

Computational Exploration on the Formation and Stability of S-Nitrosothiols: Biological Implications in Nitric Oxide Storage/Scavenging

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Abstract

Nitric oxide (NO[•]) is involved in post translational modification of cysteine (cys) residues under both physiological and pathological conditions. NO[•] formation and S-nitrosation of various proteins observed during ischemia thus alter its functions. S-nitrosothiols have been reported to be protective against cardiac reperfusion injury. In order to understand the mechanism of formation and stability of S-nitrosothiols, the adduct formation reaction of NO[•] with cys, homocysteine (Hcys), cysteamine (cysA) and N-acetylcysteine (NAC) have been investigated using 6-31G (d, p) basis set at HF method. The relative activation energies are 50.83, 404.56, 405.99, and 455.57 Kcal/mol for cys-NO, cysA-NO, NAC-NO and Hcys respectively indicates that cys-NO adduct has the least energy conformer whereas Hcys-NO has the highest energy state. The S-N bond lengths after the adduct formation are 1.8323, 1.8309, 1.8328 and 2.5267 Å for cys-NO, cysA-NO, NAC-NO and Hcys-NO, respectively and are consistent with a sulfur-nitrogen single bond in agreement with the experimental report. The relative rotational energy of CSNO dihedral angle determined for cys-NO adduct is reasonably in agreement with the already reported similar kind of adduct. Among the biologically important investigated thiols, cys-NO adduct is easily formed and very stable when compared to other cys substituted adducts. These findings shed further insight into complexity to researchers understanding of NO[•] biology and provide valuable suggestions for the design of new nitroso adducts as medicinal therapies.

Keywords: Cysteine, Nitrosothiols, Transition state, Mulliken charge, Activation energy

Introduction

Nitric oxide (NO[•]) has been shown to be an important player in virtually every step of cancer, from tumorigenesis to metastasis [1]. Recent reports highlighted the involvement of NO[•] in mitochondrial pathophysiology. NO[•] can control, modify, or even damage mitochondrial proteins depending on the concentration level and conditions [2-4]. There are multiple targets for NO[•] and its metabolites within mitochondria, including complexes I, II, and IV of the electron transport chain [5]. It has been reported that hemoglobin can act as both oxygen and NO[•] transporter. Further, deoxyhemoglobin scavenges NO[•] in post capillary vasculature, this is followed by NO[•] group transfer from heme to Cys-β₉₃ in the lung [6].

NO is not only target mitochondrial functions, and other intracellular pathways involving proteins with sensitive thiol group will also be affected. Such pathways may include the inhibition of apoptosis and glycolysis via modification of cysteine (Cys) proteases [7], or glyceraldehyde-3-phosphate dehydrogenase [8] respectively.

Indeed, NO has been shown to circulate as an S-nitroso adduct of protein cys residues and possess biological activities identical to those of NO in vitro and in vivo [11]. The pathway considered for the attack of NO on sulfur of thiolate anion is shown in Figure 1. Free NO is highly reactive in the biological environment due to its half life period of only about five seconds. Therefore, to effectively use NO, development of NO based pro-drugs, may be necessary to obtain a targeted release of NO to achieve the desired physiological effects [9]. NO reacts with reduced cys residue to form nitrosothiol, which can serve as a vehicle to transport NO in plasma thereby increasing the half-life period of NO [10]. So, the development of relatively stable NO donors with potential therapeutic value are of great importance in evaluating their physiological role.

Formation and decomposition of S-nitrosothiols are dynamic process that influenced largely by the existing redox environment, oxygen and metal ion availability and thiol reactivity [11]. Thiol group of cys has got numerous biological functions because it deprotonated to give thiolate ion which acts as nucleophile. However, as has been demonstrated, endogenous nitrosation of protein thiols appears more often selective. As a unique class of such NO-carrying vehicles, S-nitrosocysteine and its analogues are generally believed to take a most active part in many biological functions of NO, especially in the processes of NO-storage/scavenging and transport [9].

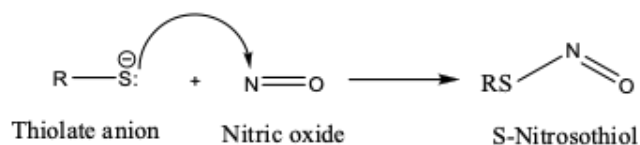


Figure 1. Schematic illustration of proposed mechanism of NO attack on cys and their analogues.

