Free Radical Research, 2002 Vol. 36 (10), pp. 1071–1077

Inhibition of Lipid Peroxidation by *S*-nitrosoglutathione and Copper

MARIA PIA RIGOBELLO, GUIDO SCUTARI, RITA BOSCOLO and ALBERTO BINDOLI*

Centro di Studio delle Biomembrane (CNR) and Dipartimento di Chimica Biologica, Università di Padova, Padova, Italy

Accepted by Dr H. Sies

(Received 22 February 2002; In revised form 22 April 2002)

The antioxidant properties of *S*-nitrosoglutathione, a nitric oxide-derived product were studied in different experimental systems. By using the crocin bleaching test, *S*-nitrosoglutathione, in the presence of copper ions, shows an antioxidant capacity about six times higher than that of Trolox c and referable to the interception of peroxyl radicals by nitric oxide. Copper alone shows a modest inhibitory action, which is about seven times lower than that of Trolox c. *S*-nitrosoglutathione prevents lipid peroxidation induced by the well-known Fe^{2+}/ascorbate system ($IC_{50} = 450\,\mu M$) and the inhibitory effect is strongly reinforced by the presence of copper ions ($IC_{50} = 6.5\,\mu M$). In addition, cumene hydroperoxide-induced lipid peroxidation is markedly decreased by *S*-nitrosoglutathione, provided that copper ions, maintained reduced by ascorbate, are present. Decomposition of *S*-nitrosoglutathione through metal catalysis and/or the presence of reducing agents and the consequent release of nitric oxide are of crucial importance for eliciting the antioxidant power. In this way, copper ions and/or reducing species with low antioxidant potency are able to promote the formation of an extremely strong antioxidant species such as nitric oxide.

Keywords: Antioxidants; Copper; Crocin bleaching test; Lipid peroxidation; Nitric oxide; *S*-nitrosoglutathione

Abbreviations: ABAP, 2,2′-azo-*bis*(2-amidinipropane) dihydrochloride; CHP, cumene hydroperoxide; GSNO, *S*-nitrosoglutathione; MDA, malondialdehyde; Trolox c, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

INTRODUCTION

Nitric oxide *per se* acts as a potent inhibitor of lipid peroxidation.[1] Its action mechanism involves the scavenging of lipid peroxyl radicals. The radical–radical reaction between nitric oxide and organic peroxyl is almost diffusion limited since the determined rate constant is $1–3 \times 10^9\,M^{-1}\,sec^{-1}$.[2] This reaction generates a transient alkyl peroxynitrite (ROONO) species whose metabolic fate is still not completely clarified.[1] Also, alkoxyl radicals are reported to be scavenged by NO.[3] Nitric oxide and NO-releasing systems are able to inhibit lipid peroxidation elicited by several different peroxidizing systems such as ferrous ions and ferrous complexes,[4] ferrous complexes in the presence of hydrogen peroxide,[5] azocompounds[6] and reactive oxygen intermediates.[7] In addition, nitric oxide prevents lipid peroxidation of low-density lipoprotein (LDL) and its scavenging effect of peroxyl radicals appears critical in the prevention of apoptosis elicited in endothelial cells by oxidized LDL.[8]

In the present paper, the effect of GSNO on lipid peroxidation was studied. Previous research has shown that GSNO decreases lipid peroxidation induced by ferrous-citrate[4] or oxygen free radicals produced by the xanthine oxidase metabolism.[7] In our system the release of NO by GSNO was stimulated by the addition of copper ions.[9–11] The latter has contrasting effect on lipid peroxidation since, according to the conditions, can act as an inhibitor[12–14] or a stimulator in a Fenton-type reaction.[15] In this paper we report that, in the proper conditions, the system GSNO/Cu(II) is a potent inhibitor of lipid peroxidation elicited *in vitro* by different peroxidizing systems.

*Corresponding author. Tel.: +39-49-827-6138. Fax: +39-49-807-3310. E-mail: labbind@civ.bio.unipd.it

ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1071576021000006680

MATERIALS AND METHODS

ABAP was purchased from Polyscience (Warrington, PA), cumene hydroperoxide and Trolox c from Fluka (Buchs, Switzerland), reduced glutathione, 2-thiobarbituric acid, and saffron, a crude source of crocin, from Sigma (St. Louis, MO). All other reagents were of analytical grade. *S*-nitrosoglutathione was prepared following the procedure of Hart.[16] The obtained product exhibits visible and ultraviolet spectra consistent with the reported data.[17] The concentration of GSNO solutions was determined using $\varepsilon_M = 15\,M^{-1}\,cm^{-1}$ at 544 nm.[17] Crocin was prepared from commercial saffron following the procedure of Friend and Meyer[18] and its concentration was estimated using an $\varepsilon_M = 1.33 \times 10^5\,M^{-1}\,sec^{-1}$ at 440 nm.[19] The crocin bleaching test was carried out essentially by following the procedure described by Bors *et al.*[19] modified according to Tubaro *et al.*[20] using the diazocompound ABAP in the presence of oxygen to produce peroxyl radicals. The test was carried out at 40°C in 5 mM phosphate buffer (pH 7.0) containing 5% ethanol and 12 μM crocin, while the antioxidant was added at increasing concentrations starting from 2.5 μM. ABAP was added from a fresh 0.5 M solution to obtain a final concentration of 5 mM. The rate of crocin bleaching was followed spectrophotometrically as decrease of absorbance at 440 nm. Bleaching rates were plotted according to the equation $v_0/v = 1 + k_a[A]/k_c[C]$ where v_0 and v are the bleaching rate of crocin in the absence and in the presence of antioxidant, respectively. [A] is the concentration of antioxidant and [C] is the concentration of crocin. k_a and k_c are the rate constants for the reaction of the peroxyl radical with the antioxidant and crocin, respectively. The plot results in a straight line intersecting the ordinate at unit and the slope of this line is the ratio of the rate constants k_a/k_c indicating the relative capacity of different antioxidant molecules to interact with the peroxyl radical. Liver microsomes were prepared according to the conventional procedures and microsomal lipid peroxidation was measured as malondialdehyde formation using the 2-thiobarbituric acid assay.[21] Oxygen uptake was measured polarographically with an oxygraph utilizing a Clark-type oxygen electrode connected to a computerized system. Proteins were measured with the biuret test. The data obtained from the various experiments and generated via the oxygraph or the spectrophotometer software were stored and utilized for averaging the various curves.

RESULTS

The peroxyl radical scavenging capacity of nitric oxide released from *S*-nitrosoglutathione was evaluated by following the competition kinetics of crocin bleaching by peroxyl radicals produced by thermal decomposition of the diazocompound ABAP. As reported in Fig. 1A (curve *a*) in the absence of GSNO and in the presence of $CuSO_4$, the alkylhydroperoxyl radicals, originating from ABAP decomposition, give rise to a linear bleaching of crocin. When GSNO is added to the system, the bleaching is markedly slowed down, but after a few minutes, depending on the concentration of added GSNO, it resumes the velocity shown by the control. The crocin bleaching velocities of the slow phase were utilized for the estimation of the relative antioxidant potency of the system GSNO/$CuSO_4$ in comparison to that of Trolox c, a hydrosoluble form of vitamin E that is considered one of the most active agents in reacting with crocin[19] and is usually taken as reference antioxidant. As apparent in Fig. 1B, the GSNO/$CuSO_4$ combination is about six times more effective than Trolox c. In the same conditions, GSNO alone is scarcely effective ($k_a/k_c = 0.07$) while a moderate effect is elicited by Cu(II) ($k_a/k_c = 0.11$). However, considering the constant ratio, the effect of copper alone is about 40-fold lower than the combination GSNO/Cu(II) and 7-fold lower than that of Trolox c.

The time required for the decomposition of GSNO is directly related to the duration of the "slow" phase of crocin bleaching. In fact, the decomposition of different concentrations of GSNO, measured at 336 nm in the presence of Cu(II) and in conditions similar to those of the crocin bleaching experiment, show a time course of the same magnitude of that exhibited by the slow phase of crocin bleaching (not shown). This indicates that the bleaching is prevented as long as a flux of NO coming from GSNO occurs.

The antioxidant properties of the system GSNO/$CuSO_4$ were directly tested as inhibition of microsomal lipid peroxidation induced by the classical combination ferrous ion/ascorbate. In this system, lipid peroxidation is both initiated and propagated by ferrous iron, which is maintained in the reduced state by the presence of ascorbate. As apparent in Fig. 2A, in the absence of copper ions, GSNO inhibits lipid peroxidation only at relatively high concentrations ($IC_{50} = 450\,\mu M$). In this case, the decomposition of GSNO and the consequent formation of NO should be elicited mainly by the presence of ascorbate that is a well-known agent able to give rise to the formation of nitric oxide from *S*-nitrosothiols.[22] Addition of copper ions to the iron/ascorbate system decreases MDA formation also in the absence of GSNO of about 14% (compare A and B of Fig. 2), in accordance with several previous reports[12–14] and also with the moderate effect of Cu(II) observed on the crocin bleaching test (Fig. 1B). However, in the presence of increasing

