Photosensitized decomposition of *S*-nitrosothiols and 2-methyl-2-nitrosopropane Possible use for site-directed nitric oxide production

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Abstract **Irradiation of *S*-nitrosoglutathione (GSNO) with light (λ = 550 nm) resulted in the homolytic decomposition of GSNO to generate glutathionyl radical (GS·) and nitric oxide (·NO), which were monitored by ESR spectrometry. Inclusion of Rose Bengal (RB) resulted in a 9-fold increase in the quantum yield for ·NO production and also an increase in the rate of thiyl radical formation. The bimolecular rate constant for the interaction of triplet RB with GSNO has been estimated to be approximately 1.2×10^9 $M^{-1}s^{-1}$ by competition with oxygen. Hematoporphyrin (HP) also enhanced the rate of ·NO production by 2–3-fold. 2-Methyl-2-nitrosopropane (MNP) decomposed on irradiation (λ = 660 nm) to form ·NO and *tert*-butyl radical. Aluminum phthalocyanine tetrasulphonate enhanced the rate of decomposition of MNP by 10-fold. These studies show that photosensitizers enhance the release of ·NO from donor compounds.**

Key words: Nitric oxide; Thiyl radical; *S*-Nitrosoglutathione; 2-Methyl-2-nitrosopropane; Rose Bengal; Hematoporphyrin; Aluminum phthalocyanine tetrasulphonate

1. Introduction

Nitric oxide (·NO) plays multiple roles in biological systems [1]. The precise biological effects of ·NO production depend largely upon the rate and localization of ·NO generation. Thus, ·NO can act both as a biological messenger and as a tumorocidal cytotoxin [2]. In endotoxic shock, for example, it has been shown to be advantageous to inhibit endogenous ·NO production while, paradoxically, administering an ·NO generating agent [3]. It is likely, therefore, that therapeutic strategies involving ·NO-donor compounds will require some specificity of both the rate and location of ·NO production. ·NO has been implicated in macrophage-mediated tumor cell killing through the inhibition of mitochondrial enzyme activity and DNA synthesis in target tumor cells [2]. ·NO donors such as *S*-nitrosothiols have been proposed as reservoirs of ·NO in vivo due to their higher stability in comparison with ·NO. These compounds exhibit a range of pharmacological properties [4–7]. The mode of action of these compounds is generally attributed to the activity of ·NO released on homolytic decomposition of the S-NO bond [8–10]. Recently, *S*-nitrosoglutathione (GSNO) has been shown to be significantly more toxic to HL-60 leukemia cells in the presence of light than in the dark [11].

Photodynamic sensitizers have been reported to produce active oxygen species by Type II and Type I mechanisms. Localized production of singlet oxygen ($^1O_2$) and superoxide radicals ($O_2^{\cdot-}$) as cytotoxic and antiviral agents has been achieved in photodynamic therapy (PDT) [12,13]. Since ·NO exhibits tumorocidal and antiviral activity [2,14], it may be possible to exploit photosensitized ·NO production in clinical PDT. In this study, we show that photosensitizers such as Rose Bengal (RB) and aluminum phthalocyanine tetrasulphonate (AlPcS) can increase ·NO production from *S*-nitrosothiols and C-nitroso compounds. Thus, localized ·NO release from photoactivation of such compounds may afford new therapeutic strategies in phototherapy.

2. Materials and methods

2.1. Chemicals

Rose Bengal (Aldrich Chemical Co., Milwaukee, WI), hematoporphyrin dihydrochloride (HP), 2-methyl-2-nitrosopropane dimer (Sigma Chemical Co., St. Louis, MO), aluminum phthalocyanine tetrasulphonate (AlPcS) (Porphyrin Products, Logan, UT) were used as supplied. *S*-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) were prepared as previously reported [15]. 2-(-*p*-carboxyphenyl)-4,4,5,5-tetramethyl-imidazolin-3-oxide 1-oxyl (NNO) was used for monitoring the rate of ·NO production [16–18]. DMPO (Aldrich Chemical Co., Milwaukee, WI) was purified according to Kotake et al. [19].