Recently, S-nitrosothiols (RSNO), such as cys-NO, have generally been used as NO donor molecules in the studies of cardio protection, and their effects have been attributed to NO release. However, some RSNO, and in particular cys-NO, can affect intracellular function and transfer of the nitroso functional group to intracellular thiols [13–15]. The inherent instability of RSNO group poses a number of challenges for the physiological characterization of these compounds. It should be believable that the stored NO in its carrier molecules has to go through certain release and capture cycles to execute its various biological functions. To understand the mechanistic details of such NO migration, it is obviously desirable to have some quantitative knowledge of the formation and stability of S-N bond and it is very difficult to experimentally determine the stability of the S-N bond as small molecule adduct.

Theoretical studies that provide mechanistic insights into the reaction between NO and thiol group as a small molecule adduct have not been conducted so far, which could help to explain the factors that favor the adduct formation. The results of such theoretical work will aid in elucidating the mechanism of formation of cys-NO adducts and similar biologically reactive adducts. So, in this article we provide a comprehensive overview of the interaction between NO with cys and its analogues by performing systematic theoretical studies.

Computational methods

The calculations described here were performed using Gaussian 03 suite of programmes [16]. The geometries of the molecules in their ground state have been optimized using 6-31G (d,p) basis set [17,18] at HF[19] method. All transition states shown in this study are traced at HF/6-31G (d, p) level and the trend in the activation energy is confirmed by computing them again using the same. Stationary points located in the potential energy surface have been characterized as either minima or transition state (TS) by computing their harmonic vibrational frequencies. Frequency calculations were carried out to ensure that the considered structures correspond to TS on the potential energy surface. The optimized TS were tested by stepping through the internal reaction coordinate to connect the TS to the products and reactants. All TS have a single imaginary frequency while minima all have real frequencies. The Mulliken charges from Mulliken population analysis are used to identify and analyse the nucleophilic sites of the cys derivatives.

Results and discussion

NO[•] is extensively synthesised in eukaryotic cells for the regulation of numerous physiological processes [20]. High levels of NO[•] produced in response to inflammation and infection, may be toxic and implications in neurodegenerative diseases [21]. The characterizations of NO[•]-dependent signalling processes are occurring through binding to heme and initiating a cGMP signalling cascade [22]. However, much of NO[•] produced in the cell does not lead to such complexes but rather to nitrosation of cys residues as signalling intermediates [23,24]. The structural and chemical insight into these protein modifications have lagged behind the biology due to its inherent instability [6]. Their inherent instability is recognized as forming the basis for physiological effects, such as release of NO[•] or the post translational chemical modification of protein cys residues. This led to considerable interest into their formation, stability and NO[•] storage as small molecule adducts. The optimized structures of the adducts are depicted in Figure 2.

Structural parameters

The calculated bond lengths between sulfur and nitrogen after the adduct formation using HF/6-31G (d, p) are listed in Table 1. The observed bond lengths of S-N for the adducts of NO[•] with cys, cysteamine (cysA), homocysteine (Hcys) and N-acetylcysteine (NAC) are 1.8323, 1.8309, 2.5267 and 1.8328 Å respectively indicating a sulfur-nitrogen single bond. Further, the observed bond lengths of N=O after the adduct formation of NO[•] with cys, cysA, Hcys and NAC are 1.1569, 1.1572, 1.1124 and 1.1571 Å respectively indicating a nitrogen-oxygen double bond. These values disclose that strengthening of N=O bond would result in lengthening of S-N bond consequently release of NO[•] from the adducts. These results suggest that the calculated bond lengths associated with the C-S-N-O group for the adducts investigated here are in good agreement with the experimental result and also with the theoretical results for the similar kind of adducts [25].

