Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2897–2902

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

^{15}N NMR AND ELECTRONIC PROPERTIES OF *S*-NITROSOTHIOLS

Kun Wang, Yongchun Hou, Wei Zhang, Mohamad B. Ksebati, Ming Xian, Jin-Pei Cheng,[a]* and Peng George Wang*

Department of Chemistry, Wayne State University, Detroit, MI 48202, U. S. A.
[a]Department of Chemistry, Nankai University, Tianjin, 300071, P. R. China

Received 12 August 1999; accepted 2 September 1999

Abstract: Investigation of the ^{15}N NMR of *S*-nitrosothiols showed that primary and tertiary RSNOs have distinct ^{15}N chemical shifts around 730 and 790 ppm, respectively. Using ^{15}N NMR technique, the equilibrium constant of NO transfer between SNAP and GSH was found to be 0.74. For primary RSNOs, linear relationships exist among ^{15}N NMR chemical shifts, reduction potentials, and the pK_as of their parent thiols.

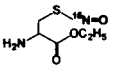
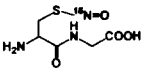
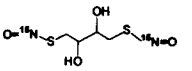
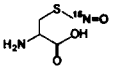
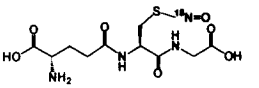
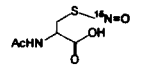
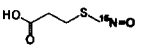
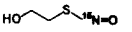
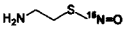
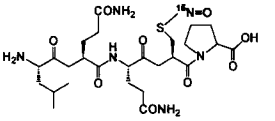
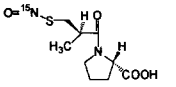
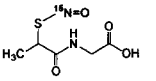
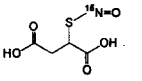
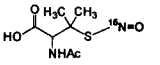
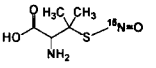
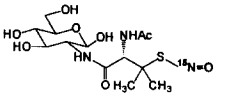
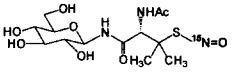
Nitric oxide (NO) has been involved in a wide variety of biological processes, including vasodilatory and antiplatelet effects, macrophage-induced cytotoxicity, and neurotransmission.[1] Specific attention has been focused on *S*-nitrosothiols (RSNO) not only because they are believed to play important roles in storing, transporting, and releasing NO in vivo,[2,3] but also due to the fact that RSNOs, such as *S*-nitroso-*N*-acetyl-penicillamine (SNAP) and *S*-nitrosocaptopril, have been used as therapeutic drugs in the treatment of angina and other circulatory diseases.[4,5] Recently, it has been reported that *S*-nitrosohemoglobin could regulate blood flow via the release of NO.[6] In addition, *S*-nitrosation of certain thiol groups on the calcium release channel might contribute to the regulation of the channel and its various functions.[7] Despite of all these important discoveries, basic aspects of RSNOs chemistry remain to be addressed.[8]

Nitrogen NMR techniques have been an invaluable tool in both organic chemistry and biochemistry. They have been used in organic structure elucidation, conformational analysis, molecular interactions, and biomolecule studies.[9] Bonnett et al.[10] used ^{15}N NMR techniques to identify a series of *S*-nitroso compounds including *S*-nitroso-*N*-acetyl cysteine and SNAP. Using a similar procedure, Stamler and Simon et al. [11,12] recently assigned a peak at 750 ppm on the ^{15}N NMR spectrum (referenced to a peak of 587 ppm of 95% enriched $Na^{15}NO_2$) to the *S*-nitroso cysteine residue on a single cysteine-containing protein Bovine Serum Albumin (BSA). Using ^{15}N NMR, we have studied the NO transfer from a NO donor to cysteine protease papain[13] and identified that *S*-nitroso papain had a single ^{15}N NMR peak at 725 ppm (using a standard of 98% enriched $Na^{15}NO_2$). In this work, seventeen RSNOs with different structures were studied for their ^{15}N NMR profiles. Equilibrium study of NO transfer between SNAP and glutathione (GSH) was also carried out based on these ^{15}N data. Moreover, linear relationships of ^{15}N NMR chemical shifts versus reduction potentials, and of ^{15}N NMR chemical shifts versus pK_as of the parent thiols were observed.

