Reaction of HSSS· radical with guanine and formation of 8-thioguanine: a computational study

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Abstract
Density functional calculations have been applied to study reactions of the HSSS· radical with guanine at the C2, C4, C5, and C8 sites of guanine that yields [GCn-SSSH] n = 2, 4, 5, and 8 adducts. Also, formation of 8-thioguanine from [GC8-SSSH]· adduct has been studied. In all the adducts [GCn-SSSH], HSSS· radical is placed on the top of the plane of the guanine ring. The results show that there is a possibility of intraresidue S-H…N hydrogen bonding in each [GCn-SSSH]· adduct. Besides, the C8 site of guanine is the most reactive site to add to HSSS· radical with appreciably high rate constant, and the adduct formed at this site would be significantly stable. According to NBO analysis, delocalization energies for all the hydrogen bondings, i.e., E(2) for nN⟶σ*SH interactions, are obtained to be in the range of 1.31–3.46 kcal mol⁻¹. The biggest E(2) and the largest amount of charge transfer is found for [GC8-SSSH]· adduct, i.e., for the S-H…N7 (Gua) interaction, in which R_N…H = 2.588 Å, to be 1.78 kcal mol⁻¹ and 0.0024e, respectively, well correlated with the prediction of most of its stability among the [GCn-SSSH]· adducts. The ZPE-corrected enthalpy changes for the reactions are obtained to be 0.2–1.4 kcal mol⁻¹. The reaction enthalpy changes and the barrier energies related to the different sites of guanine are found to be in the order of C8 < C2 < C5 < C4. Starting with the adduct [GC8-SSSH], 8-thioguanine could be achieved in two steps with two transition states (TS1 and TS2) and an intermediate complex (IM); the overall reaction is exothermic. The first reaction step, the rate-determining step, involves going from the adduct [GC8-SSSH]· to IM via TS1, in which HSS moiety gets dissociated from the HSSS moiety attached to the C8 site of guanine.

Keywords Guanine · HSSS Radical · Hydrogen bonding · NBO · DFT

Introduction
Sulfur is the third most important mineral in the body which plays an important role in biological processes [1]. Compounds containing “sulfane sulfur” have been suggested in regulation mechanisms [2]. Insulin which is an essential hormone in regulating the metabolism of carbohydrates, fats, and proteins involves disulfide bonds [3]. Moreover, sulfur is a multivalent nonmetallic element that is one of the most abundant elements in the Earth’s crust and even in the universe. Potentially, new sulfur supplies are ions and molecules related to planetary atmospheres and interstellar space, e.g., Venus and Io, in which thiozone S₃ and sulfanes H₂Sₖ are suggested as the most abundant supply of sulfur [4–10]. In atmospheric models of Venus, the reaction to produce sulfur is \( \text{H} + \text{S}_3 \rightarrow \text{HS} + \text{S}_2 \) [7]. Later, Wayne [11] suggested that in the Venusian atmosphere, S₃ may play a similar role to O₃. The mercapto HS and thiosulfeno HS₂ radicals are stable species [12, 13]. In 2008, Petris et al. [14] for the first time detected the HSSS radical, which is the sulfur analogue of HOOO', by mass spectroscopy in the gas phase. They found an open-chain bent structure for the HSSS radical, with an H atom pointing out of the SSS plane. Later, Grant et al. [15] theoretically investigated the dissociation of HSSS· radical by calculating bond dissociation energies for HS-SS, HSS-S, and HSSS-S. They found bond dissociation energy of HS-SS being 22.8 and 27.3 kcal mol⁻¹ less than those of H-SSS and HSS-S, respectively, indicating HS· radical is thermodynamically preferred to be formed. Shukla and Mishra [16] theoretically investigated radical addition and hydrogen abstraction...
reactions of HOOC radical with the guanine base of DNA and oxidation of guanine with HOOC radical to yield 8-oxoG. In the present contribution, we have investigated radical addition reactions of HSSS radical, as a sulfur analogue of HOOC, with guanine as well as the mechanisms of formation of 8-thioguanine using density functional theory to answer the following questions: (i) How reactive is HSSS radical towards guanine? (ii) Which site of guanine reacts with HSSS radical? (iii) What is the mechanism of its reaction with guanine? and (iv) Whether it can lead to the formation of 8-thioguanine?