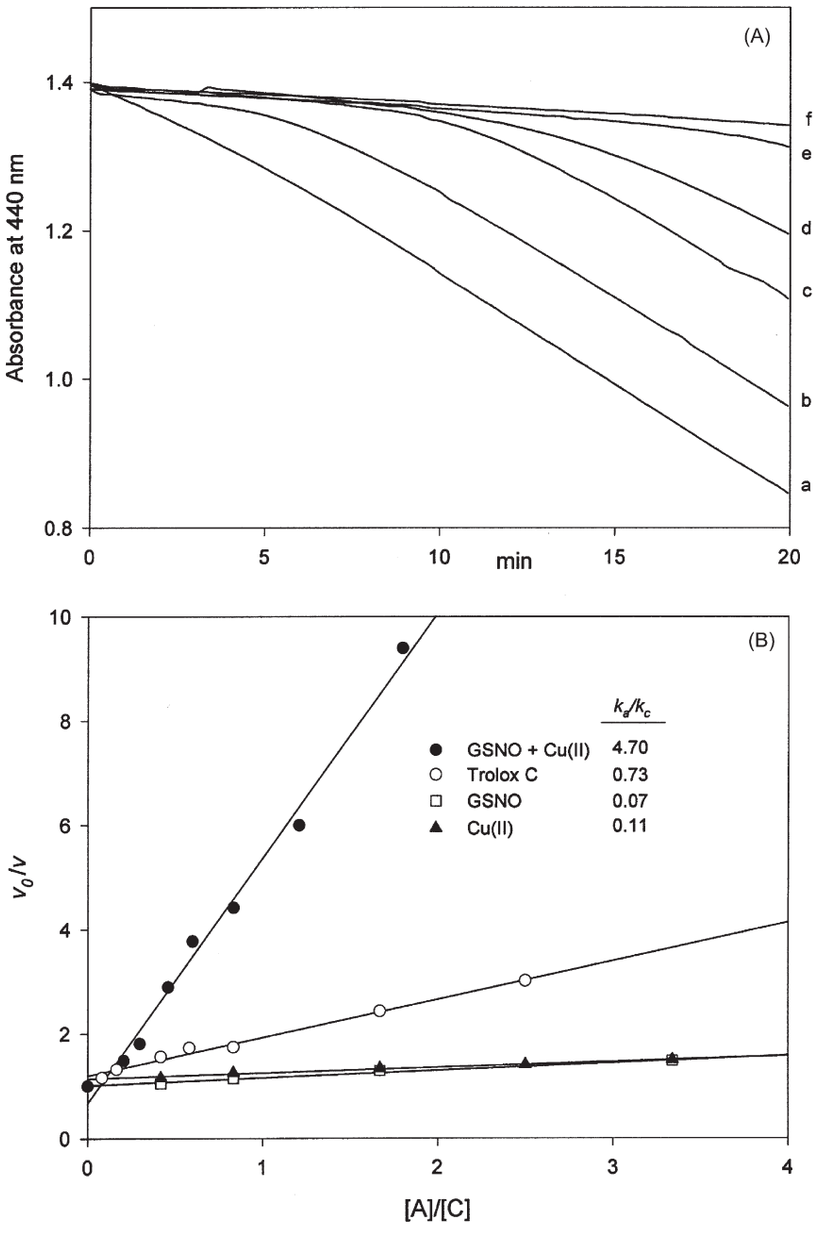

FIGURE 1 Antioxidant activity of GSNO measured with the crocin bleaching method. (A) Spectrophotometric estimation of the crocin bleaching induced by ABAP subjected to thermal decomposition. Reaction was observed at 440 nm in 5 mM phosphate buffer (pH 7.4) containing 5% ethanol, 12 μM crocin and 20 μM $CuSO_4$. After equilibration at 40°C, GSNO was added at increasing concentrations (μM): none (a), 2.5 (b), 7.5 (c), 10 (d), 15 (e), 20 (f) and the reaction was immediately initiated by 5 mM ABAP. (B) Competition plot of crocin and antioxidants for hydroperoxyl radicals derived from ABAP. Experimental conditions as in (A). See also "Materials and Methods" for further details. Data are the mean of four different experiments.

concentrations of GSNO, the addition of copper makes GSNO extremely efficient as antioxidant, at far lower concentrations (IC_{50} = 6.5 μM) if compared to iron/ascorbate alone (Fig. 2).

