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The solid-state molecular structure of the S-nitroso derivative of L-cysteine ethyl ester hydrochloride

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Abstract

Nitrosation of protein sulfhydryl groups to form thionitrites (*S*-nitrosothiols) has been reported to be important in the biochemistry of nitric oxide. Such *S*-nitrosation of protein thiol residues has been shown to alter the function of some proteins. In this brief communication, we report the X-ray crystal structure of *S*-nitroso-L-cysteine ethyl ester hydrochloride. Two rotamers with respect to the N—C—S moiety are present in the crystal: the major rotamer is in the *gauche*⁺ conformation, and the minor rotamer is in the rare *anti* (*trans*, antiperiplanar) conformation for a cysteinyl compound. Importantly, the C—S—N=O groups for both rotamers are in the *syn* (*cis*, synperiplanar) form. To the best of our knowledge, this is the first reported high-resolution solid-state structure of an *S*-nitroso derivative of a cysteine or cysteinyl-containing compound.

Keywords

Nitrosothiol; Thionitrite; Nitrosation; Cysteine; X-ray

Organic thionitrites (RS—N=O; R = alkyl or aryl) belong to the general class of *S*-nitroso compounds [1,2]. Their preparative methods, spectroscopic properties, reactions, biological activity, and coordination chemistry have been reviewed [1–6]. In general, RSNO compounds may be prepared from treatment of the precursor thiol RSH with nitrosating agents [4], or from transnitrosation reactions with other thiols/thiolates [7–10]. The decomposition of RSNO compounds in aqueous solution is catalyzed by trace copper ions [11,12].

RSNO compounds are becoming increasingly recognized as important components in the biochemistry and pharmacology of nitric oxide (NO). There are several reports that suggest that the biological actions of RSNO cannot be accounted for by a simple NO dissociation process resulting from S—N bond cleavage [13–15]. In seminal papers published over two decades ago, Ignarro et al. [16,17] proposed that the biological action of vasodilators such as organic nitrates, nitrites, and nitroprusside could be attributed, in part, to the intermediacy of unstable *S*-nitroso compounds.

Posttranslational nitrosation of sulfhydryl groups in proteins to form RSNO moieties can occur under appropriate conditions [18,19], and an attractive proposal has been put forward that this *S*-nitrosation of sulfhydryl groups may regulate the function of some proteins [20]. The possible *in vivo* relevance of RSNO compounds is intriguing. Stamler et al. [21] have suggested that nitrosation of the cysβ93 residue in Hb to form *S*-nitrosohemoglobin (Hb-SNO) is physiologically important in the overall vasodilatory action of NO. However, kinetics

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³Interestingly, theoretical calculations on RSNO compounds reveal a shortening of the C—S bond lengths in the order (CH₃)₃CSNO (1.878 Å) > CH₃CH₂SNO (1.824 Å) > CH₃SNO (1.811 Å) [28].

arguments have recently been presented that suggest that the observed high levels of Hb-SNO in the in vitro studies may be due to the choice of experimental design [22,23]. Thus, in vivo Hb-SNO formation and relevance continues to be intensely debated. The [Hb-SNO] in basal human circulation has been measured at 46 ± 17 nM in arterial erythrocytes and 69 ± 11 nM in venous erythrocytes; furthermore, Hb-SNO is unstable in the reductive environment of erythrocytes, and it was suggested that Hb-SNO is unlikely to function as a reservoir of NO [24]. Physiological levels of other RSNO compounds have been measured. For example, Loscalzo and co-workers [25] determined that the baseline *S*-nitrosocysteine concentration spans a wide range of 30–873 nM in rabbits; however, they also report that infusion of *L*-cysteine followed by administration of *S*-nitrosoalbumin increased this value to the micromolar range. Gaston and Stamler [26] identified *S*-nitrosogluthathione at 250–500 nM in human airways, and Ng et al. [27] recently determined that NO inhalation resulted in a significant increase in *S*-nitrosoalbumin levels from low nM values to 380 nM in cats.