2.2. ESR measurements

The samples were prepared in the dark on ice, taken into 50 μl capillaries (Corning, NY), and sealed with Miniseal at each end. The capillary was placed in a quartz tube and irradiated with monochromatic light inside the ESR cavity.

ESR spectra were recorded on a Varian E109 spectrometer at 9.5 GHz employing 100 kHz field modulation. ·NO formation was measured by observing the decay of NNO. This compound has a characteristic five-line ESR spectrum that changes upon reaction with ·NO to a seven-line spectrum due to imino nitroxide (INO) formation. The decay of NNO is monitored by observing the decrease in signal intensity of the low field line of the NNO spectrum. Alternatively, the rate of INO production was monitored by observing the increase in signal intensity of the low-field line of the INO spectrum. The kinetics of DMPO/·SG adduct were followed by monitoring the low-field line of the spectrum. Aqueous solutions containing MNP with and without AlPcS were similarly irradiated inside the ESR cavity at room temperature.

2.3 Irradiation procedure and quantum yield measurements

Samples were irradiated with light from a Bausch and Lomb high-intensity monochromator, using an ILC PS300–1A xenon arc source (ILC Technology, Sunnyvale, CA). Prior to ESR measurements, photon fluence rates, at approximately 1 cm from the front of the cavity, were measured using a YSI Radiometer, Model 65A (Yellow Springs Instruments, Columbus, OH). The amount of light incident on the solution inside the ESR cavity was calculated by actinometry using hematoporphyrin and 2,2,6,6-tetramethyl-4-piperidine *N*-oxyl [20]. It was assumed that $I_a = I_0\ (1-10^{-\varepsilon cl})$, where I_a is the amount of light absorbed by *S*-nitrosothiol, I_0 is the amount of light incident on the sample, ε is the molar extinction coefficient, c is the concentration of *S*-nitrosothiol, and l is the path length.

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3. Results

3.1 Rose Bengal-sensitized decomposition of S-nitrosothiols

S-nitrosothiols possess absorption maxima in the range of λ = 320–360 nm and λ = 550–600 nm [8]. GSNO absorbs at 330 nm (ε_{max} = 767 M^{-1} cm^{-1}) and has very weak absorption in the visible region at λ = 550 nm (ε_{max} = 13 M^{-1} cm^{-1}). Similarly, SNAP has absorption in the visible region at λ = 590 nm (ε_{max} = 11 M^{-1} cm^{-1}). RB has an absorption band with λ_{max} = 550 nm, which overlaps considerably with the band of *S*-nitrosothiols in the visible region (Fig. 1). RB has been widely used as a photosensitizer due to its high triplet yield (0.7) and high molar absorption coefficient (ε_{max} = 1 × 10^5 M^{-1} cm^{-1} at λ = 550 nm) [21]. This makes energy transfer between triplet state of RB and the triplet of *S*-nitrosothiols plausible. This transfer of energy may sensitize the homolytic cleavage of the S–N bond of *S*-nitrosothiols and increase the rate of ·NO production.

·NO production was not observed during incubation of GSNO (1 mM) and NNO (125 μM) in the dark. However, upon illumination with light (λ = 550 nm), the production of ·NO was observed (Fig. 2). The initial rate of decay of NNO, due to reaction with ·NO generated from photochemical decomposition of GSNO, was greatly enhanced in the presence of RB (100 μM) (Fig. 2). RB alone had no effect in the presence and absence of light indicating that neither RB nor $^1O_2$ react with NNO (data not shown). ·NO production was more rapid when the reaction was conducted under nitrogen (Fig. 2). This probably represents competition of GSNO and oxygen for triplet state RB. Neither photobleaching nor cytochrome *c* reduction was observed during irradiation (λ = 550 nm) of RB (10 μM) in the presence of GSNO (100 μM). However, irradiation of RB and NADH (20 μM) resulted in superoxide-dependent reduction of cytochrome *c* and photobleaching (data not shown). RB also enhanced the rate of ·NO production from SNAP and *S*-nitroso-*N*-acetylcysteine (SNAC) in the presence of light. The quantum yield of GSNO decomposition increased from 0.026 ± 0.002 to 0.225 ± 0.01 upon irradiation with RB. Similarly, the quantum yields for SNAP decomposition increased from 0.039 ± 0.007 to 0.4 ± 0.01 and for SNAC from 0.128 ± 0.004 to 1.03 ± 0.12. Erythrosin, a xanthine photosensitizer [21] absorbing in the visible region λ_{max} = 526 nm with a triplet yield of 0.63, also enhanced the decomposition of GSNO to almost the same extent as RB (data not shown).

