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ANTIOXIDANT PROPERTIES OF FOLIC ACID: A DFT STUDY

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ABSTRACT

Antioxidant activity of folic acid (FA) was investigated via two main antioxidant mechanisms: hydrogen atom transfer (HAT) and single electron transfer (SET) by Density Functional Theory (DFT) at the DFT/B3LYP/6-311G(d,p) level of theory. The characterizing thermochemical properties such as bond dissociation enthalpies (BDEs), ionization energy (IE) and electron affinity (EA) were calculated in the gas phase. Analyses of HOMOs distribution show that the positions which easily donate electrons to free radicals are essentially found at C ring, while the positions which easily accept electrons (LUMOs) from radicals are mainly situated at B ring. The lowest BDE values equal to 76.5 and 80.1 kcal/mol are obtained at C19–H and C9–H position, respectively. It is showed that FA represents a potential antioxidant via HAT mechanism. In addition, the IE and EA values calculated in the gas phase are equal to 5.45 eV and 1.87 eV, respectively. This result shows that FA has higher ability to donate electrons into free radicals, while the ability to receive electrons is quite weak. Finally, the HAT reaction between FA and reactive radical like HOO• was also calculated at the same level of theory. This provides more insight into its mechanism on free radicals scavenging.

Keywords: folic acid, antioxidant, density functional theory, HAT, SET.

1. INTRODUCTION

Folic acid (FA) (Fig. 1) is one of eight vitamins B that are essential for many bodily functions, especially in creating new cells and maintaining them [1]. Folate deficiency causes metabolic abnormalities, which can lead to chronic diseases and developmental disorders including neural tube defects in fetus [2]. In addition, FA is used in the treatment of cardiovascular, hematological, neurological and cancer diseases.

Among its biological activities, it has been showed that FA also has potential antioxidant properties by experimental and computational works. Indeed, Shen et al. [3] calculated bond dissociation enthalpies (BDEs) and ionization energy (IE) of different folate models to evaluate their antioxidant potential. As a result, BDE values are varied from 68.6 to 100.5 kcal/mol and

IE from 102.88 to 146.65 kcal/mol. The author argued that folates are efficient antioxidants especially in reduced forms, with strong ability to scavenge several radicals (such as DPPH$^\bullet$ radical) and protect lipid from peroxidation [3]. Ji et al. [4] elucidated the relationship between the structure and activity of the folate derivatives: tetrahydrofolate (4-HP) and 5-methyltetrahydrofolate (5-HP). DFT calculations were performed at the B3LYP/6-31+G (3,pd) level of theory. Results show that the smallest BDE of the 4-HP are 81.8 kcal/mol and the smallest IPs of the 4-HP are 143.5 kcal/mol.

Thus, the main goal of this study is to investigate the antioxidant capacity of folic acid and to elucidate the dominant antioxidant mechanism via H-atom transfer (HAT) and single electron transfer (SET) mechanism. The thermochemical properties including bond dissociation enthalpy (BDE), ionization energy (IE), electron affinity (EA) will be calculated in detail at the B3LYP/6-311G(d,p) level of theory in the gas phase. In addition, the potential energy surface of reaction between HOO$^\bullet$ radical with FA will also be established.

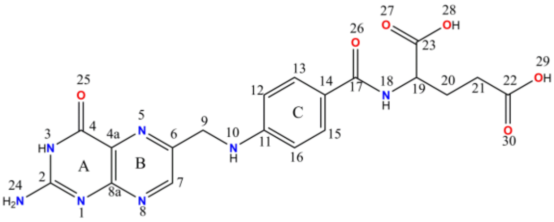

Figure 1. Structure of folic acid with numbered atom sites.