In any event, we have been very interested in the molecular structures and conformation of RSNO compounds of biological importance. This is especially relevant, since the conformation of the —SNO group has been linked not only to the spectroscopy of the RSNO compounds [28], but also to their redox chemistry [29]. While cysteine *S*-nitrosation in hemoglobin and other proteins has been demonstrated by a variety of spectroscopic techniques [19,30] and by mass spectrometry [31,32], we were surprised to find that there was no published structural information to date on *S*-nitrosocysteine.¹ In this brief communication, we report the high-resolution X-ray crystal structure of the hydrochloride salt of the lipophilic *S*-nitroso-*L*-cysteine ethyl ester.

Experimental section

L-Cysteine ethyl ester hydrochloride (98%) was purchased from Aldrich Chemical. The red *S*-nitroso derivative was prepared by nitrosation with ethyl nitrite as previously described [35, 36]. Crystals were mounted on a Bruker APEX diffractometer (SMART (version 5.625), GEMINI (version 1.0), and SAINT-plus (version 6.29), Bruker AXS, Madison, WI, USA). X-ray diffraction data were collected at 99(2) K using MoK α ($\lambda = 0.71073$ Å) radiation. Intensity data, which approximately covered the full sphere of the reciprocal space, were measured as a series of ω oscillation frames each 0.3° for 35 s/frame. The detector was operated in 512 × 512 mode and was positioned 6.12 cm from the crystal. Coverage of unique data was 99.3% complete to 54.0° (2 θ). The cell parameters were determined from a non-linear least-squares fit of 6806 reflections in the range of 3.2° < θ < 26.9°. A total of 8518 reflections were measured. The structure was solved by the direct method using SHELXTL [37], and refined by full-matrix least squares on F^2 using all reflections. All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included with idealized parameters. However, all the hydrogen atom positions were also located in the difference map, and were coincident within the given esd's with those of their idealized positions.

The S—N=O group is disordered due to the presence of two rotamers along the N—C—C—S moiety; the two S—N=O groups were refined with 70% (major) and 30% (minor) occupancies. Initial refinement was carried out with DFIX and SADI restraints, and finally without any restraints for this C—S—N=O group and this yielded a better refinement. For example, attempts to fix the C—S bond lengths at 1.80 Å to closely match the limited data from the rather small set of RSNO structures (see later) resulted in unacceptable significant increases in the *R* and *wR*₂ values. Attempts to model two positions for the C(5) atom (i.e., the carbon attached to sulfur) also resulted in significant increases in the *R* and *wR*₂ values. The

¹The assignment of electron density to an —SNO group in the crystal structure of a compound formulated as Hb-SNO [33] has been updated to include the possibility of the Hb-SNOH derivative [34].

CH₃CH₂ chain may be slightly disordered as evident from the relatively large thermal motions of the C1 and C2 atoms, however, we were not able to resolve the possible two/multiple positions of these atoms. The final $R_1 = 0.0415$ is based on 2104 “observed reflections” [$I > 2\sigma(I)$], and $wR_2 = 0.1135$ is based on all reflections (2209 reflections). Details of the crystal data are given in Table 1. Thermal ellipsoids are drawn at 35% level for Fig. 1.

The X-ray crystal structure data have been deposited with the Cambridge Crystallographic Data Center, with reference code CCDC 259476. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033.

Results and discussion

The molecular structure of *S*-nitroso-*L*-cysteine ethyl ester hydrochloride is shown in Fig. 1. Both rotamers resulting in two positions for the —S—N=O group are shown in Fig. 1A, and the major occupancy component is shown in Fig. 1B. Selected metrical data are included in Table 1.

Rotamers

As can be seen in Fig. 1A, two rotamers with respect to the N—C—C—S moiety are present in the crystal structure. The Newman projections of these rotamers are shown in Fig. 2. The major rotamer is in the *gauche*⁺ (synclinal)² conformation with a N—C—C—S torsion angle χ_1 of 83.7(1)°, and it was refined with 70% occupancy. The minor rotamer with 30% occupancy is in the *anti* (*trans*, antiperiplanar) conformation with χ_1 of 176.1(1)°. The backbone N(1)—C(4)—C(3)—O(1) torsion angle is 166.3(1)°.