^{15}N NMR Chemical Shifts and UV Absorption of *S*-Nitrosothiols. ^{15}N NMR chemical shifts of seventeen *S*-nitrosothiols were studied in D_2O/CD_3CN (3/1) solution referenced to ^{15}N spectrum of $Na^{15}NO_2$ (Table 1). The use of D_2O/CD_3CN (3/1) as the solvent is to dissolve all the listed compounds. It was found that the ^{15}N

0960-894X/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(99)00499-0

Table 1. ^{15}N NMR chemical shifts, UV absorption maxima of *S*-nitrosothiols

Entry	*S*-nitroso Compounds	Structure	^{15}N Chemical Shift (ppm)	UV Absorption Maxima (nm)
1	*S*-nitroso-L-cysteine ethyl ester		726.2	544
2	*S*-nitroso-Cys-Gly		726.7	546
3	*S*-nitroso-D,L-dithiothreitol		727.8	546
4	*S*-nitroso-L-cysteine		728.2	544
5	*S*-nitroso-glutathione		728.8	546
6	*S*-nitroso-*N*-acetyl-cysteine		729.3	546
7	*S*-nitroso-3-mercaptopropionic acid		730.4	547
8	*S*-nitroso-2-mercaptoethanol		730.6	547
9	*S*-nitroso-2-aminoethanethiol		730.8	547
10	NH_2-Leu-Gln-Gln-Cys(S-NO)-Pro-OH		731.0	544
11	*S*-nitrosocaptopril		732.9	547
12	*S*-nitroso-*N*-(2-mercaptopropionyl)glycine		728.6	549
13	*S*-nitroso-mercaptosuccinic acid		715.4	540
14	SNAP		790.8	590
15	*S*-nitroso-D-penicillamine		789.2	591
16	Glucose-2-SNAP		792.2	590
17	Glucose-l-SNAP		791.3	590

chemical shifts in pure D_2O did not change much from that in D_2O/CD_3CN (3/1). For example, ^{15}N chemical shift of *S*-nitrosocaptopril in D_2O is 730.0 ppm, and it is 732.9 ppm in D_2O/CD_3CN (3/1).

As shown in Table 1, the ^{15}N NMR chemical shifts of primary RSNOs (**1–11**) range from 726.2 to 732.9 ppm while tertiary RSNOs (**14–17**) have ^{15}N NMR peaks around 790 ppm. Compared to primary RSNOs, tertiary RSNOs are downfield by 60 ppm on the ^{15}N NMR spectra. The chemical shift variations among different compounds within each group are very small. Bonnett et al.[10] showed that primary RSNOs, such as *S*-nitroso-*N*-acetyl cysteine, had a ^{15}N signal at 748.3 ppm, and tertiary RSNOs, such as SNAP, showed a ^{15}N NMR at 812 ppm. The difference of the chemical shifts between *S*-nitroso-*N*-acetyl cysteine and SNAP was about 60 ppm. Our results are consistent with their observations. The chemical shift difference may be due to the different solvent and $Na^{15}NO_2$ standard used. For simple RSNOs, it is observed that substituents on the α position of *S*-nitroso group has a significant effect on the ^{15}N chemical shifts. An electron-withdrawing neighboring group, such as carboxylic group in compound **13**, moves the chemical shift upfield, and an electron-donating group, such as methyl group in compound **12**, moves it downfield. For tertiary RSNO **14-17**, the two methyl groups on the α position of SNAP move the ^{15}N chemical shift downfield to 790 ppm.

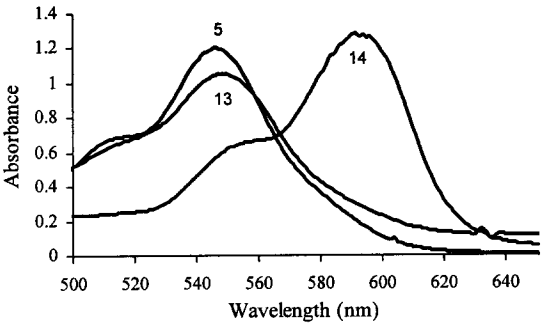


Figure 1 UV absorption of *S*-nitrosothiols.