HP, a well studied photodynamic photosensitizer, has a Soret band at 390 nm with ε_{max} = 1.4 × 10^5 M^{-1} cm^{-1}, but has relatively low molar extinction coefficient in the 500–600 nm region (Fig. 1). The triplet yield for HP has been reported to be 0.36 in aqueous medium [22]. HP in the dark or light (λ = 550 nm) had no effect on the ESR spectrum of NNO (data not shown). However, HP (100–350 μM) enhanced the rate of NNO decay by 2–3-fold in the presence of GSNO (1 mM) and light (λ = 550 nm) (Fig. 2). Under anaerobic conditions, the rate of NNO decay was further enhanced (data not shown).

Glutathionyl radical (GS·) formed from photodecomposition of GSNO was trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) to produce the characteristic ESR signal for the DMPO/·SG adduct (a_N = 14.9 G; a_H = 15.4 G) (Fig. 3, inset) [23]. The DMPO/·SG adduct increased in intensity to reach a maximum within 7 min. This maximum represents a steady-state level of DMPO/·SG adduct concentration where the adduct is both forming and decaying at the same rate. The rate of DMPO/·SG adduct formation increased by 5–6-fold in the presence of RB (100 μM) (Fig. 3). The bimolecular rate constant for the interaction between RB* and GSNO has been determined from the slope of the plot of

$$1/\frac{d[\cdot NO]}{dt} \text{ vs. } 1/[GSNO]$$

(Fig. 4) according to the following equation:

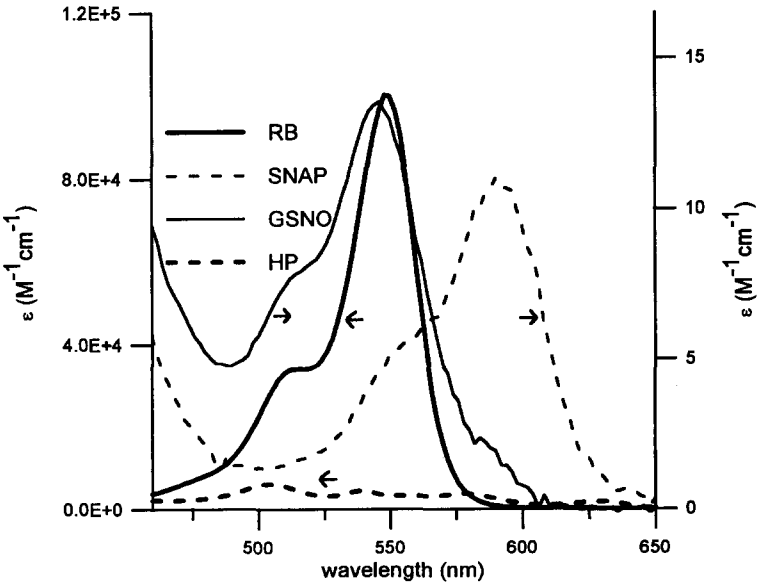

Fig. 1. Wavelength dependance of extinction coefficients for RB, HP, GSNO, and SNAP. The y-axis on the left is for RB and HP, and the y-axis on the right is for SNAP and GSNO as indicated by arrows.

$$\frac{\frac{d[\cdot NO]_{max}}{dt}}{\frac{d[\cdot NO]}{dt}} = \frac{k_{O_2}[O_2]}{k_{NO}[GSNO]} + 1$$

where k_{O_2} and k_{NO} are the bimolecular rate constants between RB* and O_2 or GSNO, respectively. This calculation assumes that oxygen competes for RB* with GSNO. d[·NO]/dt is the initial rate of ·NO formation for a particular concentration of GSNO, and

$$\frac{d[\cdot NO]_{max}}{dt}$$

is the maximum rate of ·NO formation at a saturating concentration of GSNO. All rates of ·NO formation were corrected for ·NO release in the absence of RB. This analysis gives $k_{NO} = 1.2 \times 10^9\ M^{-1}s^{-1}$ using a value of $1.6 \times 10^9\ M^{-1}s^{-1}$ for k_{O_2} [24].