Görbitz [40] has shown that crystal structures of cysteine and cysteinyl-containing small molecules reveal predominantly *gauche*⁺ conformations. In addition, MacArthur and Thornton [41] have analyzed the conformations of protein side chains and have shown that cysteinyl groups display predominantly *gauche* conformations. The orthorhombic polymorph of *L*-cysteine [42] with a disordered sulfur atom displays a *gauche*⁺ conformation ($\chi_1 = 60.23^\circ$ and 69.01° for the two sulfur positions). Interestingly, the monoclinic polymorph [43] contains two independent molecules in the asymmetric unit; one of these displays a *gauche*⁺ conformation ($\chi_1 = 74.39(10)^\circ$), and the other displays the then-unprecedented *anti* conformation ($\chi_1 = -170.15(7)^\circ$) for a cysteine group. Thus, the *anti* conformer for *S*-nitroso-*L*-cysteine ethyl ester hydrochloride reported in our current work presents, to the best of our knowledge, only the second high-resolution example of this conformation for cysteine-containing small molecules.

The RSNO group

The thionitrite functionality in RSNO compounds may exist in either the *syn* or *anti* conformations (Fig. 3); the *syn* conformation has a C—S—N=O torsion angle φ close to 0°, whereas the *anti* conformation has a torsion angle close to 180°.

As shown in Fig. 1A, the alkyl thionitrite functionality is present in the *syn* (*cis*, synperiplanar) conformation for *both* rotamers of the compound, with C—S—N=O torsion angles of $-2.4(3)^\circ$ for the major rotamer, and $3.3(5)^\circ$ for the minor rotamer. This experimentally observed *syn* conformation for the C—S—N=O group in *S*-nitroso-*L*-cysteine ethyl ester hydrochloride is in accord with hybrid density functional theory calculations that show that the *syn* conformers

²We have adopted the *syn* (*cis*, synperiplanar), *gauche* (synclinal), and *anti* (*trans*, antiperiplanar) nomenclature proposed by the International Union of Pure and Applied Chemistry to describe the A—B—C—D torsion angles [38], where *gauche*⁺ refers to a positive sign of the torsion angle [39].

are more stable than the *anti* conformers for isolated molecules of CH₃SNO (by 0.78 kcal/mol) and CH₃CH₂SNO (by 0.45 kcal/mol) [28]. A similar *syn* over *anti* preference (by 0.15 kcal/mol) was calculated for isolated Ph₃CSNO [44], although the crystal structure of the compound displayed the *anti* conformation [29].

Only five solid-state RSNO structures have been reported to date, and selected structural parameters are listed in Table 2. As is seen from the table, four of the previously reported structures contain C—S—N=O moieties in the *anti* conformation (i.e., with ϕ angles close to 180°); the first example of a *syn* group is for *S*-nitrosocaptopril (with a primary carbon attached to sulfur) [28], and the second is for a disordered component of Ar₃CSNO (with a tertiary carbon bound to sulfur; Ar = a 3,5-disubstituted bulky aryl group) [44].

As can be observed from Table 2, the S—N and N—O metrical parameters of the SNO group of *S*-nitroso-*L*-cysteine ethyl ester hydrochloride are similar to those previously reported for other RSNO compounds, and these will not be discussed further.

The C—S bond of the major occupancy rotamer is 1.734(2) Å, and this bond length is slightly shorter than that determined for orthorhombic *L*-cysteine (1.790(1) and 1.810(1) Å) [42], and for *L*-cysteine ethyl ester hydrochloride-urea (1.772(16) Å) [48]. However, the C—S bond length may be compared with the 1.739–1.873 Å range previously determined for related —C—S—(N)-containing compounds (Cambridge Structural Database, 2004 release; 27 entries), and is similar to the 1.749–1.814 Å range in compounds containing the —CH₂—S(N) moiety [28,49–52].³ We believe that the lower occupancy of the —S(1')—N(2')—O(3') group (only 30%) probably compromises the accuracy of the determined metrical parameters for this minor component, hence we are hesitant to over-interpret these bond lengths and angles. The C(4)—C(5)—S(1) bond angle of 117.6(1)°, although it deviates from the ideal tetrahedral angle, is similar to the corresponding angles in *L*-cysteine (114.8(3)°; orthorhombic form) [42], *L*-cysteine ethyl ester hydrochloride (115.7(1)°) [53], and the cysteinyl group of glutathione (114.7(4)°) [54].

Finally, the NO moiety in the major component of *S*-nitroso-*L*-cysteine ethyl ester hydrochloride eclipses a β -CH bond (i.e., at C(5)), and a similar preferential eclipsing was found in the molecular structure of *S*-nitrosocaptopril in accord with theoretical predictions [28].