2. COMPUTATIONAL DETAILS

Gaussian 09 revE.01 package is employed for all calculations [5]. The geometry optimization and vibrational frequency calculation are performed at the B3LYP/6-311G(d,p) level of theory. Two antioxidant mechanisms are considered including Hydrogen atom transfer (HAT) and single electron transfer (SET). These two mechanisms consist in the common ones that have been found in a large number of potential antioxidant compounds [6, 7]:

HAT mechanisms are characterized by bond dissociation enthalpies (BDE). BDE values are calculated in the gas phase [8, 9]:

$$\text{R–H} \rightarrow \text{R}^\bullet + \text{H}^\bullet \quad || \quad \text{BDE(R–H)} = H(\text{R}^\bullet) + H(\text{H}^\bullet) - H(\text{R–H})$$

SET mechanisms are characterized by ionization energies (IE) and electron affinities (EA) [8, 9]:

$$\text{R–H} \rightarrow \text{RH}^{+\bullet} + e^- \quad || \quad \text{IE} = H(\text{RH}^{\bullet+}) + H(e^-) - H(\text{R–H})$$

$$\text{R–H} + e^- \rightarrow \text{RH}^{\bullet-} \quad || \quad \text{EA} = H(\text{RH}^{\bullet-}) + H(e^-) - H(\text{R–H})$$

where H is the total enthalpy of the studied species at 298.15 K, 1 atm.

Full-Electron Donor-Acceptor Map (FEDAM) is also established based on pairs of RIE and REA values of the considered compounds to evaluate the electron transfer reactions between FA and free radicals. RIE and REA correspond to the relative values IE and EA in comparing with the ones of Na and F, respectively [10]. Finally, reaction enthalpies (ΔH) and Gibbs free energies (ΔG) of the SET reaction between FA and the HOO• radical are considered in order to study the associated energy evolution. The electron-donating/-accepting processes between folic acid (FA) and HOO$^\bullet$ radical may occur as follows [10]:

$$\mathrm{FA} + \mathrm{HOO}^{\bullet} \rightarrow \mathrm{FA}^{\bullet+} + \mathrm{HOO}^{-}$$

and ΔH and ΔG are calculated by the following equations:

$$\Delta \mathrm{H} = [H(\mathrm{FA}^{\bullet+}) + H(\mathrm{HOO}^{-})] - [H(\mathrm{FA}) + H(\mathrm{HOO}^{\bullet})]$$

$$\Delta \mathrm{G} = [G(\mathrm{FA}^{\bullet+}) + G(\mathrm{HOO}^{-})] - [G(\mathrm{FA}) + G(\mathrm{HOO}^{\bullet})]$$

3. RESULTS AND DISCUSSION

3.1. Structural and electronic properties

Figure 2a displays the optimized structure of FA calculated in gas phase at the B3LYP/6-311G(d,p) level of theory.

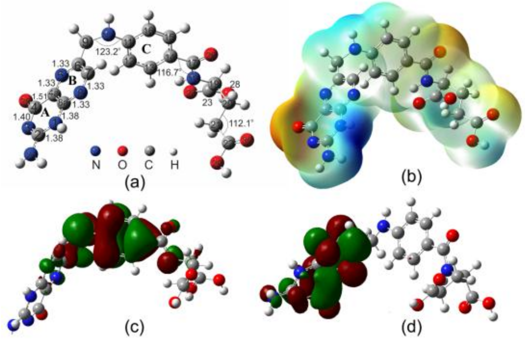

Figure 2. Optimal structure of folic acid (a), ESP (b), HOMO (c) and LUMO (d).

As can be seen, several double bonds are distributed along rings A, B, C to form a conjugated system that produces an electron-absorbing effect. Figure 2b shows electrostatic potential (ESP) maps of FA in three dimensional diagrams. Different colors represent different levels of electrostatic potential: the red one as the most negative electrostatic potential energy whereas the blue one as the most positive potential [11]. As a result, the most negative electrostatic potential regions are found at O-atom and N-atom positions. The blue area contains the C atoms of rings A, B. Figures 2c, 2d show distributions of frontier orbitals consisting of the highest occupied molecular orbitals (HOMOs) and the lowest occupied molecular orbitals (LUMOs) of FA. HOMOs are concentrated at the ring C of the molecule, and some ketone and amine groups (Figure 2c). Meanwhile, LUMOs are mainly found in the rings A and B (Figure 2d). Therefore, the electron transfer processes take place at rings.