3.2. *Aluminum phthalocyanine tetrasulphonate sensitized decomposition of 2-methyl 2-nitrosopropane*

AlPcS was used to photosensitize the release of ·NO from MNP. Specifically, AlPcS absorbs in the visible region at $\lambda = 660$ nm (Fig. 5) with a molar absorption coefficient of $2 \times 10^5\ M^{-1}\ cm^{-1}$ and has triplet yield of 0.4 [25]. MNP in the monomeric form absorbs in the visible region at $\lambda = 660$ nm with a molar absorption coefficient of only $15\ M^{-1}\ cm^{-1}$ [26]. The rate of ·NO generation from MNP due to its photolytic decomposition with light of $\lambda = 660$ nm was monitored by following the decay of NNO in aqueous solution. A slow release of ·NO from MNP was observed on irradiation; however, the rate of ·NO formation increased markedly in the presence of AlPcS (100 μg/ml) (Fig. 6). A three-line ESR spectrum

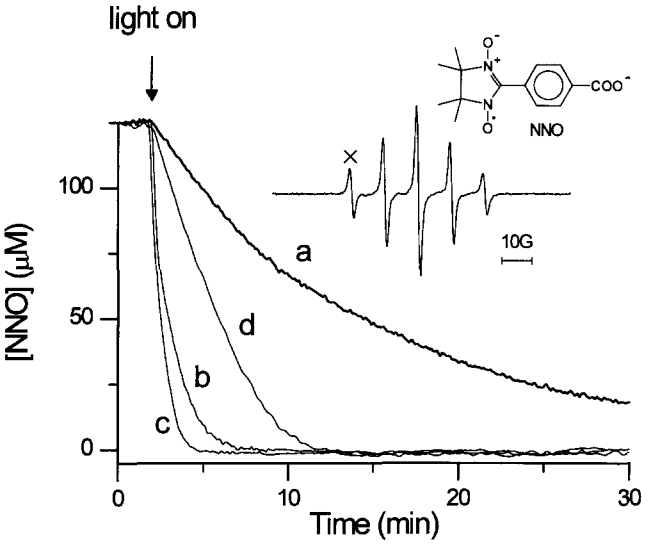

Fig. 2. ·NO generation during photodecomposition of GSNO. Samples were prepared in the dark on ice, placed inside the ESR cavity and the decay of the low field line (marked 'x' in the inset spectrum) was monitored on irradiation (↓ with light ($\lambda = 550$ nm, $I_0 = 85$ watts/meter^2) at 20° C. (a) The decay of NNO (125 μM) in the presence of GSNO (1 mM) in air saturated PBS; (b) as (a) but in the presence of 100 μM RB; (c) as (b) but under nitrogen; (d) as (a) but, in the presence of 250 μM HP. Spectrometer conditions: time constant, 1 s; scan time, 30 min; modulation amplitude, 1 G; microwave power, 1 mW.

