Thionitroxides, RSNHO·: The Structure of the SNO Moiety in "*S*-Nitrosohemoglobin", a possible NO Reservoir and Transporter

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Considerable evidence suggests that the *S*-nitrosation of cysteine residues, thought to regulate the activities of many proteins, is involved in the uptake and intracellular trafficking of NO.[1] Indeed, to absorb the neutral NO with thiols, a redox reaction is needed to form *S*-nitrosothiols (RSNOs).[1-3]

A structure of *S*-nitrosocysteine in hemoglobin proteins was first reported by Arnone and coworkers in 1998.[4a] Interestingly, the resulting protein structure obtained under non-biological conditions (NO pressure, anaerobic) exhibits unexpected C-S-N-O dihedral angles of 88° and -76° for *Cys*93 of β1 and β2 subunits. The crystallographic results contradict the planar geometries of all RSNOs in small molecule crystallographic data,[5] and from quantum mechanical calculations.[6] Recently, Arnone *et al.* refined the X-ray data again and reported that the electron density of the SNO-*Cys*98(F9)β could not be fit with a planar *syn* or an *anti* *S*-nitrosothiol model. They found C-S-N-O dihedral angles between 70° and 90° for SNO-*Cys*93(F9)β1 and SNO-*Cys*93(F9)β2.(Fig. 1)[4b] To explain this, they proposed that a *N*-centered radical, *Cys*-S-N·-OH, was present and stabilized by hydrogen bonding with *Val*98. Such a radical was previously proposed as an intermediate in reactions of NO with thiols.[7] However, the SNO moiety in the *Cys*93(F9)β2 subunit is unlikely to be stabilized in this way, because the shortest O---O distance between the oxygen atom of the studied SNO species and the nearest oxygen of *Lys*144 is about 3.84 Å, a distance too long for significant OH---O hydrogen bonding interactions.

By contrast, Montfort and coworkers have reported that the nitric oxide transport protein, nitrophorin cNP,[8] couples reduction and oxidation of a heme moiety with reversible *S*-nitrosation of a cysteine residue (PDB: 1Y21). This *S*-nitrosothiol species has a C-S-N-O dihedral angle of 0°, coinciding with a planar CSNO geometry in small molecule crystallographic data. The cysteine sulfide coordinating to ferric heme is the key to the *S*-nitrosothiol formation in this protein. Unlike nitrophorins, the NO-bound cysteine in hemoglobin is 10 Å (Fe---S) from the closest heme.

To determine the identity of the ~90° CSNO moiety in the purported *S*-nitrosohemoglobin, we have explored all four types of SNO species (RSNO, RS-N·-OH, RS-NH-O·, and RS-NH-OH) that could be candidates for the observed SNO species, using simple models (R = Me) that can be computed at a high level. By comparison of the calculated structures to the experimental crystallographic data, we have discovered that the thionitroxide radical (*Cys*-S-NH-O·) is consistent with the observed SNO structures in both β1 and β2 subunits.

Quantum mechanical calculations were carried out with GAUSSIAN 03,[9] using the (U)B3LYP density functional theory[10] and the 6-31+G* basis set. The lowest energy minima were verified by harmonic vibrational frequency analysis. Geometries and energies were re-evaluated using the CBS-QB3 and G3 methods,[11,12] expected to predict experimental energies to 1 kcal/mol. The solvation energies in water were calculated with the B3LYP (CPCM) /6-31+G* method with Pauling (Merz-Kollman) atomic radii to mimic a polar environment.[13]

The global minima of these species (**1**-**5**) are given in Fig. 2. Relative energies and selected geometrical parameters of energy minima and transition states, with CBS-QB3 and G3, are collected in Supporting Information.

The *S*-nitrosothiol, MeSNO, has two planar energy minima on the potential surface, **1** and **2**. The *cis*-isomer is more stable, but bulky alkyl groups make the *trans*-isomer more stable.[5,6] Partial S-N double-bond character is manifested by a bond length of 1.79-1.80 *Å* and an S-N rotational barrier of 12 *kcal/mol* (**6**).