Cumene hydroperoxide induces lipid peroxidation in microsomes even in the absence of a free metal catalysis. Lipid peroxidation is mediated by cytochrome P-450 and may proceed through either a hemolytic or heterolytic cleavage of the O–O bond of the hydroperoxide. In the first case, the cumyloxyl radical may be the initiator of lipid peroxidation, while, in the second case the oxoferryl form of cytochrome P-450[23] could propagate the lipoperoxidative process possibly reacting with other molecules of cumene hydroperoxide. As apparent in Fig. 3 (curve *b*) the addition of CHP to rat liver microsomes gives rise to oxygen uptake strictly linked to lipid peroxidation as revealed by the MDA formation determined at the end of the experiment. In the absence of a reducing system and/or copper, i.e. in

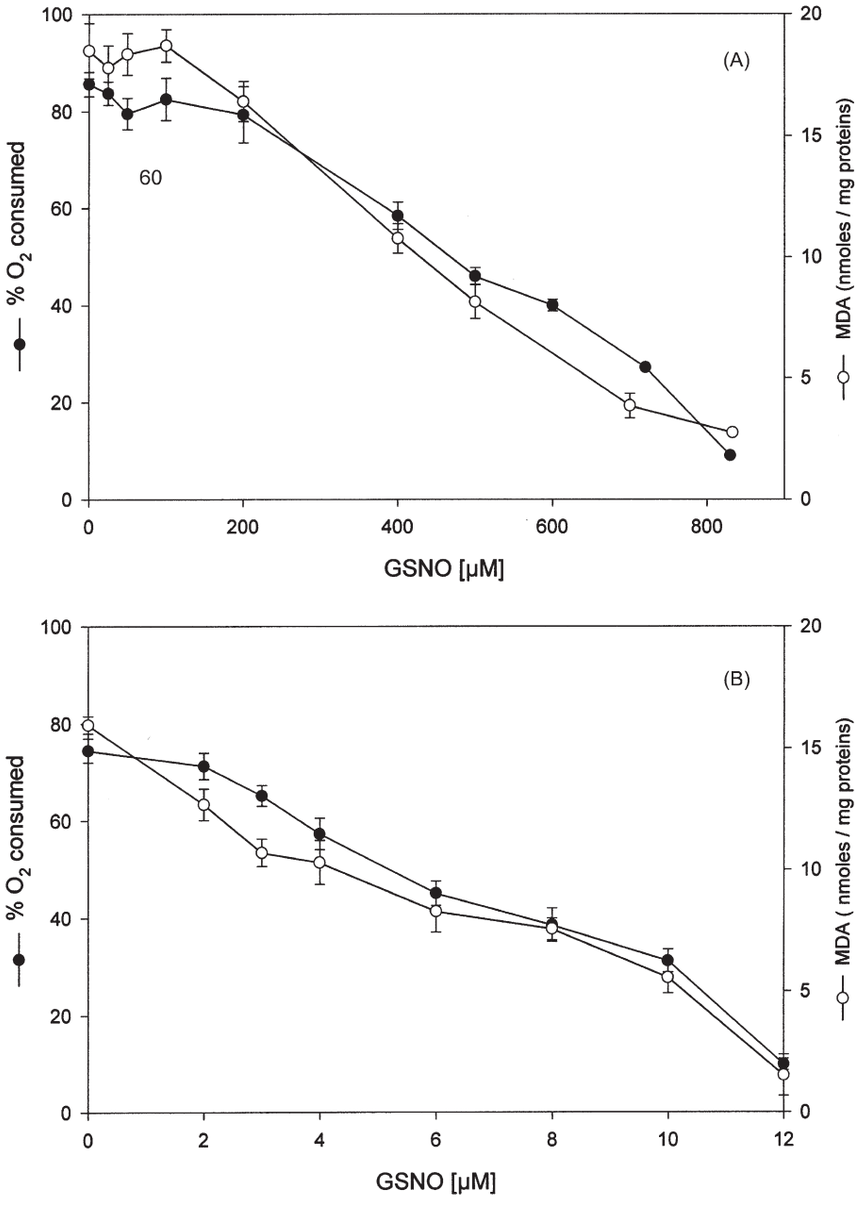

FIGURE 2 Inhibition by increasing concentrations of GSNO of microsomal lipid peroxidation induced by iron/ascorbate. Effect of copper. Oxygen uptake (–●–) and MDA formation (–○–) were estimated after 10 min of incubation at 25°C in 125 mM KCl, 20 mM Hepes/Tris (pH 7.4) in the presence of 1 mg/ml of rat liver microsomes, 0.2 mM ascorbate and 20 μM $FeSO_4$. In B, 20 μM $CuSO_4$ was also present. GSNO was added at increasing concentrations as indicated. 100% oxygen, indicated in the ordinate axis, corresponds to 278 nanomoles/ml of oxygen dissolved in the vessel. MDA formation was measured as indicated under "Materials and Methods" section. Data are expressed as mean ± SD ($n = 4$).