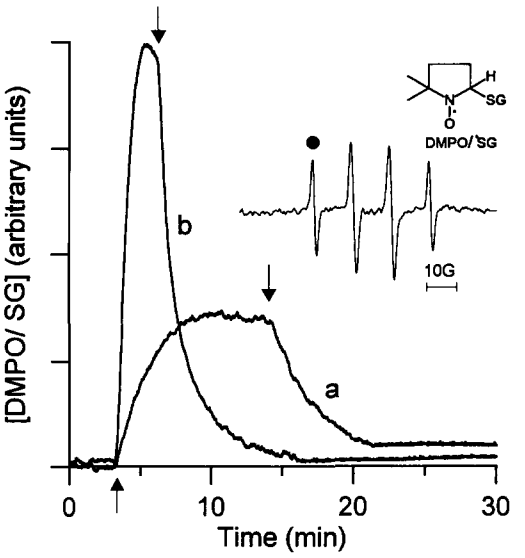

Fig. 3. DMPO/·SG adduct formation and decay during photodecomposition of GSNO. The ESR spectrum of the DMPO/·SG adduct was recorded after irradiation ($\lambda = 550$ nm) of DMPO (20 mM) and GSNO (1 mM) in PBS (inset). The formation and decay of the adduct was monitored by following the change in the low-field line of the spectrum (marked '●' in the inset spectrum). (a) GSNO (1 mM) and DMPO (20 mM) in PBS during (↑) and after (↓) irradiation; (b) as (a), but in the presence of RB (100 μM). Spectrometer conditions: time constant, 1 s; scan time, 30 min; modulation amplitude, 5 G; microwave power, 20 mW.

($a_N = 17$ G), corresponding to di-*tert*-butyl nitroxide, was obtained on photolysis ($\lambda = 630$–660 nm) of MNP (20 mM) in PBS. The yield of di-*tert*-butyl nitroxide increased approximately 10-fold on irradiation of MNP (20 mM) in the presence of AlPcS (100 μg/ml) (data not shown).

4. Discussion

S-nitrosothiols have previously been reported to generate ·NO on irradiation with light of 330 nm by reaction 1 [27–28].

$$2GSNO \xrightarrow{h\upsilon} GSSG + 2\cdot NO \quad (1)$$

S-nitrosothiols absorb in the visible region in the range of $\lambda = 550$–600 nm though the molar absorption coefficients are low. The excitation of GSNO with 550 nm radiation also leads to the formation of the same products as in Reaction **(1)**, none of which absorbs light in this range. The excitation of GSNO in the presence of RB with $\lambda = 550$ nm radiation resulted in significant increase in the yields of GS· and ·NO. This implies that triplet RB, with a quantum yield of 0.75, transfers energy to the triplet state of *S*-nitrosothiols leading to efficient homolytic decomposition (Reactions **(2)** and **(3)**)

$$RB^* + RSNO \rightarrow RSNO^* + RB \quad (2)$$

$$RSNO^* \rightarrow RS\cdot + \cdot NO \quad (3)$$

It is possible to envisage electron transfer to RB* from GSNO as occurs with reducing agents such as NADH and

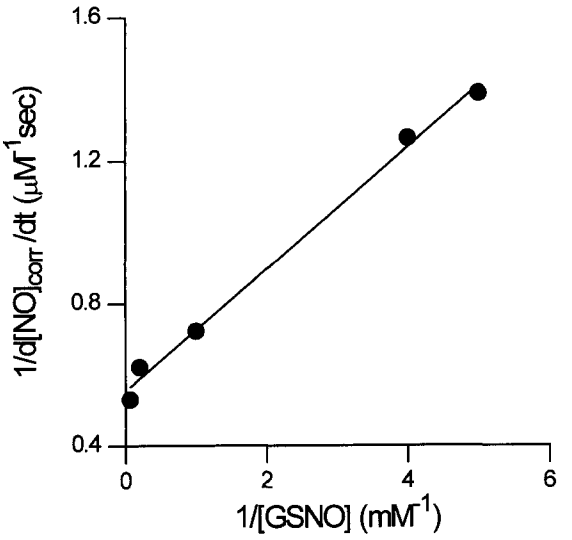

Fig. 4. Plot of $\frac{1/d[\cdot NO]_{corr}}{dt}$ vs. 1/[GSNO] for the determination of the rate constant between GSNO and RB*. The rate of · NO production was calculated from the decay of the NNO spectrum during photoirradiation of 0.2, 0.25, 1, 5, and 15 mM GSNO in the presence of RB (100 μM).

