hszieh, R. and Chaudhuri, G. (1992) Biochem. Pharmacol. 43, 607–613.
[17] Singh, R.J., Hogg, N. and Kalyanaraman, B. (1995) Arch. Biochem. Biophys. 324, 367–373.
[18] Joseph, J., Hyde, J.S. and Kalyanaraman, B. (1992) Biochem. Biophys. Res. Commun. 192, 926–934.
[19] Maragos, C.M., Morley, D., Wink, D.A., Dunams, T.M., Saavadra, J.E., Hoffman, A., Bove, A.A., Isaac, L., Hrabie, J.A. and Keefer, L.F. (1991) J. Med. Chem. (1991) 34, 3242–3247.
[20] Akhtar, M.J., Bonner, F.T. and Hughes, M.N. (1985) Inorg. Chem. 24, 1934–1935.
[21] DeMasters, E.G., Quast, B.J., Redfern, B. and Nagasawa, H.T. (1995) Biochemistry 34, 11494–11499.
[22] Oae, S., Kim, Y.H., Fukushima, D. and Shinhama, K. (1978) J. Chem. Soc. Perkin I, 913–917.
[23] Turk, T. and Hollacher, T.C. (1992) Biochem. Biophys. Res. Commun. 183, 983–988.
[24] Arnelle, D.R. and Stamler, J.S. (1995) Arch. Biochem. Biophys. 218, 279–285.
[25] Fukuto, J.M., Hobbs, A.J. and Ignarro, L.J. (1993) Biochem. Biophys. Res. Commun. 196, 707–713.
[26] Donald, C.E., Hughes, M.N., Thompson, J.M. and Bonner, F.T. (1986) Inorg. Chem. 25, 2676–2677.

[27] Radi, R., Beckmand, J.S., Bush, K.M. and Freeman, B.A. (1991) J. Biol. Chem. 266, 4244–4250.
[28] Augusto, O., Gatti, R.M. and Radi, R. (1994) Arch. Biochem. Biophys. 310, 118–125.
[29] Karoui, H., Hogg, N., Fréjaville, C., Tordo, P. and Kalyanaraman, B. (1996) J. Biol. Chem., in press.
[30] Stamler, J.S., Simon, D.I., Osborne, J.A., Mullins, M.E., Jaraki, O., Michel, T., Singel, D. and Loscalzo, J. (1992) Proc. Natl. Acad. Sci. USA 89, 444–448.
[31] Donald, C.E., Hughes, M.N., Thompson, J.M. and Bonner, F.T. (1986) Inorg. Chem. 25, 2676–2677.
[32] Czapski, G. and Goldstein, S. (1995) Free Radical Biol. Med. 19, 785–793.