conditions where the decomposition of GSNO is limited, almost no inhibition of lipid peroxidation is apparent (curve *c*) indicating that GSNO *per se* is ineffective as antioxidant. Furthermore, when lipid peroxidation is elicited by CHP in the presence of a chelator such as EDTA no effect at all of GSNO on lipid peroxidation was observed both in the presence or absence of Cu(II) (not shown). When Cu(II)/ascorbate are added to the CHP-peroxidizing systems the rate of consumption of oxygen is strongly enhanced (curve *a*). However, in this case, oxygen uptake is not a reliable indication of CHP-stimulated lipid peroxidation since a large part of oxygen is utilized in copper-stimulated ascorbate oxidation and, in addition, the presence of CHP further stimulates oxygen depletion. Therefore, MDA estimation is a more direct index of GSNO effects. In fact, comparing curves *a* and *b* of Fig. 3, it is apparent that, in the absence of Cu(II)/ascorbate, the rate of oxygen uptake is strongly diminished, but MDA measured at the end of the experiment is about twice as that measured in the presence of

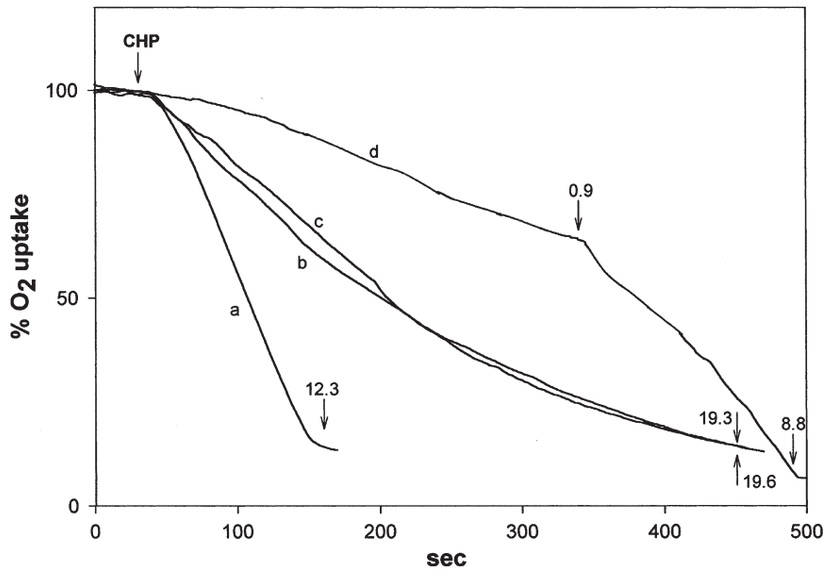

FIGURE 3 Inhibition by GSNO of microsomal lipid peroxidation induced by cumene hydroperoxide. Effect of copper and ascorbate. Oxygen uptake was estimated at 25°C in 0.125 M KCl, 20 mM Hepes/Tris (pH 7.4) in the presence of 1 mg/ml of rat liver microsomes; reaction was initiated by the addition of 0.5 mM CHP. When indicated, 20 μM $CuSO_4$, 0.5 mM ascorbate and 0.15 mM GSNO were also present in the incubation medium before CHP addition. a: $CuSO_4$ + ascorbate; b: control; c: GSNO; d: GSNO + $CuSO_4$ + ascorbate. The values of MDA formed (nanomoles/mg protein) are indicated by each trace at the arrow. 100% oxygen, indicated in the ordinate axis, corresponds to 278 nanomoles/ml of oxygen dissolved in the vessel. MDA formation was measured as indicated under "Materials and Methods" section.

Cu(II)/ascorbate in front of the same extent of oxygen consumption. When GSNO is added in the presence of Cu(II)/ascorbate, a strong inhibition of lipid peroxidation is observable (Fig. 3, curve *d*). Oxygen consumption is markedly reduced in a biphasic curve characterized by a slow phase of about 5 min followed by a fast one. During the slow phase, the formation of MDA is almost totally inhibited, while, after the fast phase, a moderate concentration of MDA is apparent reinforcing the concept that the splitting of GSNO is of crucial importance for eliciting protection from lipid peroxidation.

DISCUSSION

Nitric oxide is enzymatically produced in almost all cell types and exerts a fundamental physiological role as a messenger after binding to guanylyl cyclase. However, it might also secondarily act as a cytotoxic agent[24] if formed in relatively high concentration as it can interact with oxygen and superoxide anion producing several reactive derivatives such as nitrogen dioxide (NO_2), nitrous anhydride (N_2O_3) and peroxynitrite anion ($ONOO^-$). These derivatives exhibit a high reactivity with GSH that is present to a large extent in biological systems, and, therefore, the formation of GSNO can easily take place. *S*-nitrosothiols were reported to occur naturally in tissues, plasma and other body fluids as *S*-nitrosoalbumin[25] and GSNO.[26,27] Therefore, glutathione can be considered an efficient scavenger of the nitrogen oxides, playing a critical role in prevention of the nitrosative stress.[24] *S*-nitrosoglutathione exhibits different roles and properties[1,28] since it acts essentially as a carrier of NO. A major function of *S*-nitrosothiols is in fact linked to their ability to delivering NO and forming the corresponding disulfide by photochemical and thermal pathways.[29] The rate and extent of NO formation from *S*-nitrosothiols depends on the presence of transition metal ions and, in particular, of copper ions[9–11] or copper-containing enzymes.[30] Copper acts in a catalytic way and its cuprous form is the active one that cleaves the S–N bond.[9–11]

