

COMPUTATIONAL STUDY OF MOLECULAR STRUCTURES AND ANTIOXIDANT MECHANISM OF OVOTHIOLS

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ABSTRACT

In this paper, the molecular structure and antioxidant activity of ovothiols (OSH) have been studied by using four DFT functionals, namely B3LYP, B3PW91, X3LYP, M06 with the basis set of 6-311++G(2df,2p). Two major antioxidant mechanisms, namely, hydrogen atom transfer (HAT) and stepwise electron transfer-proton transfer (SET-PT) have been investigated and applied on three optimized conformations of ovothiols. Bond dissociation enthalpy (BDE), vertical ionization energy (IE), proton dissociation enthalpy (PDE), chemical potential (μ), chemical hardness (η) and global electrophilicity (ω), have been calculated and discussed in the gas phase.

Keywords: ovothiols, antioxidant, HAT, SET-PT, BDE, IE, PDE, density functional theory.

1. INTRODUCTION

Sulfur containing functional groups have been found to play an important in both primary and secondary metabolites [1]. The maintenance of cellular redox homeostasis, the maintenance of protein thiol-disulfide ratios and the protection of cells from reactive oxygen species occur under the presence of thiols in living systems. Ovothiols (1-methyl-4-mercaptohistidines) are present in millimolar concentrations in sea urchin eggs [2]. Such high concentrations of these heterothiols might be needed to protect the fertilized sea-urchin egg from free radical induced damage. Recently, there has been a renewed interest in ovothiols arising from the identification and characterization of a 5-histidylcysteine sulfoxide synthase (OvoA), the enzyme that catalyzes the first step of their biosynthesis [3]. Experimentally, OSH has been proposed to be one of the more powerful natural antioxidants and to be comparable to that of ascorbic acid and water-soluble vitamin E analogue [3]. Although, the scavenging reactions of radicals dissociated from ovothiols were investigated by pulse and gamma radiolysis techniques, how do ovothiols act as a H-atom and/or electron donors need to be illuminated. Obviously, the antioxidant activity

of ovothiols was experimentally determined and can be predicted based on their optimized structures and the proved mechanisms. To the best of our knowledge, detailed computational study for antioxidant activity of OSH based on hydrogen atom transfer (HAT) and stepwise electron transfer-proton transfer (SET-PT) have not been reported elsewhere, encouraging us to fully examine the antioxidant reactivity of OSHs using DFT methods.

2. THEORETICAL MODELS AND COMPUTATIONAL METHODS

Antioxidants are functionally divided into two major groups, preventive and chain-breaking, according to whether they reduce the rate of chain-initiation or capture the alternating alkyl (R^\bullet) and peroxy (ROO^\bullet) radicals responsible for the oxidative propagation in organic matter. The mechanism of hydrocarbon autoxidation and antioxidant protection is illustrated in Figure 1 [4].

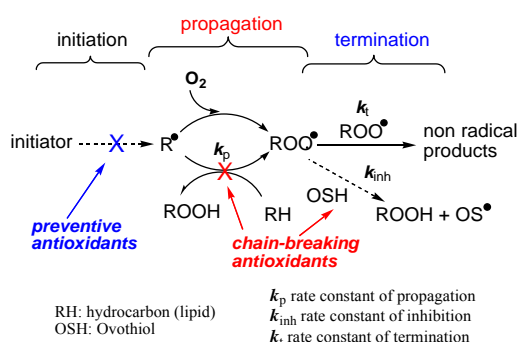


Figure 1. Simplified mechanism of hydrocarbon autoxidation and antioxidant protection.

According to the reaction pathways of antioxidant protection in Figure 2, two acceptable mechanisms are described hereafter:

i) *Hydrogen atom transfer (HAT) mechanism*: In this mechanism, the antioxidant capacity of ovothiols (OSH) can be explained via the hydrogen transfer process (**R1**) to the reactive oxygen/nitrogen species. The bond dissociation enthalpy of S-H bond is a key parameter.



ii) *Single electron transfer followed by -proton transfer (SET-PT)*



In Reaction **R2**, ionization energy (IE) of ovothiols (OSH) is a key thermo-parameter. The experimental and theoretical data have proved that the first step in this mechanism is significant to control the rate of reaction. Reaction **R3** is characterized by proton dissociation enthalpy (PDE).