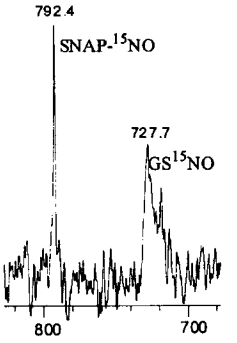


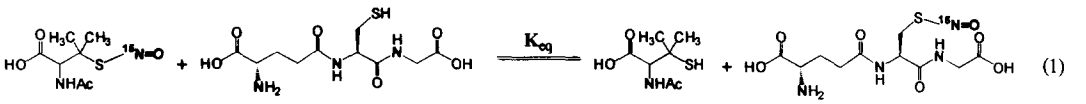
Figure 2 ^{15}NO tranfer Between SNAP and GSH

UV-visible measurements were carried out and the UV absorption maxima (λ_{max}) of the RSNOs are listed in Table 1. It is interesting to note that λ_{max} of these RSNOs show a similar trend as that of the ^{15}N chemical shifts. The λ_{max} for tertiary RSNOs, such as compound **14**, are around 590 nm, while that of primary and secondary RSNOs, such as compounds **5** and **13**, are about 540 nm (Figure 1), which is consistent with the published results.[14] Again, there is little variation among compounds within each group. The λ_{max} studied belongs to $n \rightarrow \pi^*$ transition, which is quite sensitive to the substituents. Substituents located at the α position of the *S*-nitroso group affect the λ_{max} of the absorption band. Investigation of the λ_{max} with structure changes revealed that an electron-withdrawing group such as a carboxylic group has a red-shift effect on the λ_{max}, while an electron-donating group such as a methyl group has a blue-shift effect. The substituent effect on λ_{max} is quite obvious in the tertiary RSNOs, such as SNAP, in which the two methyl groups on the α position move the λ_{max} to 590 nm.

It has been reported[15] that the ^{15}N deshielding of the nitroso compounds could be attributed to the

presence of a low-energy n → π* transition, the lower the n → π* transition energy, the greater the deshielding. In other words, a larger λ_{max} (lower n → π* energy) of RSNO can translate to a further downfield shift (larger ppm) of ^{15}N NMR due to stronger deshielding. Thus, our experiment results are in good agreement with previous observations on the ralationships between ^{15}N NMR and λ_{max} of nitroso compounds.

The ^{15}N NMR chemical shifts of RSNO provide one of the fundamental data for this class of compounds. As an example of the application of this data, the transnitrosation equilibrium of SNAP and GSH was investigated using the ^{15}N NMR technique (Eq. 1). Previously, such equilibria have been studied based on specific UV-visible absorptions of RSNO.[16] In the present study, SNAP-^{15}NO and GSH were mixed in 0.7 mL D_2O/CD_3CN (3/1), after the equilibrium was reached, ^{15}N NMR was recorded as show in Figure 2. The equilibrium constant was calculated based on the ^{15}N signal integration of SNAP-^{15}NO and GS^{15}NO to be 0.74, which is very close to the published result of 0.8 based on UV-Visible absorptions.[15] We envision that this technique can be a general approach to study a variety of transnitrosation reactions.



SNAP GSH NAP GSNO

Linear Relationships among ^{15}N Chemical Shifts, the Reduction Potentials (E_{red}) of *S*-Nitrosothiols and the pK_a of the Parent Thiols. Correlation of the reduction peak potentials[8b] with ^{15}N chemical shifts of these RSNOs gave a linear relationship as shown in Figure 3.

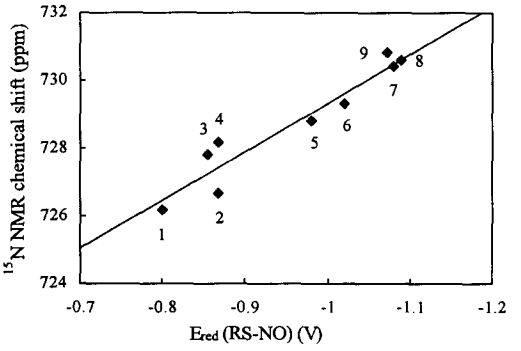


Figure 3 Correlation of ^{15}N NMR chemical shift with reduction potential of RSNO [(Ered(RSNO,V):1: -0.80; 2: -0.86; 3: -0.86;4. -0.87; 5: -0.98; 6: -1.02; 7: -1.09; 8: -1.08; 9: -1.07]