In the crocin bleaching test the system Cu(II)/GSNO proves to be extremely potent in preventing the crocin bleaching and appears to be far more effective than Trolox c that is usually taken as reference antioxidant in this test. Since the antioxidant effect should be mostly referred to the NO released from GSNO, this is consistent with previously reported data, indicating that NO reacts with peroxyl radicals at near diffusion limit.[2] The presence of copper is essential for the inhibition of the bleaching since GSNO alone is scarcely effective, while copper(II) alone shows only a moderate effect. In the crocin bleaching experiments no reducing agent such as ascorbate was added, since GSH generated from GSNO serves as reductant for copper (II).[9]

Copper catalyzes the peroxidation of low-density lipoproteins acting, like iron, as a promoter of hydroperoxide decomposition in a Fenton-like reaction.[15] However, depending on the conditions, it might act as an inhibitor of lipid peroxidation.[12–14] In fact, according to Wills,[12] in microsomal suspensions Cu(II) strongly inhibits both ascorbate- and NADPH-induced lipid peroxidation measured as malondialdehyde formation. The mechanism of inhibition of lipid peroxidation by copper ions might be due, at least in part, to a direct interception of peroxyl radicals. In fact, similarly to other metal ions,[31] copper shows a moderate effect in preventing crocin bleaching, giving a k_a/k_c of 0.11. This figure, although lower than that of Mn(II) (0.5) and Co(II) (0.3) that are good inhibitors of lipid peroxidation,[31] indicates a moderate scavenging action of copper(II) towards peroxyl radicals. However, the free radical scavenging effect of copper ions towards peroxyl radicals cannot be the only explanation for the protective effect of the metal, since both Cu(I) and Cu(II) are able to intercept both oxidizing and reducing radicals, respectively.[32] As an alternative to its potential free radical scavenging capability, the effect of Cu(II) can be referred to a displacing action on transition metals, such as iron, from their binding sites. However, when microsomal lipid peroxidation was stimulated by GSH in the presence of iron, contrasting effects were observed in the presence of copper,[13] since it inhibits lipid peroxidation at low concentrations of glutathione, while strongly stimulates it at higher concentrations. This potentiation of iron-induced lipid peroxidation by copper appears to depend on the reduction of ferric ions by the cuprous form.[13] In our system, Cu(II) inhibits microsomal lipid peroxidation induced by iron/ascorbate (Fig. 2) and by cumene hydroperoxide (not shown). In addition, by stimulating the release of NO from GSNO, it unmasks the latent antioxidant potential of the latter molecule.

Copper ions appear to play an essential role in the decomposition of *S*-nitrosothiols[9–11] where the reduction of Cu(II) to its cuprous form appears necessary to speed up the reaction.[11] In addition, the various cellular reducing systems are important in the NO release from GSNO. For instance, the thioredoxin system is active in forming NO from GSNO.[33] Also ascorbate, glutathione and other thiols stimulate the release of NO from GSNO.[22]

Copper is an essential trace element and exists almost entirely complexed with small molecules or proteins. Consequently, in living cells, a direct interaction between free copper ions and GSNO appears improbable. Also, not only copper, but also copper-containing enzymes such as Cu-Zn-superoxide-dismutase[30] and a copper-dependent GSNO lyase[34] found in fibroblasts and platelets actively decompose GSNO. However, a NADH-dependent GSNO reductase evolutionary conserved from bacteria to humans and able to degrade GSNO to GSSG and ammonia has been recently described.[35]

In conclusion, the antiperoxidative action of GSNO appears to depend on the rate of its decomposition and, consequently, on the rate of NO delivery. This occurs either as a metal catalysis, particularly copper, or taking advantage of the several reducing systems present in the cell. The ability of reducing agents to displace NO from GSNO or from other *S*-nitrosothiols, results in the conversion of a substance with scarce antioxidant power to a very potent inhibitor of lipid peroxidation such as nitric oxide. However, the power of NO as an antioxidant seems to be restricted to its action towards lipid peroxidation since, for instance, in the presence of oxygen, it appears to act as a rather effective stimulator of catecholamine oxidation.[36] In addition, the NO-derived product, peroxynitrite, is reported to stimulate lipid peroxidation.[7] The specific and different action of NO and its derivatives on well-defined molecules can rationalize the conflicting reports regarding their action considered to be either cytotoxic or cytoprotective.[28] In the vascular, nervous and immune systems, the control of the rate and extent of NO production is exerted, according to the conditions, by the action of NO synthase. In turn, reducing systems and metal catalysis might exert a control on NO release from GSNO.