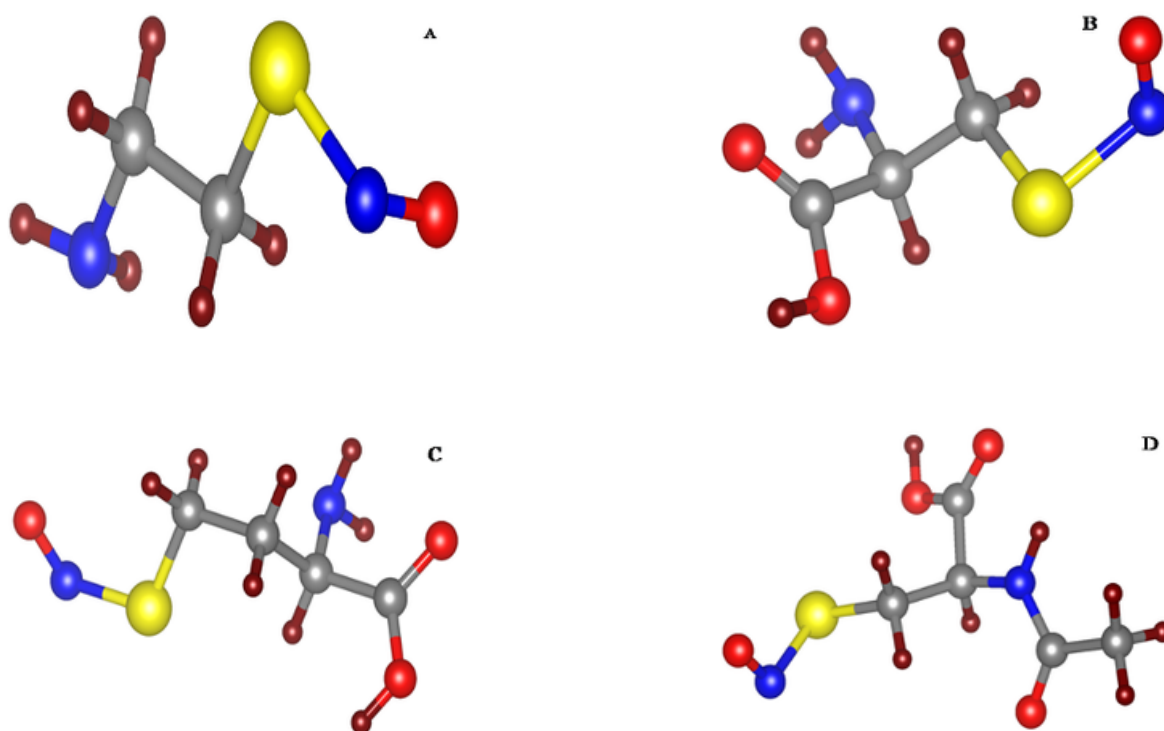


Figure 2. HF/631-G (d, p) Optimized structures A) cysteine-NO, B) cysteamine-NO, C) homocysteine-NO and D) N-acetylcysteine-NO adducts. Gray: Carbon, Blue: Nitrogen, Red: Oxygen, Yellow: Sulfur and Brown: Hydrogen.

It has been reported that conformational flexibility will influence compounds stability as there is an increase in the S-N bond length in the transition state [25]. Lengthening of S-N bond is also an initial step in the reductive elimination of NO[•] from the adducts.

Table 1. Bond lengths of cys/analogues-NO adducts computed using HF/6-31G (d, p)

Adducts	Bond length S-N (Å)	Bond length N-O (Å)
Cys-NO	1.8323	1.1569
cysA-NO	1.8309	1.1572
Hcys-NO	2.5267	1.1124
NAC-NO	1.8328	1.1571
anti-MeSNO ^a	1.8653	1.1746
anti-{MeSNO} ^a	2.4300	1.1950

^aJ. Am. Chem. Soc., Vol.121 No.30 1999

The lengthening of S-N bond possibly due to the antibonding character of S-N orbital leading to structural degradation of the adducts [25]. Among the investigated adducts, HCys-NO adduct has the larger lengthening of S-N bond and supports the experimental data that show reduction of RSN=O rapidly leads to degradation of the compound under anaerobic conditions. This shows the antibonding character of S-N orbital resulting in reductive elimination of NO[•] from the adducts and confirming that HCys-NO adduct strongly influences protein stability.

The bond angles and dihedral angles associated with the C-S-N-O group for the adducts are presented in Table 2 and Table 3 respectively. From Table 3, it is evident that cys-NO, cysA-NO, Hcys-NO and NAC-NO adducts are found to have a C-S-N-O dihedral angle of 84.42°, 85.69°, -102.82° and 83.38° respectively indicating non-planarity of Hcys-NO adduct. Therefore, it hinders the effective π -orbital conjugation and resulting in higher energy conformer in Hcys-NO when compared to other adducts.