GSH [29]. The result of this is the production of $RB^{\cdot}$, which decays by either disproportionation, resulting in photobleaching of RB, or by reaction with oxygen generating superoxide. No photobleaching was observed during irradiation of RB with GSNO and the superoxide-dependent cytochrome *c* reduction was observed only during irradiation (λ = 550 nm) of RB (10 μM) and NADH (20 μM) and not during irradiation of RB (10 μM) and GSNO (100 μM). This suggests that electron-transfer from GSNO to RB* is negligible. HP also enhanced the rate of GSNO decomposition by an energy transfer mechanism leading to a 2- to 3-fold enhanced rate of · NO production as shown by NNO decay studies.

Thiyl radicals can initiate oxidation/reduction reactions in biological systems. Thiol radicals such as GS· can react with O_2 to form GSOO· or with GS^- to form the glutathione disulfide radical anion that can reduce O_2 to $O_2^{\cdot-}$ [30]. The latter reaction is favored in an intracellular milieu because of the high intracellular concentration of glutathione. Thus, photosensitized decomposition of GSNO in cells can, in principle, result in the formation of · NO and $O_2^{\cdot-}$. The simultaneous production of $O_2^{\cdot-}$ and · NO is clearly detrimental to cells due to formation of peroxynitrite, a potent oxidant [31].

The excitation of MNP with light of λ = 660 nm results in the formation of *tert*-butyl radical and · NO in very low yield. The *tert*-butyl radical is trapped by the parent compound MNP resulting in the formation of di-*tert*-butyl nitroxide radical [32]. The yield of di-*tert*-butyl nitroxide and · NO increased approximately 10 times when MNP was irradiated in the presence of AlPcS.

We propose Reactions (4–6) as the mechanism for photosensitized decomposition of MNP. Energy transfer from the triplet state of AlPcS to the triplet state of MNP leads to the enhanced homolytic decomposition to generate · NO and *tert*-butyl radical (Reaction 5). The *tert*-butyl radical is trapped by MNP to yield di-*tert*-butyl nitroxide (Reaction 6).

$$AlPcS^* + (CH_3)_3\,C{-}N = 0 \rightarrow [(CH_3)_3\,C{-}N = 0]^* + AlPcS \quad (4)$$

$$[(CH_3)_3\,C{-}N = 0]^* \rightarrow (CH_3)_3C\cdot + \cdot NO \quad (5)$$

$$(CH_3)_3C\cdot + (CH_3)_3\,C{-}N = 0 \rightarrow ((CH_3)_3C)_2\,N\,{-}O \quad (6)$$

Previously, photosensitizers have been used to increase the yield and specificity of products formation as compared with *direct photochemical* reactions [33]. The mechanism for this effect has been reported to be energy transfer from the triplet state of the photosensitizer to the triplet state of an acceptor. Photosensitized decomposition of · NO donors as shown in the present study may provide a mechanism for localized release of · NO in high yield. It is known that during PDT, rapid tumor

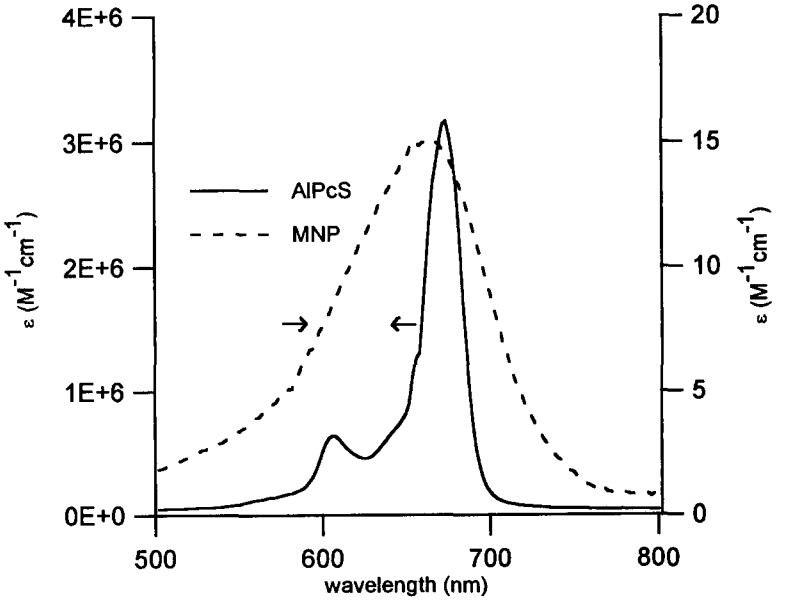

Fig. 5. Wavelength dependence of extinction coefficient for AlPcS and MNP. The y-axis on the left is for AlPcS and on the right is for MNP as indicated by arrows.

