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Thionitroxides, RSNHO•: The Structure of the SNO Moiety in "*S*-Nitrosohemoglobin", A Possible NO Reservoir and Transporter

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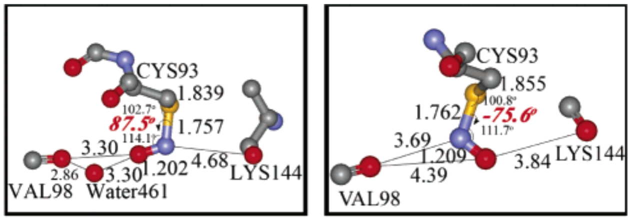
Considerable evidence suggests that the *S*-nitrosation of cysteine residues, thought to regulate the activities of many proteins, is involved in the uptake and intracellular trafficking of NO.[1] Indeed, to absorb the neutral NO with thiols, a redox reaction is needed to form *S*-nitrosothiols (RSNOs).[1−3]

A structure of *S*-nitrosocysteine in hemoglobin proteins was first reported by Arnone and co-workers in 1998.[4a] Interestingly, the resulting protein structure obtained under nonbiological conditions (NO pressure, anaerobic) exhibits unexpected C−S−N−O dihedral angles of 88 and −76° for Cys93 of $\beta1$ and $\beta2$ subunits, respectively. 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-Cys98(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-Cys93(F9)$\beta1$ and SNO-Cys93(F9)$\beta2$, respectively (Figure 1).[4b] To explain this, they proposed that a N-centered radical, Cys−S−N•−OH, was present and stabilized by hydrogen bonding with Val98. Such a radical was previously proposed as an intermediate in reactions of NO with thiols.[7] However, the SNO moiety in the Cys93(F9)$\beta2$ 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 Lys144 is about 3.84 Å, a distance too long for significant OH···O hydrogen bonding interactions.



"SNO"-Cys93β1 **"SNO"-Cys93β2**

Figure 1. The crystallographic C−S−N−O structures in purported *S*-nitrosohemoglobin (distances in angstroms, unexpected dihedral angles in red).

By contrast, Montfort and co-workers 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 $\beta1$ and $\beta2$ 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 Figure 2. Relative energies and selected geometrical parameters of energy minima and transition states, with CBS-QB3 and G3, are collected in Supporting Information.

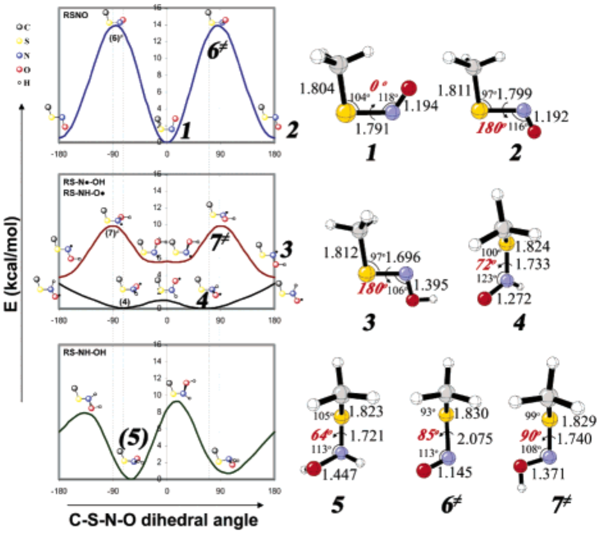


Figure 2. Rotational energy profiles of RSNO, RS−NH−O•, RS−N•−OH, and RS−NH−OH (R = Me) with the B3LYP/6-31+G* method (two dashed lines represent the dihedral angles of the observed SNO species in the proteins), and the CBS-QB3 calculated global minima of *cis*-RSNO (**1**), *trans*-RSNO (**2**), RS−N•−OH (**3**), RS−NH−O• (**4**), and RS−NH−OH (**5**), and rotational transition states for RSNO (**6**) and RS−N•−OH (**7**).

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,

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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. $RS-N^{\bullet}-OH$ and $RS-NH-O^{\bullet}$ are less stable than the stable radical, TEMPO$^{\bullet}$ (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 Figure 2, the S−N rotational barrier of MeSNO is the highest among the four SNO species (12 kcal/mol). The nitrogen inversion of $RS-N^{\bullet}-OH$ is facile with a small barrier height (<2 kcal/mol), but the S−N rotation requires 8−9 kcal/mol (**7**). The thionitroxide radical, $RS-NH-O^{\bullet}$, 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 $RS-N^{\bullet}-OH$ 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, $RS-NH-O^{\bullet}$. Geometrical parameters, such as 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), the C−S−N bending angle (ca. 100°), and the 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$^{\bullet}$ concentration increases, thionitroxide formation will be enhanced; thionitroxides can be readily reversible carriers of NO$^{\bullet}$. $MeSH + NO^{\bullet} \rightarrow MeS-NH-O^{\bullet}$ is endothermic by about 10 kcal/mol in the gas phase but is thermoneutral in aqueous solution or potentially in the hydrogen-bonding environment of a protein, such as hemoglobin. Furthermore, the reaction $MeS-NH-O^{\bullet} + NO^{\bullet} \rightarrow MeSNO + HNO$ 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.

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Supporting Information Available: Complete ref 9, the NO-modified hemoglobin, and the detailed structures and energetics of stationary points on potential energy surfaces of the four SNO species. This material is available free of charge via the Internet at http://pubs.acs.org.

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