The global minimum of RS-N·-OH, **3**, is a planar *trans, trans*-geometry. There are two other near-planar energy local minima, higher in energy by a few *kcal/mol* (Supporting Information).

The global minimum of the thionitroxide radical, **4**, has a twisted 72° C-S-N-O geometry. The *O*-centered radical is more stable by 3 *kcal/mol* (B3LYP) in the gas phase than the *N*-centered radical, **3**. Higher level calculations (CBS-QB3 and G3) result in very similar stabilities for the two radicals; **4** is stabilized more than **3** by 0.4 kcal/mol in aqueous solution.

For the *S*-hydroxylamine species, RS-NH-OH (**5**), with a 62° dihedral angle is the global minimum among four local minima.

RS-N·-OH and RS-NH-O· are less stable than the stable radical, TEMPO· (*2,2,6,6-tetramethyl-1-piperidyloxyl*). Using the bond dissociation energy (BDE) of the O-H bond in TEMPOH (69.8 *kcal/mol*),[14] the BDEs of O-H and N-H in RS-NH-OH are predicted to be 77.9 and 77.5 *kcal/mol* (CBS-QB3), respectively.

According to the energy profiles in Fig. 2, the S-N rotational barrier of MeSNO is the highest among the four SNO species (12 *kcal/mol*). The nitrogen inversion of RS-N·-OH is facile with a small barrier height (<2 *kcal/mol*), but the S-N rotation requires 8-9 *kcal/mol* (**7**). The thionitroxide radical, RS-NH-O·, has a shallow C-S-N-O potential energy surface. The RS-NH-OH has S-N rotational barrier of 7 - 8 *kcal/mol* in the gas phase and water.

The *S*-nitrosothiol itself, RSNO, is the least probable for the crystallographic SNO species, not only due to the large barrier of 12 *kcal/mol* needed to distort into the observed geometry, but also due to the long S-N distance (2.08 *Å*, **6**) that deviates substantially from the observed S-N bond length (1.76 *Å*). Similarly, the geometry of the nitrogen radical RS-N·-OH that corresponds to the crystallographically determined geometry is an energy maximum, 7 *kcal/mol* above the planar minimum, and with a N-O bond length of 1.37 *Å*.

The observed twisted SNO species is most likely to be a thionitroxide radical, RS-NH-O·. Geometrical parameters, like the N-O bond length (*ca.* 1.27 *Å*), the S-N bond length (*ca.* 1.73 *Å*), the C-S bond length (*ca.* 1.82 *Å*), C-S-N bending angle (*ca.* 100°), and C-S-N-O dihedral angle (*ca.* 72°), are all in good agreement with the observed SNO structures (N-O: 1.21 *Å*; S-N: 1.76 *Å*; C-S: 1.84-1.86 *Å*; C-S-N: 101-103°; C-S-N-O: -76, 88°).[4] Furthermore, the shallow potential surface of thionitroxide radical is consistent with the considerable

difference between two observed C-S-N-O dihedral angles. The fully reduced species, RS-NH-OH, also has a dihedral angle matching that of the crystal structure, but with a longer N-O bond length of 1.45 Å.

Persistent nitroxide radicals, such as TEMPO, have been used as spin-labels for biophysical probes[15] and as stable intermediates in living radical polymerizations.[16] Numerous compounds containing the nitroxide radical have been synthesized and characterized by EPR, including thionitroxides generated from reactions of nitroso-compounds and thiyl radical.[17] These *N*-aryl or alkyl, *N*-thioalkyl nitroxides undergo bimolecular decompositions, a pathway not available in protein-bound species. Examples of thionitroxides with a proton on nitrogen have not been isolated, but probably already detected by McMahon *et al*.[17c]

The thionitroxide could be responsible for some of the biological effects attributed to *S*-nitrosothiols.[18] In particular, when NO· concentration increases, thionitroxide formation will be enhanced; thionitroxides can be readily reversible carriers of NO·. MeSH + NO· → MeS-NH-O· is endothermic by about 10 *kcal/mol* in the gas phase, but thermoneutral in aqueous solution or potentially in the hydrogen-bonding environment of a protein such as hemoglobin. Furthermore, the reaction MeS-NH-O· + NO· → MeSNO + HNO, is feasible;[19] thionitroxide could be a precursor of *S*-nitrosothiol when excess NO is present.