3.2. Characteristic thermochemical properties for antioxidant acitivity

3.2.1. Antioxidant capacity via HAT mechanism

Bond dissociation enthalpy (BDE) of C–H and N–H values is calculated in the gas phase at the B3LYP/6-311G(d,p) level of theory. This result is showed in the Table 1. As can be seen, BDE is varied from 76.5 to 112.3 kcal/mol. The antioxidant capacity via HAT mechanism of FA is decided by the C19–H donating site with the lowest BDE value equal to 76.5 kcal/mol. The easiest H-atom donor is observed at C19–H position, because of the presence of different ketone groups like C17=O, C23=O and C17–N18 bond, and it makes this bond extremely polarized and H-atom is easily released. In comparison with BDE values of some popular antioxidant compounds, BDE value of FA (76.5 kcal/mol) is lower than gallic acid (78.1 kcal/mol), trolox (78.5 kcal/mol), caffeic acid (78.8 kcal/mol), quercetin (79.4 kcal/mol) as well as trans-resveratrol (82.5 kcal/mol) [12]. Thus, FA may be considered as strong antioxidant via HAT mechanism.

Table 1. Bond dissociation enthalpies (BDEs) of folic acid calculated in the gas phase at the B3LYP/6-311G(d,p) level of theory.

Bonds	**BDE (kcal/mol)**
C19–H	**76.5**
C20–H	96.0
C21–H	89.3
C9–H	**80.1**
C13–H	112.3
C15–H	108.0
C12–H	110.6
C16–H	109.5
C7–H	105.1
N10–H	86.2
N14–H	97.8
N18–H	97.8

3.2.2. *Antioxidant capacity via SET mechanism*

Single electron transfer corresponds to one of the most important actions of antioxidant [8, 9]. Ionization energy (IE), and electron affinity (EA) characterize for this mechanism. The lower the IE value is, the easier electron donation is, while the higher the EA is, the easier electron acception is.

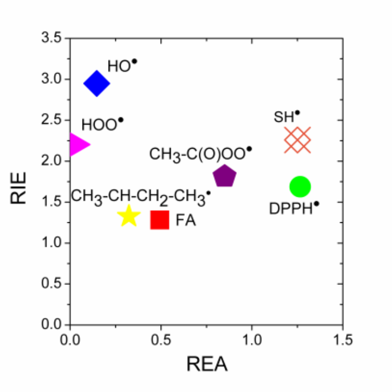

Figure 3. FEDAM for folic acid and selected free radicals calculated at the B3LYP/6-311G(d,p) level of theory.

The semi-empirical PM6 method was used to calculate IE and EA values. The IE of FA is equal to 5.45 eV. Compared to IE values of some common antioxidants such as vitamin A (6.41 eV), β-carotene (6.50 eV), caffeine (7.95 eV), coumarin (8.72 eV) [13], this demonstrates FA as a good antioxidant by via electron-donating mechanism. Whereas, the electron-accepting ability of FA is low with an EA value being 1.87 eV. Figure 3 displays the electron-donating and electron-accepting trends of FA with some common radicals such as $HO^{\bullet}$, $HOO^{\bullet}$, $DPPH^{\bullet}$, $SH^{\bullet}$, $CH_3–C(O)OO^{\bullet}$ and $CH_3–CH–CH_2–CH_3^{\bullet}$ using FEDAM. In terms of RIE value, RIE of FA is lower than the ones of all the considered free radicals. This indicates the high electron donating capacity of FA to the free radicals. In addition, REA of FA is also lower than REA of $DPPH^{\bullet}$, $SH^{\bullet}$, $CH_3–C(O)OO^{\bullet}$. Thus, FA can scavenge these free radicals in accepting electron from them.

3.2.3. Interaction of folic acid with $HOO^{\bullet}$ radical

The H-atom transfer reactions of FA at two positions with the lowest BDE value, including C19–H and C9–H with $HOO^{\bullet}$ radicals were investigated in this study to clarify the mechanism of action.

Potential energy surfaces (PES) reaction between the FA at the positions C19–H and C9–H with $HOO^{\bullet}$ are displayed in Figure 4. The reaction consists of three steps: firstly, formation of intermediate state 1 (INT-1), followed by the H-atom transfer passing through the transition state (TS) and finally, formation of the intermediate product (INT-2). The geometries of the intermediate states and the transition state at C19–H (left) and C9–H (right) positions are displayed in Figure 5.