All thermoparameters (BDE, IE and PDE) are calculated *via* the reaction enthalpy in the gas phase at 298.15 K and 1 atm of each reaction from **R1** to **R3**.

$$\text{BDE}(\text{S-H}) = H_f(\text{OS}^\bullet) + H_f(\text{H}^\bullet) + H_f(\text{OSH}) \quad (1)$$

$$\text{IE}(\text{OSH}) = H_f(\text{OSH}^{*+}) + H_f(e^-) - H_f(\text{OSH}) \quad (2)$$

$$\text{PDE} = H_f(\text{OS}^\bullet) + H_f(\text{H}^+) - H_f(\text{OSH}^{*+}) \quad (3)$$

where H_f is the total enthalpy of the studied species at the temperature of 298.15 K and usually estimated from the expression below:

$$H_f = E_0 + ZPE + H_{trans} + H_{rot} + H_{vib} + RT. \quad (4)$$

The H_{trans} , H_{rot} , and H_{vib} are the translational, rotational, and vibrational contributions to the enthalpy, respectively. E_0 is the total energy at 0 K and ZPE is the zero-point vibrational energy. Finite differences method proposed by Parr et al. allows approximating chemical potential as $\mu = -\frac{1}{2}(IE + EA)$, chemical hardness as $\eta = \frac{1}{2}(IE + EA)$ and global electrophilicity as $\omega = \frac{\mu^2}{2\eta}$ [5].

All computational calculations were performed by using Gaussian 09 program [6]. The structures of three OSHs, the corresponding radicals and anions were fully optimized by using the B3LYP/6-311G(d,p) level of theory. Single point calculations were carried out using several DFT methods at the basis set of 6-311++G(2df,2p). Restricted open shell (RO) procedures were applied for radical species at the same methods.

3. RESULTS AND DISCUSSION

3.1. Optimized structure of ovothiols

Ovothiols with chemical formula of $C_7H_8O_2N_2SNH_{3-x}(CH_3)_x$ ($x = 0, 1, 2$) are abbreviated as OSH_A, OSH_B and OSH_C respectively. It should be mentioned that there are more than one conformation for each ovthiol. Due to this reason, the calculated thermoparameters depend on the optimized conformations that we obtained. Therefore the examination for finding the most stable conformation for each OSH is necessary.

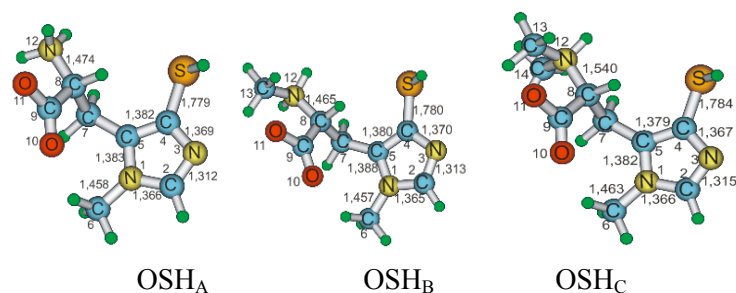


Figure 2. Optimized structures of ovothiols at the B3LYP/6-311G(d,p) level of theory.

The structure conformations described in Figure 2 correspond to the most stable one of OSH_A or OSH_B or OSH_C. The hydrogen bond between oxygen of carbonyl and amino groups has been observed for all three OSHs. This leads to conclude that three optimized conformations given in Figure 2 are considered as the most stables ones. By comparing the bond lengths in three conformations, we recognized that the replacement of one or two methyl groups (CH_3) for H of NH_3 group, the deviations of bond lengths in the five membered ring are not significant.

