Arch. Pharm. Res. 12(4), 257-258 (1989)

An Unusually Stable S-Nitrosothiol from Glutathione

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(Received August 22, 1989)

Abstract□Glutathione was converted by HNO_2 into a S-nitrosothiol which was stable in solution and atypically so even as a solid. FAB/MS and IR data have been obtained for the confirmation of structure of S-nitrosoglutathione in the crystalline state.

Keywords□Bacteriostatic agent, hypotensive drug, stability, homolytic decomposition.

S-Nitrosothiols with a general structure of RSN = O formed from thiols in the presence of N_2O_4[1], nitrite[2], and NO gas[3] under appropriate conditions and are structural analogs of nitrous acid (HON = O) in which the hydroxyl group is replaced by an RS group. S-Nitrosothiols are known bacteriostatic agents which interfere with the development of bacterial spores into vegitative forms[4,5]. They also have been implicated to involve relaxation of vascular smooth muscle[6]. Recently, S-nitrosoglutathione (GSNO) has been known to be a hypotensive drug as effective as sodium nitroprusside[7], the most effective nitrovasodilator. These widespread biomedical applications of S-nitrosothiols enhance the interest in the synthesis of stable S-nitrosothiols.

Solutions of S-nitrosothiols have been considered unstable and solid forms of S-nitrosothiols have been reported much more unstable than solution forms. By Far, only S-nitroso-N-acetyl-D,L-penicillamine crystal has been isolated as a stable S-nitrosothiol crystal[8]. Among the several S-nitrosothiols synthesized, GSNO revealed unusual stability in the crystalline state. The yield of GSNO solid, pink powder, in the aqueous solution was determined by a high-performance liquid chromatography (HPLC) with monitoring at 215 nm or by visible absorption spectroscopy at λ_{max} = 544 nm (ε = 15.0 $M^{-1}cm^{-1}$) was greater than 90%. The only other product was identified as an oxidized glutathione. The decomposition of S-nitrosothiols occur spontaneously, and radical mechanism has long been suspected. Decomposition gives nitric oxide and the corresponding disulfide, which is considered to be formed by the intemolecular reaction of the S-nitrosothiol with a thiyl radical by homolytic fission of the sulfur-nitrogen bond[9].

From the IR Spectrum of GSNO crystal, the peak characteristic of the sulfhydryl moiety in the glutathione (~2540 cm^{-1}) was absent from the GSNO and the nitroso moiety was identified as a sharp peak at 1450 cm^{-1}. FAB/MS has been used in an attempt to analyze nanomole amount of GSNO solid with high molecular weight without the need of prior chemical derivatization which may destroy sensitive functional groups. The FAB/MS spectrum of GSNO solid dissolved in glycerol exhibits abundant molecular ion $[M + H]^+$ at m/z 337, sodium cationized molecular ion $[M + Na]^+$ at m/z 359, and $[M + H - NO]^+$ at m/z 307 as a characteristic fragment. The spectrum contains numerous intense peaks related to oligomers of glycerol and its related sodium cationized ions, which is a normal background for FAB/MS. It is the first mass spectrum data obtained from S-nitrosothiol solid.

Although some S-nitrosothiols in the crystalline state except S-nitroso-N-acetyl-D,L-penicillamine have been reported highly unstable in air and at room temperature, GSNO solid was very stable in air at 4 °C as shown in Fig. 1. Even at room temperature, 75% of original absorbance at 544 nm retained after 32 days (Fig. 1). Exclusion of light and air had virtually no noticeable effect on the stability of GSNO solid.

The problem in the stability of S-nitrosothiols in

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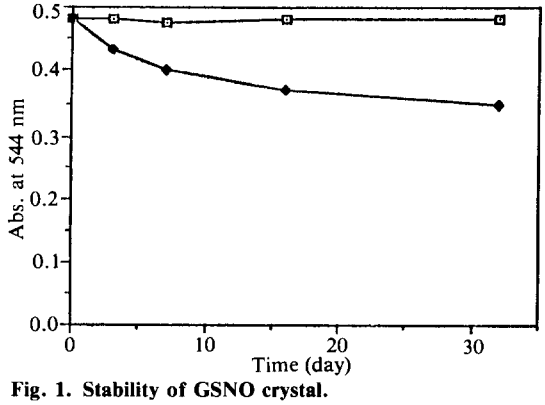

Fig. 1. Stability of GSNO crystal.
Monitored at 544 in water with [GSNO] = 30 mM at 4 °C(-⊡-) and 23 °C(-■-)

the crystalline state may be resulted in the process of its preparation. Because self-decomposition of S-nitrosothiols follows a second-order kinetics[10], increased concentration resulted in the evaporation of solvent causes rapid decomposition. Decomposition of the S-nitrosothiols is makedly faster at higher concentrations in solution. GSNO in the aqueous solution decomposes with a second-order rate constant, k_2, of $3 \times 10^{-4} M^{-1}sec^{-1}$ at 37 °C and pH 7.4. In contrast, other S-nitrosothiols such as S-nitrosocysteine and S-nitrosocysteamine in the same conditions, exhibit k_2 of 0.11 and 0.3 $M^{-1}sec^{-1}$, respectively.

EXPERIMENTAL METHODS

The S-nitroso derivative of glutathione crystal was prepared by adding equimolar amount of glutathione and sodium nitrite to the minimum amount of water enough to dissolve both reagents and then, added HCl to attain pH 1.5. The resulting solution was rapidly freezed by dry ice-acetone and washed with cold absolute ethanol several times. The residue was dried under vacuum. HPLC chromatogram was obtained from the freshly prepared GSNO in solution which was injected to reversed-phase Lichroscorb RP-18 column (0.46 × 25 cm, 5 μm) with a linear gradient from 0.1% phosphoric acid to 0.1% phosphoric acid containing 50% acetonitrile over 20 minutes. The flow rate was 1 ml/min. The IR spectra were recorded on a Beckman Model 4220 IR spectrophotometer with NaCl cell and Nujol as a mulling agent. FAB/MS spectra were obtained on a Kratos MS-30 mass spectrometer fitted with an Ion Tech B-11 NF Saddle field atom gun. The FAB source was operated at room temperature with a xenon gas flow of about 0.5 cm^3min^{-1} at 10 psi, giving a beam at 8 keV and 1 mA of current. Crystal form of GSNO was mixed with glycerol and then coated on the probe.

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