The conclusion that thionitroxides are formed from proteinic cysteines when NO is present at appreciable pressure suggests a variety of ways that thionitroxides might be involved in the already remarkably diverse chemistry of NO.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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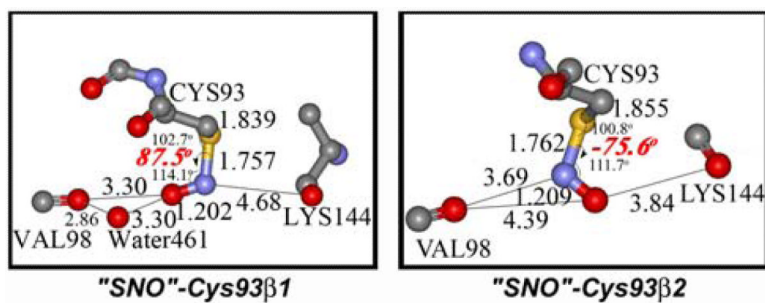
Figure 1.
The crystallographic C-S-N-O structures in purported *S*-nitroso-hemoglobin (distances in angstrom, unexpected dihedral angles in red).
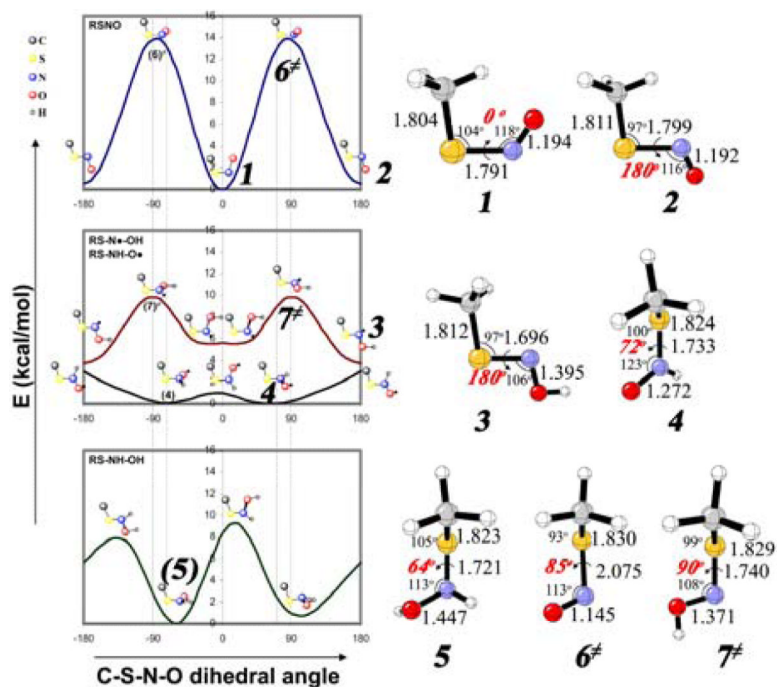
Figure 2.
Rotational energy profiles of RSNO, RS-NH-O·, RS-N·-OH, and RS-NH-OH (R = Me) with the B3LYP/6-31+G* method (two dashed lines stand for the dihedral angles of the observed SNO species in the proteins), and the CBS-QB3 calculated global minima of *cis*-RSNO (**1**), *trans*-RSNO (**2**), RS-N·-OH (**3**), RS-NH-O· (**4**), and RS-NH-OH (**5**), and rotational transition states for RSNO (**6**$^{\neq}$) and RS-N·-OH (**7**$^{\neq}$).