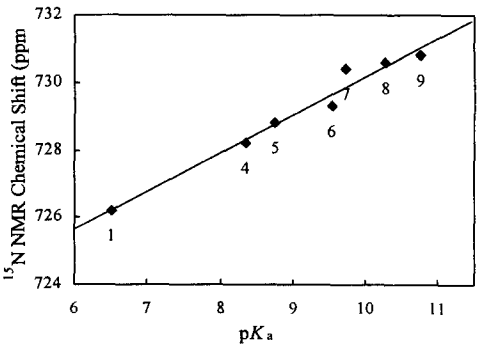


Figure 4 Correlation of ^{15}N NMR chemical shift with pK_a of RSH [pK_a(RSH): 1: 6.50: 4: 8.35; 5: 8.75; 6: 9.52;7: 9.72; 8: 10.27; 9: 10.75]

In our previous electrochemical studies on *S*-nitrosothiols,[8b] we discovered that a linear relationship existed between the reduction potentials of RSNOs and the pK_as of their parent thiols. Here we also observed that plotting of the chemical shifts of primary RSNOs against pK_as[17] of their parent thiols gave a very good linear relationship (Figure 4). As shown in Figure 4, ^{15}N chemical shifts move downfield with increased pK_as.

For primary RSNOs, the pK_as of their parent thiols are influenced by electronic property of the β substituents due to field effects, an electron-donating group will increase the pK_a, while an electron-withdrawing group will decrease it. Thus, a smaller pK_a indicates a lower electron density on S of the parnet thiol as well as the corresponding RSNO. This electron "deficiency" causes easy reduction (lower E_{red}) of the RSNO and higher n $\rightarrow \pi^*$ transition energy (poor electron conjugation), leading to smaller ppm of ^{15}N chemical shifts. Similarly, a larger ^{15}N chemical shifts of RSNO indicates a larger pK_a of the parent thiols and higher reduction potential of RSNO.

In summary, ^{15}N chemical shifts and λ_{max} of seventeen *S*-nitrosothiols have been investigated. It was found that for primary RSNOs, ^{15}N chemical shifts and λ_{max} were around 730 ppm and 540 nm, respectively, while for tertiary RSNOs, ^{15}N chemical shifts and λ_{max} moved to about 790 ppm and 590 nm, respectively. As an example of the application of the ^{15}N NMR of RSNO, equilibrium study of NO transfer between SNAP and GSH were also carried out using ^{15}N NMR technique. For primary *S*-nitrosothiols, linear relationships were also found to exist among ^{15}N NMR chemical shifts, reduction potentials of RSNOs, and the pK_as of the parent thiols.

Experimental

General. All the reagents were purchased from commercial suppliers and were used as received. ^1H NMR spectra were recorded on a Varian Unity 500 MHz NMR instrument and UV-visible measurements were carried out on a HP 8453 UV-Visible Spectrometer (Hewlett Packard Co.).

Glucose-l-SNAP,[18] Glucose-2-SNAP,[18] SNAP,[19] and GSNO[20] were synthesized according to previously published methods using $Na^{15}NO_2$ (98% enriched) as nitrosation reagent.

Peptide NH_2-Leu-Gln-Gln-Cys-Pro-OH was synthesized using standard solid phase peptide synthesis techniques.

Synthesis of other *S*-nitrosothiols. *S*-nitrosothiols were prepared by mixing equimolar amount of thiol, $Na^{15}NO_2$ (98% enriched) and HCl in D_2O/CD_3CN (3/1) solution. All the experiments were carried out with 100 mM *S*-nitrosothiols without further purification. The formation of the *S*-nitroso group was confirmed by characteristic absorption maxima at 330 - 370 nm.

^{15}N NMR measurements. ^{15}N NMR spectra were measured on a Unity 500 MHz Spectrometer and were referenced to a ^{15}N signal of $Na^{15}NO_2$ (98% enriched) in D_2O/CD_3CN (3/1) at 570 ppm. All the spectra were recorded at 50.67 MHz, 200 transients were collected with a 35° pulse width and a 5-s relaxation delay for compounds **1** - **9** and **11** - **17** while 20,000 transitions were collected with the same condition for compound **10**.