**Computational details**

The equilibrium geometries of all the stationary points (reactants, products, intermediates, and transition states) are optimized at the restricted or unrestricted M06-2X/6-311 + G(d,p) level of theory in the gas phase. The M06-2X functional developed by Truhlar and Zhao [17] belongs to hybrid meta-generalized-gradient-approximation exchange-correlation functionals that include an accurate treatment of dispersion energy and reported to be a reliable density functional in the calculation of activation energies of reactions involving radicals and the hydrogen-bonded systems [18]. The single-point energy calculations were done in aqueous media using the polarizable continuum model (PCM) [19], at the same level of theory, where the solute molecule is placed into a cavity surrounded by the solvent as a continuum medium with a given dielectric constant. [19] The dielectric constant of H2O as the solvent is applied.

Vibrational frequency analysis at the M06-2X/6-311 + G(d,p) level of theory in the gas phase was performed on all the optimized species to ensure the genuineness of minima and TSs, as well as to determine their zero-point energy (ZPE) and enthalpy corrections at 298.15 K and 1 atm. Zero-point energy and enthalpy corrections were also applied to the total energies obtained by single-point calculations in both gas phase and aqueous media. All DFT calculations were performed using the GAMESS suite of programs [20, 21]. Wave functions calculated at the M06-2X/6-311 + G(d,p) level of theory were analyzed by the natural bond orbital (NBO) method [22, 23].

**Results and discussion**

It is previously shown that molecular structure of polyoxides such as hydrogen trioxide (HOOC) radical is very complicated; some methods like (SDQ) and QCISST(T) and density functionals like PBE0 and B3LYP predict cis-conformer to be the most stable [16, 24]. Therefore, finding the preferred conformer of HSSS radical is an important step.

To this aim, the dihedral angle S1S2S3H3 is changed from 0° to 360° by the step size of 5 degree, and then relative stabilities are investigated through the calculation of relative energies of the conformers of HSSS radical obtained in each step. For illustration, the relative energies are depicted versus the dihedral angles $\theta$ in Fig.1. As seen relative energy gradually decreases from $\theta = 0^\circ$ to 0.00 kcalmol$^{-1}$ in HSSS with $\theta = \pm76.15^\circ$. Therefore, it seems that the bent structure of SSS with the H atom pointing out of the SSS plane with S1S2S3H3 dihedral angle $\theta = 76.15^\circ$ is the preferred conformer of HSSS radical; see Fig.2.

The standard atomic numbering scheme for guanine has been followed, as shown in Fig. 2, to study the addition of HSSS radical to C2, C4, C5, and C8 sites of guanine:

$$\text{GCn} + \text{HSSS} \rightarrow [\text{TSn}] \rightarrow [\text{GCn-SSSH}], \text{where n} = 2, 4, 5, \text{and 8.}$$

The geometrical characteristics of optimized structures of guanine at the M06-2X/6-311 + G(d,p) level of theory are in excellent agreement with the previously reported experimental values. The optimized structures of the adducts [GCn-SSSH], formed by the addition of HSSS radical to the different sites of guanine, and their stationary points (transition states) are shown in Fig. 3. As seen in all the adducts [GCn-SSSH], the HSSS radical is placed above the plane of the rings of guanine, and the S1-Cn ($n = 2, 4, 5, 8$) distances are found to be in the range of 1.834–1.880 Å. The plane of rings is distorted due to binding to the HSSS radical.