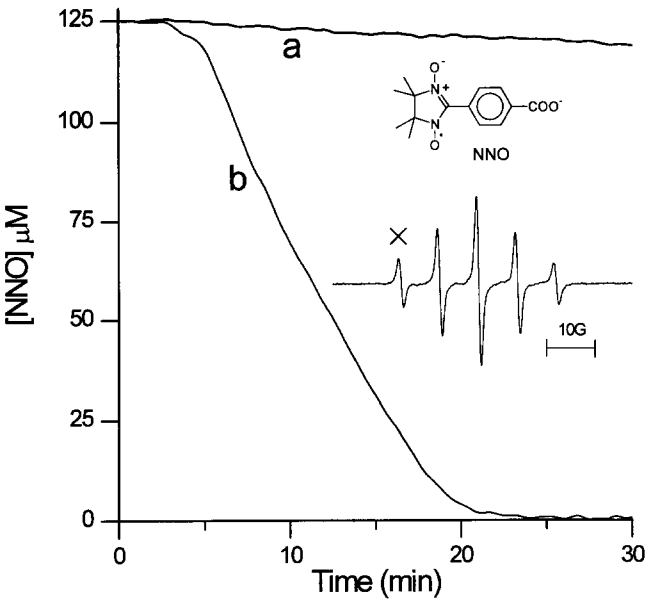

Fig. 6. ·NO generation during photodecomposition of MNP (λ = 660 nm). (a) The decay of low-field line (marked 'x' the inset) NNO (125 μM) during the photodecomposition of MNP (20 mM) was monitored in air-saturated PBS on irradiation (λ = 660 nm); (b) as (a) but in the presence of 100 μg/ml AlPcS. Spectrometer conditions were as in Fig. 2.

hypoxia is achieved secondarily to microvascular stasis and thrombosis [34–35]. Furthermore, clinical PDT is apparently limited by the presence of preexisting hypoxic areas within the tumor. Therefore, these nitroso compounds might act as light-dependent hypoxic sensitizers by augmenting tumor-cell killing through generation of toxic levels of ·NO. The present modality may also be used to exploit the antimicrobial and antiviral activity of ·NO in phototherapy [15].

In conclusion, we show that the photodecomposition of *S*-nitroso and C-nitroso compounds to generate ·NO occurs by homolytic cleavage to form thiyl and carbon-centered radicals, respectively. ·NO release can be dramatically enhanced in the presence of photodynamic sensitizers such as RB and AlPcS.