References

[1] Hogg, N. and Kalyanaraman, B. (1999) "Nitric oxide and lipid peroxidation", *Biochim. Biophys. Acta* **1411**, 378–384.
[2] Padmaja, S. and Huie, R.E. (1993) "The reaction of nitric oxide with organic peroxyl radicals", *Biochem. Biophys. Res. Commun.* **195**, 539–544.
[3] O'Donnell, V.B., Chumley, P.H., Hogg, N., Bloodsworth, A., Darley-Usmar, V.M. and Freeman, B.A. (1997) "Nitric oxide inhibition of lipid peroxidation: kinetics of reaction with lipid peroxyl radicals and comparison with α-tocopherol", *Biochemistry* **36**, 15216–15223.
[4] Rauhala, P., Lin, A.M.Y. and Chiueh, C.C. (1998) "Neuroprotection by *S*-nitrosoglutathione of brain dopamine neurons from oxidative stress", *FASEB J.* **12**, 165–173.
[5] Kanner, J., Harel, S. and Granit, R. (1991) "Nitric oxide as an antioxidant", *Arch. Biochem. Biophys.* **289**, 130–136.
[6] Hayashi, K., Noguchi, N. and Niki, E. (1995) "Action of nitric oxide as an antioxidant against oxidation of soybean phosphatidylcholine liposomal membranes", *FEBS Lett.* **370**, 37–40.
[7] Rubbo, H., Radi, R., Trujillo, M., Telleri, R., Kalyanaraman, B., Barnes, S., Kirk, M. and Freeman, B.A. (1994) "Nitric oxide regulation and peroxynitrite-dependent lipid peroxidation", *J. Biol. Chem.* **269**, 26066–26075.
[8] Kotamraju, S., Hogg, N., Joseph, J., Keefer, L.K. and Kalyanaraman, B. (2001) "Inhibition of oxidized low-density lipoprotein-induced apoptosis in endothelial cells by nitric oxide", *J. Biol. Chem.* **276**, 17316–17323.
[9] Williams, D.L.H. (1996) "*S*-nitrosothiols and role of metal ions in decomposition to nitric oxide", *Meth. Enzymol.* **268**, 299–308.