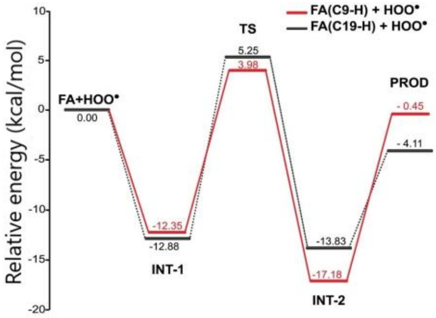

Figure 4. Potential energy surface of reaction between folic acid and $HOO^{\bullet}$ radicals at C19–H and C9–H positions calculated at the B3LYP/6-311G(d,p) level of theory.

Firstly, H-atom is transferred from C19–H bond of FA to $HOO^{\bullet}$ radical. In HAT reaction, $HOO^{\bullet}$ radicals and FA can form reactant complex at lower energy than the separated reactants of -12.88 kcal/mol. At this state, the hydrogen bond is formed between the O-atom of $HOO^{\bullet}$ radical and the H-atom at C19–H of FA. The HOO···H–C19 bond length is 2.21 Å (Figure 6A). Then, H-atom of FA tends to form a chemical bond with O-atom of $HOO^{\bullet}$ radical through the transition state (TS) with a higher total energy than the separated reactants 5.25 kcal/mol. HOO···H and H–C19 bond lengths are 1.20 and 1.33 Å, respectively (Figures 6B and 6C). After this state, the reaction produces a product complex with a lower total energy than the original reactant -13.83 kcal/mol. Finally, the reaction produces a separated product with a total energy lower than the one of the initial reactants by -4.11 kcal/mol. The similar observation is found for HAT reaction at the C9−H position.

The result of the ability to scavenge free radicals of FA is coherent with the previous study [3-14].

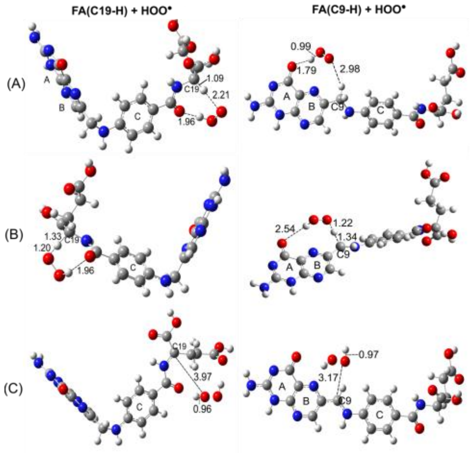

Figure 5. Optimized structure of (A) Reactant complex (INT-1), (B) Transition state (TS) and (C) Product complex (INT-2) of the HAT reaction between folic acid and $HOO^\bullet$ radical at two sites: C19−H (left) and C9−H (right).

Table 2. Gibbs Free Energy and reaction enthalpies of folic acid and $HOO^\bullet$ radical.

HAT reactions	ΔH, kcal/mol	ΔG, kcal/mol
C19−H + $HOO^\bullet$	-4.11	-4.14
C9−H + $HOO^\bullet$	-0.44	-0.17

Finally, Gibbs free energy and reaction enthalpies of reaction between FA and $HOO^\bullet$ radical at the C19−H, C9−H position were also calculated (Table 2). We see that reaction enthalpies (ΔH) at C9−H and C19−H positions is equal to -4.11 and -0.44 kcal/mol. Gibbs free energies (ΔG) at these two positions are -4.14 and -0.17 kcal/mol, respectively. Thus, it is showed that HAT reactions between FA and $HOO^\bullet$ radical are all feasible and slightly exergonic.

4. CONCLUSIONS

In this research, antioxidant capacity of folic acid was investigated in the gas phase via HAT and SET mechanisms. Density functional theory (DFT) at the B3LYP/6-311G(d,p) was used to calculate the characterizing thermochemical properties including BDE, IE and EA. The results show that FA represents as a very powerful antioxidant via the HAT mechanism. The lowest bond dissociation enthalpy being 76.5 kcal/mol is found at C19−H which is located near the strong electron affinity groups. According to the SET mechanism, FA is a potential antioxidant via its electron-accepting capacity. The potential energy surface (PES) between FA and $HOO^\bullet$ radical was also established to further clarify the reaction mechanism. Finally, the

Gibbs free energy variation and enthalpy of the reactions between FA and $HOO^\bullet$ radical show that these reactions are all feasible and slightly exergonic.

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