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References

[1] Moncada, S. (1992) Acta Physiol. Scand. 145, 201–227.
[2] Stuehr, D.J., Gross, S.S., Sakuma, I., Levi, R. and Nathan, C.F. (1989) J. Exp. Med. 169, 1011–1020.
[3] Nava, E., Palmer, R.M.J. and Moncada, S. (1992) J. Cardiovasc. Pharmacol. 20, S132–134.
[4] Venturini, C.M., Palmer, R.M.J. and Moncada, S. (1993) J. Pharmacol. Exp. Therapeut. 266, 1497–1500.
[5] Mathews, W.R. and Kerr, S.W. (1993) J. Pharmacol. Exp. Therapeut. 267, 1529–1532.
[6] Stamler, J.S. and Loscalzo, J. (1991) J. Am. Coll. Cardiol. 18, 1529–1536.
[7] 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.
[8] Keaney Jr., J.F., Simon, D.I., Stamler, J.S., Jaraki, O, Scharfstein, J., Vita, J.A. and Loscalzo, J. (1993) J. Clin. Invest. 91, 1582.
[9] Barrett, J., Fitzgibbones, T.J., Glauser, J., Still, R. H., Young, R.W. (1966) Nature 211, 848.
[10] Stamler, J.S., Simon, D.I., Osborne, J.A., Mullins, M.E., Jaraki, O., Michel, T., Singel, D.J. and Loscalzo, J. (1992) Proc. Natl. Acad. Sci. USA 89, 444–448.
[11] Sexton, D.J., Muruganandam, A., Mckenny, D.J. and Mutus, B. (1994) Photochem. Photobiol. 59, 463–467.
[12] Henderson, B.W. and Dougherty, T.J. (1992) Photochem. Photobiol. 55, 145–157.
[13] Seiber, F., Krueger, G.J., O'Brien, J.M., Schober, S.L., Sesenbrenner, L.L. and Sharkis, S.J. (1989) Blood 73, 345–300.
[14] Karupiah, G., Xie, O., Buller, R.M.L., Nathan, C., Duarte, C. and MacMicking, J.D. (1993) Science 261, 1445–1448.
[15] Field, L., Dilts, R.V., Ravichandran, R., Lenhert, P. G. and Carnahan, G.F. (1978) J. Chem. Soc. Chem. Commun. 249–250.
[16] Akaike, T., Yoshida, M., Miyamato, Y., Sato, K., Kohno, M., Sasamoto, K., Miyazaki, K., Ueda, S. and Maeda, H. (1993) Biochemistry, 32, 827–832.
[17] Joseph, J., Kalyanaraman, B. and Hyde, J.S. (1993) Biochem. Biophys. Res. Commun. 192, 926–934.
[18] Hogg, N., Singh, R.J., Joseph, J., Neese, F., Kalyanaraman, B. (1995) Free Rad. Res. Commun. (in press).
[19] Kotake, Y., Reinke, L.A., Tanigawa, T. and Koshida, H. (1994) Free Rad. Biol. Med. 17, 215–223.
[20] Moan, J., Hovik, B. and Wold, E. (1979) Photochem. Photobiol. 80, 623–624.
[21] Neckers, D.C. (1989) J. Photochem. Photobiol. 47, 1–29.
[22] Blum, A. and Grossweiner, L.I. (1985) Photochem. Photobiol. 41, 27–32.
[23] Josephy, P.D., Rehorek, D. and Janzen, E.G. (1984) Tetrahedron Lett. 25, 1685–1688.
[24] Lee, P.C.C. and Rodgers, M.A.J. (1987) Photochem. Photobiol. 45, 79–86.
[25] Sonoda, M., Krishna, C.M. and Riesz, P. (1987) Photochem. Photobiol. 46, 625–631.
[26] Calvert, J.G. and Pitts Jr., J.N. (1966) In: Photochemistry, John Wiley, 454.
[27] Butler, A.R. and Williams, D.L.H. (1993) Chem. Soc. Rev. 22, 233.
[28] McAninly, J., Williams, D.L.H., Askew, S.C., Butler, A.R. and Russell, C. (1993) J. Chem. Soc. Chem. Commun. 1758–1759.
[29] Sarna, T., Zajac, J., Bowman, M.K. and Truscott, T.G. (1991) J. Photochem. Photobiol. A: Chem. 60, 295–310.
[30] Winterbourn, C.C. (1993) Free Rad. Biol. Med. 14, 85–90.
[31] Radi, R., Beckman, J.S., Bush, K.M. and Freeman, B.A. (1991) Arch. Biochem. Biophys. 288, 481–487.
[32] Chamulitrat, W., Jordan, S.J., Mason, R.P., Saito, K. and Cutler, R.G. (1993) J. Biol. Chem. 268, 11520–11527.
[33] Hammond, G.S., Salteil, J., Lamola, A.A., Turro, N.J., Bradshaw, J.S., Cowan, D.O., Counsell, R.C., Vogt, V. and Dalton, C. (1964) J. Am. Chem. Soc. 86, 3197–3217.
[34] Chaudhuri, K., Keck, R.W. and Selman, S.H. (1987) Photochem. Photobiol. 46, 823–827.
[35] Gillisen, M.J., Wit, L.E.A., Star, W.M., Koster, J. F. and Sluiter, W. (1993) Cancer Res. 53, 2548–2552.