Table 2. Bond angles of cys/analogues-NO adducts computed using HF/6-31G (d, p)

Adducts	S-N-O (Å)	C-S-N (Å)
cys-NO	113.4	94.1
cysA-NO	111.4	95.4
Hcys-NO	124.1	65.6
NAC-NO	113.4	94.3
anti-MeSNO ^a	116.7	94.4
anti-{MeSNO} ^a	118.3	85.5

^aJ. Am. Chem. Soc., Vol.121 No.30 1999

Table 3. C-S-N-O dihedral angles of cys/analogues-NO adducts computed using HF/6-31G (d, p)

Adducts	Atoms	Angle After TS (Å)
Cys-NO	C-S-N-O	84.42
cysA-NO	C-S-N-O	85.69
Hcys-NO	C-S-N-O	-102.82
NAC-NO	C-S-N-O	83.38

Charge population analysis

Mulliken atomic charges of sulfur, nitrogen and oxygen atoms for the adducts are shown in Table 4. Investigation of the electronic structure calculation indicates that the adduct formation reaction accompanied by the charge transfer from nitrogen of NO[•] to sulfur of cys and their analogues. Mulliken atomic charge is computed with HF/631-G (d, p) level shows the nature of charge transfer between NO[•] and cys moieties. The stability of the geometry is determined by formation and decomposition of S-N bonds which are arranged tetrahedrally around the central nitrogen atom with planar S-N-O conformation. These arrangements leading to a predicted transition state with most of the charge reside on sulfur atoms, rather than the S-N-O oxygen atom [26]. The positive charge on nitrogen atom indicates a stronger attraction with the negatively charged sulfur atom which favours the adduct formation. So most of the nitrogen atom charge residing on sulfur atom would strongly support the adduct formation [26].

In the optimized geometries of NO[•] and cys, the Mulliken charge calculated on nitrogen of NO[•] is 0.255, oxygen of NO[•] is -0.255 and sulfur of cys is -0.731 respectively. As seen in Table 4, the charge on the oxygen atom of Hcys-NO adduct is significantly increased to -0.125 from -0.255 and a negligible decrease in the charge on nitrogen (0.251) bonding to sulfur. This increase in the charge on oxygen atom in the transition state of Hcys-NO adduct would result in breaking of S-N bond thus causing protein damage.

Table 4. Mulliken atomic charges calculated by HF/6-31G (d, p)

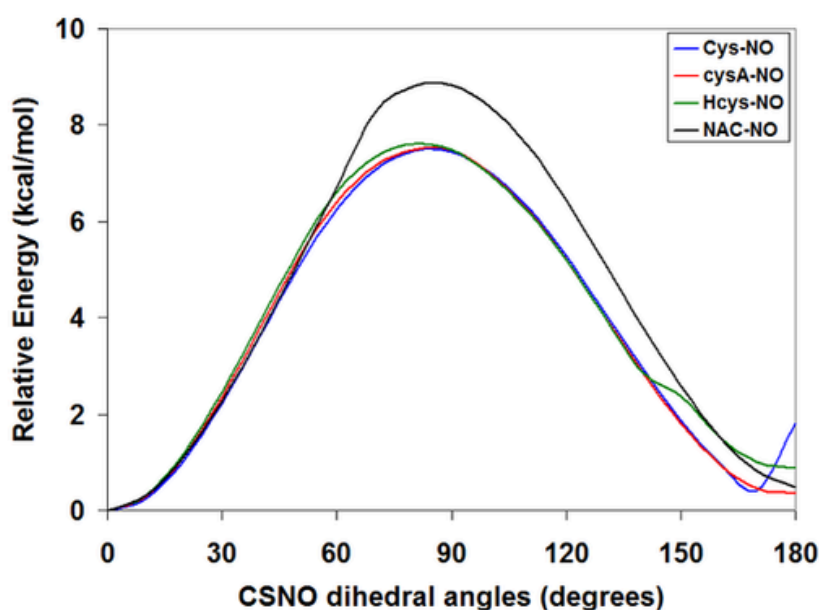
	Sulfur	Nitrogen	Oxygen
Optimized	-0.731	0.255	-0.255
Adducts	Sulfur	Nitrogen	Oxygen
Cys-NO	0.245	-0.007	-0.209
cysA-NO	0.220	-0.004	-0.213
Hcys-NO	-0.199	0.251	-0.125
NAC-NO	0.254	-0.005	-0.212

These observations are consistent with our conclusion from structural parameters analysis. But all of the other adducts, the charge on nitrogen considerably decreases thus favouring the adduct formation. The Mulliken charge calculated on nitrogen for cys-NO, cysA-NO, Hcys-NO, and NAC-NO adducts are found to be -0.007, -0.004, 0.251, -0.005 respectively.