3.2. Antioxidants of ovothiols

3.2.1. HAT mechanism

By examining the structures of three OSHs, one can recognize that although there are several X-H bonds in OSHs, but only two bonds namely, S-H and C2-H at the imidazole ring need to be evaluated their BDEs. The comparison of bond strength between S-H and C2-H

bonds for three OSHs using the B3LYP/6-311++G(2df,2p) level of theory shows that S-H bond strength is the more weaker than C2-H in twice. For example, in case of OSH_A, BDE(S-H) and BDE(C2-H) are 72.8 kcal/mol and 117.69 kcal/mol, respectively. To ensure the reliability of the calculated results, four DFT functionals namely, B3LYP, B3PW91, X3LYP and M06 were applied for determining the BDE(S-H) of three OSHs. The results are given in Table 1.

Table 1. Calculated BDE(S-H) of ovothiols using four DFT functionals in kcal/mol.

Compounds	B3LYP	B3PW91	X3LYP	M06
OSH _A	72.18 (0.00)	73.40 (1.22)	70.72 (-1.46)	71.95 (-0.27)
OSH _B	73.55 (0.00)	74.78 (1.23)	72.10 (-1.45)	73.62 (0.07)
OSH _C	69.87 (0.00)	70.98 (1.11)	68.46 (-1.41)	69.78 (-0.09)
Data in parenthesis: $\Delta BDE = BDE(S-H)_{DFT} - BDE(S-H)_{B3LYP}$				

The computed BDEs of (S-H) bond are consistent in four DFT functionals, the deviations among them are very slightly as can be seen in Table 3 (data in parenthesis). Among three ovothiols, the calculated BDE(S-H) can be arranged in the sequence: BDE(S-H) of OSH ovothiol C < BDE(S-H) of ovothiol A < BDE(S-H) of ovothiol B. It should be noted that the BDE(S-H)'s of the ovothiols are smaller than the BDE(O-H) of phenol (88.60 kcal/mol). As mentioned above, hydrogen atom transfer takes place at the weakest bond whose BDE value is the smallest.

3.2.2. SET-PT mechanism

SET-PT reactions are usually slow and require long times to reach completion, so antioxidant capacity calculations are based on percent decrease in product rather than kinetics. In general, the first reaction (**R1**) plays more important role than the second one (**R2**). The calculated data for IE and PDE using DFT/6-311++G(2df,2p) are presented in Tables 2 and 3. The result at the PM6 is also given for the sake of the comparison.

Table 2. Calculated IE in kcal/mol at different DFT/6-311++G(2df,2p) and PM6 methods.

Ovothiols	B3LYP	B3PW91	X3LYP	M06	PM6
OSH _A	167.26 (0.00)	166.85 (-0.41)	166.43 (-0.16)	167.10 (0.64)	180.75 (13.48)
OSH _B	171.92 (0.00)	172.18 (0.26)	171.05 (-0.86)	171.98 (0.06)	177.57 (5.65)
OSH _C	172.17 (0.00)	172.14 (-0.03)	171.91 (-0.26)	174.01 (1.84)	179.67 (7.50)

Obviously, the results obtained by using B3PW91, X3LYP and M06 at the same basis set are quite similar with the deviations are smaller than 1 kcal/mol (See data in bracket of Table 2). The largest deviation of 1.84 kcal/mol was observed for OSH_C when using M06 method. IE value computed at PM6 method in in the last column is larger than IEs generating from DFT methods with amount of 6 to 13.5 kcal/mol. The lower IE value the higher antioxidant activity.

Data in Table 3 shows the calculated results at four DFT methods are converged and the PDE at S-H bond of three ovothiols can be placed in order: OSH_C \approx OSH_B < OSH_A.

Table 3. Calculated PDE(S-H) bond using DFT/6-311++G(2df,2p), kcal/mol.