It is known that hydrogen bonding formation is probable when the distance of H…X, X = F, O, or N is shorter than the sum of their van der Waals radii. Therefore, there is a possibility of intraresidue SH…N hydrogen bonding in the adducts [GCn-SSSH], as S-H…N9 (Gua), S-H…N7 (Gua), S-H…N3 (Gua), and S-H…N7 (Gua) in [GC2 − SSSH], [GC4 −...
SSSH], [GC5 − SSSH], and [GC8 − SSSH], respectively, because their lengths are obtained to be in the range of 2.188–2.559 Å which is shorter than the sum of their van der Waals radii of nitrogen and hydrogen (2.72 Å). However, based on Jeffrey’s classification [25], hydrogen bondings with the bond lengths (H…X) larger than 2.2 Å are considered as weak bonds. It would be interesting to conduct NBO analysis which is a better alternative to investigate hydrogen bondings than geometrical parameters. In terms of NBO analysis theory [22], S-H…N hydrogen bonding can be attributed to the delocalization of electron density from the occupied lone pair of the electron donor N (nN) into the unoccupied antibond orbital of electron acceptor S-H (σ*SH). The strength of the S-H…N hydrogen bonding interactions could be assessed by delocalization energies, E(2), estimated by second-order perturbation theory [23]. Table 1 lists the calculated delocalization energies, E(2), as well as the amount of transferred charge (q_nN → σ*SH) and occupancies (e) of nN (oxygen lone pair) and σ*SH (SH antibond). As seen, the delocalization energies obtained for nN → σ*SH interactions of all the mentioned intraresidue hydrogen bondings range from 1.26 to 1.77 kcalmol⁻¹. One can notice that the largest value of E(2) (1.77 kcalmol⁻¹) is obtained for the adduct of [GC8 − SSSH], i.e., for the interaction of S-H…N7(Gua) with R_N…H = 2.188 Å which forms one six-membered ring. The next one belongs to E(2) (1.62 kcalmol⁻¹) of S-H…N9(Gua) hydrogen bonding with R_N…H = 2.390 Å in [GC2 − SSSH]. It has been shown by extensive NBO studies that nN → σ*SH charge transfer (CT) plays a unique role in the energetic stabilization of hydrogen-bonded systems [26]. According to this theoretic basis, the amount of charge transfer between acceptor-donor atoms can be used to evaluate the strength of hydrogen bonding; the larger the amount of CT, the stronger the hydrogen bonding. For example, charge transfers q_nN → σ*SH S-H…N7(Gua) in [GC8 − SSSH] and [GC4 − SSSH] are obtained to be 0.0024e and 0.0019e, respectively, suggesting that the former leads to stronger hydrogen bonding interaction.

Some structural parameters of the transition states obtained for these reactions are also shown in Fig. 3. The S1–S2 and S2–S3 bond lengths in TSs are found to be in the range of 2.041–2.170 Å and 2.030–2.100 Å, respectively, and the S1-Cn (n = 2, 4, 5, 8) bond lengths lie in the range of 2.211–2.240 Å. The ring plane of guanine in TSs gets distorted as a result of binding to HSSS radical. The potential profiles of addition of HSSS radical to Cn sites, n = 2, 4, 5, 8, of guanine are depicted in Fig. 4, which includes the calculated ZPE-corrected enthalpy changes (kcal/mol) and the corresponding barrier energies (E_b in kcal/mol) of the addition reactions. The results revealed that only the adduct, [GC8-SSSH], formed due to the addition of HSSS radical to C8 site is

![Fig. 2](image-url) The standard atomic numbering scheme for guanine and trisulfanyl radical

![Fig. 3](image-url) The optimized structures of the adducts [GCn-SSSH], formed by the addition of HSSS radical to the different sites of guanine and their stationary points (transition states). The geometrical parameters are shown in Å.
thermodynamically favorable as the corresponding ZPE-corrected enthalpy change is negative ($-15.36$ kcal/mol), while the enthalpy changes related to the other $[GCn-SSSH]$ adducts are obtained to be appreciably positive; see Fig. 4. The $E_b$ values for the addition reactions are found to be $9.15$–$49.97$ kcal/mol, which follow the order of $C8 < C2 < C5 < C4$, similar to the order found for enthalpy changes. It is noted that the $E_b$ corresponding to the addition to $C8$ site is significantly smaller (by $\sim 15$ kcal/mol) than the value found for the addition to $C2$ site. The rate constant ($=K_b T/h e^{-E_b/RT}$) is predicted to be $1.20 \times 10^6$ s$^{-1}$, indicating the addition to $C8$ site would occur appreciably fast. All the results show that the $C8$ site is the most reactive site for the addition reaction with HSSS radical, in accordance with the theoretical results of Jena and Mishra [24], Kumar et al. [27], and Bhattacharjee and Shukla [28] on the reactions of the OH, OOH, and OOOH radicals with guanine.