Ab initio torsional energies of CS-NO and CC-SN

The torsional energy profile of C-S-N-O in α -conformation is calculated using an ab initio method and is shown in Figure 3. A series of conformers with the C-S-N-O dihedral from 0° to 180° at an interval of 10° were subjected to geometry optimization and the resulting energies were plotted to obtain the theoretical profile. The S-N bond lengths in cys-NO adduct and their analogues were elongated. The N-O bond shortened as the C-S-N-O dihedral angle increases from 0° to 90°. This is in agreement with the already reported result for the similar kind of adduct CH₃SNO by Arulsamy et al [6].

Figure 3. The rotational energy barrier for cys/analogues-NO adducts.



The rotational energy barrier determined for cys-NO adduct (7.463 kcal/mol) is reasonable in agreement with the already reported value for the similar kind of adduct Nitrosomethylthiol (12.86 kcal/mol) [6]. The rotational energy barrier calculated for cysA-NO, Hcys-NO and NAC-NO are 7.49, 7.62 and 8.83 kcal/mol respectively. From these results, it is concluded that among the cys substituted analogues cys-NO adduct is more likely to be formed. Rotation around C-S-N-O dihedral angle leads to substantial weakening of S-N bond length and a contraction of both N-O bond length and S-N-O bond angle to reach transition state. This is in agreement with the already reported works [6,27].

Activation energy

The activation energy barrier determined for cys-NO, cysA-NO, NAC-NO and Hcys-NO adducts are of the order of 50.83, 404.56, 405.99 and 455.57 kcal/mol respectively. These calculations show that NO[•] adduct with Hcys-NO has the highest activation energy than the other adducts and cys-NO adduct has the lowest activation energy. These observations predict that cys-NO adduct is more likely to be formed and also very stable than the other adducts. However, irrespective of the higher activation energy required for the attack of NO[•] with Hcys. The rotational energy barrier and activation energy for the adducts are listed in Table 5.

Table 5. Rotational energy barrier and activation energy for the reaction between NO[•] and cys, cysA, NAC and Hcys calculated at HF/6-31G (d, p) level

Adducts	CSNO-Rotational energy barrier (kcal/mol)	Activation energy (kcal/mol)
Cys-NO	7.46	50.83
cysA-NO	7.49	404.56
Hcys-NO	7.62	455.57
NAC-NO	8.83	405.99

Conclusions

The adduct formation reactions of NO[•] with cys, cysA, NAC and Hcys have been investigated by theoretical calculations to analyze their structural and electronic properties quantitatively, to understand the formation and stability of the adducts that can be used to explain the role of these adducts in NO[•] scavenging/antioxidant activities. Hcys-NO exhibits considerable differences in its structure and electronic properties compared to other adducts, such as the larger weakening of S-N bond, a negligible decrease in the charge on N atom bonded to S and the higher activation energy, result in affecting its inability to take part in NO[•] scavenging and antioxidant activities. The activation energies are 50.83, 404.56, 405.99, and 455.57 Kcal/mol for cys-NO, cysA-NO, NAC-NO and Hcys respectively. Further, Hcys-NO adduct is not in-plane as indicated by the dihedral angle of -102°. The relative rotational energy barrier calculated for cys-NO adduct (7.463 kcal/mol) is reasonably in agreement with the already reported value for the similar kind of adduct. Taken together these results may provide some insight into the elucidation of mechanisms governing NO[•] scavenging of cys and their analogues and act guide to model the cys-SNO conformation in proteins more accurately, and to decrease their reactivities in the biological systems.

Declarations

Ethics approval and consent to participate

The authors would like to declare that the work described was original research that has not been published previously, and is not under consideration for publication elsewhere, in whole or in part.

Consent for publication: All authors agree to publish the presented work

Availability of data and materials: The data and material that support the finding of this study are reported within the article.

Competing interest: The authors declare no competing interests.

Author Contributions: G.M. performed the calculations and wrote the manuscript. K.M. did the analysis and C.K. suggested the idea with supervision and wrote the manuscript.

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