[10] Singh, R.J., Hogg, N., Joseph, J. and Kalyanaraman, B. (1996) "Mechanism of nitric oxide release from *S*-nitrosothiols", *J. Biol. Chem.* **271**, 18596–18603.
[11] Gorren, A.C.F., Schrammel, A., Schmidt, K. and Mayer, B. (1996) "Decomposition of *S*-nitrosoglutathione in the presence of copper ions and glutathione", *Arch. Biochem. Biophys.* **330**, 219–228.
[12] Wills, E.D. (1969) "Lipid peroxide formation in microsomes", *Biochem. J.* **113**, 315–332.
[13] Beckman, J.K., Borowitz, S.M., Greene, H.L. and Burr, I.M. (1988) "Promotion of iron-induced rat liver microsomal lipid peroxidation by copper", *Lipids* **23**, 559–563.
[14] Ciuffi, M., Cellai, C., Franchi-Micheli, S., Failli, P., Zilletti, L., Ginanneschi, M., Chelli, M., Papini, A.M. and Paoletti, F. (1998) "An *in vivo*, *ex vivo* and *in vitro* comparative study of activity of copper oligopeptide complexes vs Cu(II) ions", *Pharmacol. Res.* **38**, 279–287.
[15] Esterbauer, H., Gebicki, J., Puhl, H. and Jürgens, G. (1992) "The role of lipid peroxidation and antioxidants in oxidative modification of LDL", *Free Radic. Biol. Med.* **13**, 341–390.
[16] Hart, T.W. (1985) "Some observations concerning the *S*-nitroso and *S*-phenylsulphonyl derivatives of L-cysteine and glutathione", *Tetrahedron Lett.* **26**, 2013–2016.
[17] Park, J.W. (1988) "Reaction of *S*-nitrosoglutathione with sulfhydryl groups in protein", *Biochem. Biophys. Res. Commun.* **152**, 916–920.
[18] Friend, J. and Mayer, A.M. (1960) "The enzymic destruction of carotenoids by isolated chloroplasts", *Biochim. Biophys. Acta* **41**, 422–429.
[19] Bors, W., Michel, C. and Saran, M. (1984) "Inhibition of the bleaching of the carotenoids crocin. A rapid test for quantifying antioxidant activity", *Biochim. Biophys. Acta* **796**, 312–319.
[20] Tubaro, F., Micossi, E. and Ursini, F. (1996) "The antioxidant capacity of complex mixtures by kinetic analysis of crocin bleaching inhibition", *J. Am. Oil Chemists Soc.* **73**, 173–179.
[21] Buege, J.D. and Aust, S.D. (1978) "Microsomal lipid peroxidation", *Meth. Enzymol.* **52**, 302–310.
[22] Holmes, A.J. and Williams, D.L.H. (2000) "Reaction of ascorbic acid with *S*-nitrosothiols: clear evidence for two distinct reaction pathways", *J. Chem. Soc.-Perkin Transactions* **2**, 1639–1644.
[23] Barr, D.P., Martin, M.V., Guengerich, F.P. and Mason, R.P. (1996) "Reaction of cytochrome P450: ESR spin trapping evidence for the homolytic scission of the peroxide O–O bond by ferric cytochrome P450 lA2", *Chem. Res. Toxicol.* **9**, 318–325.
[24] Wink, D.A., Hanbauer, I., Grisham, M.B., Laval, F., Nims, R.W., Laval, J., Cook, J., Pacelli, R., Liebmann, J., Krishna, M., Ford, P.C. and Mitchell, J.B. (1996) "Chemical biology of nitric oxide: regulation and protective and toxic mechanisms", *Curr. Top. Cell. Regul.* **34**, 159–187.
[25] Stamler, J.S., Jaraki, O., Osborne, J., Simon, D.I., Keaney, J., Vita, J., Singel, D., Valeri, C.R. and Loscalzo, J. (1992) "Nitric oxide circulates in mammalian plasma primarily as an *S*-nitroso adduct of serum albumin", *Proc. Natl Acad. Sci. USA* **89**, 7674–7677.
[26] Do, K.Q., Benz, B., Grima, G., Gutteck-Amsler, U., Kluge, I. and Salt, T.E. (1996) "Nitric oxide precursor arginine and *S*-nitrosoglutathione in synaptic and glial function", *Neurochem. Int.* **29**, 213–224.
[27] 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) "Endogenous nitrogen oxides and bronchodilator *S*-nitrosothiols in human airways", *Proc. Natl Acad. Sci. USA* **90**, 10957–10961.
[28] Krönke, K.D., Fehsel, K. and Kolb-Bachofen, V. (1997) "Nitric oxide: cytotoxicity versus cytoprotection—How, why, when and where?", *Nitric oxide: Biol. Chem.* **1**, 107–120.
[29] Butler, A.R. and Rhodes, P. (1997) "Chemistry, analysis and biological roles of *S*-nitrosothiols", *Anal. Biochem.* **249**, 1–9.
[30] Singh, R.J., Hogg, N., Goss, S.P.A., Antholine, W.E. and Kalyanaraman, B. (1999) "Mechanism of superoxide dismutase/H_2O_2-mediated nitric oxide release from *S*-nitrosoglutathione. Role of glutamate", *Arch. Biochem. Biophys.* **372**, 8–15.
[31] Coassin, M., Ursini, F. and Bindoli, A. (1992) "Antioxidant effect of manganese", *Arch. Biochem. Biophys.* **299**, 330–333.
[32] Gilbert, B.C., Silvester, S. and Walton, P.H. (1999) "Spectroscopic, kinetic and mechanistic studies of the influence of ligand and substrate concentration on the activation by peroxides of Cu^{I}-thiolate and other Cu^{I} complexes", *J. Chem. Soc.-Perkin Trans.* **2**, 1115–1121.
[33] Nikitovic, D. and Holmgren, A. (1996) "*S*-nitrosoglutathione is cleaved by the thioredoxin system with liberation of glutathione and redox regulating nitric oxide", *J. Biol. Chem.* **271**, 19180–19185.
[34] Gordge, M.P., Addis, P., Noronha-Dutra, A.A. and Hothersall, J.S. (1998) "Cell-mediated biotransformation of *S*-nitrosoglutathione", *Biochem. Pharmacol.* **55**, 657–665.
[35] Liu, L., Hausladen, A., Zeng, M., Que, L., Heitman, J. and Stamler, J.S. (2001) "A metabolic enzyme for *S*-nitrosothiol conserved from bacteria to humans", *Nature* **410**, 490–494.
[36] Rigobello, M.P., Scutari, G., Boscolo, R. and Bindoli, A. (2001) "Oxidation of adrenaline and its derivatives by *S*-nitrosoglutathione", *Nitric Oxide: Biol. Chem.* **5**, 39–46.