Ovothiols	B3LYP	B3PW91	X3LYP	M06
OSH _A	205.93	207.15	204.48	205.66
OSH _B	235.56	236.79	234.11	235.63
OSH _C	204.71	205.82	203.30	204.62

3.2.3. Antioxidant mechanism and chemical reactivity of ovothiol radicals

As mentioned above, the question raised here that do ovothiols act as an H-atom and/or electron donors. To answer this question, two factors need to be analyzed ($\Delta\text{BDE} = \text{BDE}(\text{S-H})_{\text{ovothiol}} - \text{BDE}(\text{O-H})_{\text{phenol}}$ and $\Delta\text{IE} = \text{IE}_{\text{ovothiol}} - \text{IE}_{\text{phenol}}$) in order to decide which mechanism is favor [7]. The BDE gap of OSH_A, OSH_B and OSH_C referred to BDE(O-H) of phenol of 88.30 ± 0.70 kcal/mol are -16.12, -14.75 and -18.43 kcal/mol. In comparison with IE of phenol at the PM6 (193.4 kcal/mol), the difference between IE of three OSHs and phenol are within the range of -12 to -16 kcal/mol. Relative reactivity in HAT is determined by the BDE of the H-donating group in the potential antioxidant, dominating for compounds with the absolute values of ΔBDE are around 15 kcal/mol and the absolute values of ΔIE are not larger 36 kcal/mol [7].

Moreover, to predict the antioxidant capacity of three OSHs, we also examined the reactivity of the radicals formed from ovothiols by hydrogen removal at S-H bond via the global and local theoretical reactivity descriptors such as chemical potential (μ), chemical hardness (η) and global electrophilicity (ω). The estimated values of these global descriptors are shown in Table 4.

Table 4. Global reactivity descriptors in kcal/mol.

Ovothiol radicals	μ	η	ω
OS [•] _A	-140.11	64.82	151.43
OS [•] _B	-121.46	60.60	122.46
OS [•] _C	-132.44	64.45	136.08

In principle, the compound with the lowest value of hardness (η) and global electrophilicity (ω) is predicted to have the highest reactivity. Comparing the data from Table 4, the OS[•]_B and OS[•]_C radicals possess η and ω lower than that of OS[•]_A, as a result they display less reactivity. This leads to the same conclusion stated above.

4. CONCLUSIONS

In this work, the antioxidant activity of OSH_A, OSH_B and OSH_C has been computationally. Several thermo-parameters including BDE, IE, PDE and quantum chemical indexes η , ω and μ were calculated using DFT/6-311++G(2df,2p) basis sets show that the antioxidant capacities of OSH_A and OSH_C are better than that of OSH_B. In terms of theoretical reactivity descriptors, the radicals generated from OSH_A and OSH_C are more stable than from OSH_B. The analysis of BDE and IE have led to a conclusion that for ovothiols, HAT is a major antioxidant mechanism and the BDE(S-H) for OSHA, OSHB, OSHC are 72.18, 73.55 and 69.87 kcal/mol.

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TÓM TẮT

NGHIÊN CỨU BẰNG PHƯƠNG PHÁP TÍNH TOÁN CẤU TRÚC VÀ CƠ CHẾ CHỐNG OXY HÓA CỦA CÁC HỢP CHẤT OVOTHIOL

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Trong bài báo này, cấu trúc phân tử và hoạt tính chống oxy hóa của các ovothiol (OSH) đã được nghiên cứu bằng cách sử dụng bốn hàm mật độ DFT là B3LYP, B3PW91, X3LYP, M06 tại bộ hàm cơ sở 6-311++G(2df,2p). Hai cơ chế chống oxy hóa chính bao gồm cơ chế chuyển hydro (HAT) và cơ chế chuyển tuần tự điện tử - chuyển proton (SET-PT) đã được nghiên cứu và áp dụng trên ba cấu trúc tối ưu của ovothiol. Năng lượng phân li liên kết (BDE), năng lượng ion hóa (IE), năng lượng phân li proton (PDE), thế hóa học (μ), độ cứng hóa học (η) và ái lực điện tử toàn phần (ω), đã được tính toán và thảo luận đối với các phân tử ovothiol trong pha khí.

Từ khóa: ovothiols, chống oxy hóa, HAT, SET-PT, BDE, IE, PDE, density functional theory.