Conclusion

We report the first high-resolution crystal structure determination of an *S*-nitrosocysteine derivative. Given the importance of *S*-nitrosation of cysteine residues in biology, we anticipate that knowledge of the solid-state conformations of the *S*-nitroso-*L*-cysteine group will allow for more complete predictions of the conformations of *S*-nitroso-*L*-cysteinyl groups in nitrosated peptides and biomolecules. Such work is ongoing in our laboratories.

Note added in proof

During the review of the manuscript, an excellent report describing the reaction of NO with the proximal cysteine ligand of the heme in *Cimex lectularius* nitrophorin to generate a nitrosocysteine moiety was published; the product was characterized by X-ray crystallography to 1.75-Å resolution [55].

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Fig. 1.

(A) The crystal structure of *S*-nitroso-*L*-cysteine ethyl ester hydrochloride showing both rotamers with respect to the N—C—C—S moiety. The major CSNO component is displayed with solid bonds. The H atoms attached to C5 are deleted for clarity. The N...Cl distance is 3.114(1) Å. (B) A different view of the compound showing only the major component.

Fig. 2. Newman projections showing the *gauche⁺* and *anti* rotamers with respect to the N—C—C—S moiety.

Fig. 3.
The *syn* and *anti* forms of alkyl thionitrites (*S*-nitrosothiols).

Table 1

Crystal data and structure refinement

Formula (fw)	C ₅ H ₁₁ Cl ₁ N ₂ O ₃ S (214.67)
<i>T</i> (K)	99(2)
Crystal system	Orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	
<i>a</i> (Å), α (°)	5.000(1), 90
<i>b</i> (Å), β (°)	8.348(1), 90
<i>c</i> (Å), γ (°)	24.164(2), 90
<i>V</i> , <i>Z</i>	1008.60(11) Å ³ , 4
Reflections collected	8518
Independent reflections	2209 [<i>R</i> _{int} = 0.0217]
Data/restraints/parameters	2209/24/138
<i>R</i> indices (all data)	<i>R</i> ₁ = 0.0438, <i>wR</i> ₂ = 0.1135
Final <i>R</i> indices (<i>I</i> > 2σ (<i>I</i>))	<i>R</i> ₁ = 0.0415, <i>wR</i> ₂ = 0.1118
GOF on <i>F</i> ²	1.091
Largest difference peak and hole	0.864 and −0.229 e Å ^{−3}

Table 2

Selected data for structurally characterized alkyl thionitrites

Compound	N—O (Å)	S—N (Å)	C—S (Å)	S—N—O (°)	C—S—N (°)	ϕ^a	References
ONSC(Me) ₂ CH ₂ NHC(O)Me	1.206(2)	1.754(2)	1.829(2)	114.9(2)	99.44(8)	178.5	[45]
S-Nitroso-N'-acetylpenicillamine ^b	1.199(2)	1.763(2)	1.833(1)	113.99(11)	100.80(7)	176.3	[46,47]
S-Nitroso-N'-acetylpenicillamine ^c	1.206(3)	1.762(3)	1.842	114.9(2)	96.56(8)	179.7	[29]
Ph ₃ CSNO	1.177(6)	1.792(5)	1.867(3)	114.0(4)	102.1(2)	175.7	[29]
S-Nitrosocaptopril	1.206	1.766	1.800	117.7	103.7	0.68	[28]
Major	1.191(4)	1.769(3)	1.734(2)	117.6(2)	105.6(1)	2.4(3)	This work
S-Nitroso-L-cysteine ethyl ester hydrochloride							
Minor	1.183(7)	1.728(5)	1.638(2)	116.4(4)	109.5(2)	3.3(5)	This work
Ar ₃ CSNO ^d							
Major	1.205 (6)	1.781(5)	1.841(4)	111.4(6)	104.2(3)	179.6	[44]
Minor	1.189(12)			123.6(7)		7.3	[44]

^a Defined as the magnitude of the O—N—S—C torsion angle.

^b Crystallized from methanol/water.

^c Crystallized from acetonitrile.

^d Ar = 3,5-bis(2,6-dimethylphenyl)phenyl; the nitroso O-atom is disordered over two positions to give a mixture of *anti* and *syn* conformers in the ratio of 0.67:0.33.