Starting from $[GC8-SSSH]$, as the most stable adduct, we now intend to investigate the formation of 8-thioguanine. Two steps are considered passing through two transition states (TS1 and TS2) and an intermediate complex (IM). The optimized geometries of the intermediate (IM), transition states (TS1 and TS2), and the product (8-thioguanine) along with some geometric parameters are shown in Fig. 5. The first step involves the adduct $[GC8-SSSH]$ transferring to TS1 and then to IM, in which the hydrogen atom of HSSS moiety moves and is attached to the N7 site of guanine. HSS of the HSSS moiety attached to the C8 of guanine is getting distant from SC8(G), so that HSS2-S1C8(G) distance in TS1 stretches to $2.472$ Å and is eventually broken in IM. So, in IM an open ring derivative of guanine is formed, in which a sulfur atom is attached to the C8 site, the N9C8 bond is broken, and the N9 site is complexed with HSS moiety through hydrogen bonding. In the second step, the intermediate IM, in which abstracts the H8 atom of guanine derivative, forming 8-thioguanine radical and H$_2$S$_2$, transforms to TS2, in which the C8-H8 and H8-SSH distances become $1.277$ and $2.084$ Å, respectively, finally to the products. The barrier energies for the first and second steps are calculated to be $56.07$ and $22.14$ kcal/mol, respectively, indicating that the first step is a determining step. The overall reaction of the formation of 8-thioguanine radical from the adduct $[GC8-SSSH]$ is exothermic by $14.99$ kcal/mol.

### Conclusion

This density functional investigation has been carried out on the reactions of HSSS radical with different sites of guanine, suggesting that $[GC8-SSSH]$ is the most stable adduct. Then a suggested mechanism was studied for the formation of 8-thioguanine from the adduct $[GC8-SSSH]$. According to the obtained results, the following points are emphasized. First, it is found that in the preferred conformer of HSSS radical, the H atom points out of the bent plane of SSS where the S1S2S3H3 dihedral angle is $\theta = 76.15^\circ$. Second, there is a

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**Table 1** NBO analysis of donor–acceptor interactions in adducts $[GCn-SSSH]$ showing stabilization energy $E^{(2)}$ values (kcal/mol), charge transfer $Q_{nN} \rightarrow \sigma^*_{SH}$, and occupancies ($e$) of $nN$ (nitrogen lone pair) and $\sigma^*_{SH}$ (SH antibond) NBOs.

| Adduct     | Hydrogen bonding | $R$ (SH…N)/Å | $E^{(2)}$ (kcal/mol) | $Q/e$ | Occu. acceptor | Occu. donor |
|------------|------------------|--------------|----------------------|-------|----------------|-------------|
| $[GC2-SSSH]$ | S-H…N9           | 2.390        | 1.62                 | 0.0029| 0.0213         | 1.9786      |
| $[GC4-SSSH]$ | S-H…N7           | 2.559        | 1.26                 | 0.0023| 0.0134         | 1.9869      |
| $[GC5-SSSH]$ | S-H…N3           | 2.456        | 1.44                 | 0.0026| 0.0191         | 1.9810      |
| $[GC8-SSSH]$ | S-H…N7           | 2.188        | 1.77                 | 0.0035| 0.0245         | 1.9759      |

Fig. 4  Schematic potential energy for the addition of HSSS radical to C2, C4, C5, and C8 sites of guanine.

Fig. 5  Optimized geometries of the intermediate (IM), transition states (TS1 and TS2), and the product (8-thioguanine) along with some geometric parameters.
possibility of intraresidue S-H...N hydrogen binding in all the [GCn-SSSH] adducts, based on geometrical parameters. Third, the strength of the suggested intraresidue S-H...N hydrogen bindings was inspected based on the delocalization energies $E^{(2)}$ and charge transfers for $n_N \rightarrow \sigma_{SH}^*$ which were obtained from NBO analysis. The largest value of $E^{(2)}$ (1.77 kcalmol$^{-1}$) and charge transfer (0.0035e) was obtained for the interaction of S-H...N7(Gua) in the adduct of [GC8-SSSH]. Forth, for this adduct, i.e., the addition of SSSH radical to the C8 site of guanine, ZPE-corrected enthalpy change is obtained to be negative, and the corresponding $E_b$ is appreciably smaller than that for the other adducts. Fifth, the formation of 8-thioguanine from the [GC8-SSSH] adduct occurs in two steps via two transition stats and an intermediate complex. Finally, while the formation of 8-thioguanine from the adduct [GC8-SSSH] is exothermic, the first step, i.e., going to the intermediate is a rate-determining step.

Fig. 5 Formation of 8-thioguanine radical from the adduct [GC8-SSSH]

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the manuscript.

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