Equilibrium study. GSH (11.3 mg, 0.034 mmol) and SNAP-^{15}NO (12.1 mg, 0.055 mmol) were mixed in 0.7 mL D_2O/CD_3CN (3/1). After the equilibrium was reached, the ^{15}N signal integration of SNAP-^{15}NO and GS^{15}NO was obtained to calculate the K_{eq}.

Acknowledgements: This work is generously supported by research grants from the American Heart Association, FL Affiliate (9701760), NIH (GM 54074), National Science Foundation of China (NSFC No. 29832040) and Ministry of Education of China.

References and Notes

1. (a) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109. (b) Beckman, J. S. In *The Physiological and Pathological Chemistry of Nitric Oxide: Nitric Oxide, Principles and Actions;* Lancaster, J. Jr., Ed.; Academic: San Diego; 1996; pp 1 - 69. (c) Fukuto, J. M. In *Chemistry of Nitric Oxide: Biologically Relevant Aspects, Nitric Oxide, Biochemistry, Molecular Biology, and Therapeutic Implications*; Ignarro, L., Murad, F.; Eds.; Academic: San Diego; 1995; p 1.
2. Williams, D. L. H. *Chem. Soc. Rev.* **1985**, 171.
3. Stamler, J. S. In *S-Nitrosothiols and the Bioregulatory Actions of Nitric Oxides through Reactions with Thiol Groups: The Role of Nitric Oxide in Physiology and Pathophysiology*; Koprowski, H.; Maeda, H., Eds.; Springer-Verlag: New York; 1993; p 19.
4. Radomski, M. W.; Rees, D. D.; Dutra, A.; Moncada, S. *Br. J. Pharmacol.* **1992**, *107*, 745.
5. (a) Jia, L.; Blantz, R. C. *Eur. J. Pharmacol.* **1998**, *354*, 33. (b) Nakae, I. *J. Pharmaco;. Exp. Ther.* **1995**, *274*, 40.
6. (a) Stamler, J. S.; Jia, L.; Eu, J. P.; McMahon, T. J.; Demchenko, I. T.; Bonaventura, J.; Gernet, K.; Piantadosi, C. A. *Science* **1997**, *276*, 2034. (b) Mannick, J. B.; Hausladen A.; Liu L.; Hess, D. T.; Zeng, M.; Miao, Q. X.; Kane, L. S.; Gow, A. J.; Stamler, J. S. *Science* **1999**, *284*, 651.
7. Xu, L.; Eu. J. P. Meissner, G.; Stamler, J. S. *Science* **1998**, *279*, 235.
8. (a) Le, M.; Zhang, H.; Means, G. E. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1393. (b) Hou, Y. C.; Wang, J. Q.; Arias, F.; Echegoyen, L.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3065.
9. Levy, G. C.; Lichter, R. L. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; John Wiley & Sons: New York; 1979; pp 167 - 213.
10. Bonnett, R.; Holleyhead, R.; Johnson, B. L.; Randall, E. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2261.
11. Stamler, J. S.; Simon, D. I.; Osborne, J. A.; Mullins, M. E.; Jaraki, O.; Michel, T.; Singel, D. J.; Loscalzo, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 444.
12. Simon, D. I.; Mullins, M. E.; Jia, L.; Gaston, B.; Singel, D. J.; Stamler, J. S. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 4736.
13. Guo, Z. M.; Ramirez, J.; Li, J.; Wang, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 3726.
14. Williams, D. L. H. *Chem. Commun.* **1996**, *10*, 1085
15. Levy, G. C.; Lichter, R. L. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; John Wiley & Sons: New York; 1979; pp 83 - 91.
16. Meyer, D. J.; Kramer, H.; Ozer, N.; Coles, B.; Ketterer, B. *FEBS Lett.* **1994**, *345*, 177
17. Jencks, W. P.; Regenstein, J. In *The Handbook of Biochemistry-Selected Data for Molecular Biology;* Sober, H. A. Ed.; The Chemical Rubber Co.; Cleveland, 1968, p186.
18. Ramirez, J.; Yu, L. B.; Li, J.; Braunschweiger, P. G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2575.
19. Field, L.; Dilts, R. V.; Ravichandran, R.; Lenhert, P, G.; Carnahan, G. *J. Chem. Soc. Chem. Commun.* **1978**, 249.
20. Hart, T. W. *Tetrahedron Lett.* **1985**, 2013.