Density functional theory study of possible mechanisms of folic acid photodecomposition

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ABSTRACT

Density functional theory (DFT) calculations were performed at the B3LYP/6-31+G*//B3LYP/6−31G* level of theory on a series of folic acid, pterin, and *p*-aminobenzoic acid (PABA) models to further understand the anaerobic mechanism of folic acid photodecomposition. After initial excitation of the pterin ring at 350 nm, an energetically favored intramolecular electron-transfer reaction occurs from the PABA ring to the pterin (-11.9 kcal/mol; PCM using water as the dielectric constant). The resulting zwitterionic species can then undergo an exothermic heterolytic bond cleavage which results in bond fragmentation between the PABA and pterin rings (-15.9 kcal/mol). The pterin radical is then hypothesized to undergo oxidation to produce the reported 6-carboxypterin. Natural population analyses (NPA) were performed on all radical and radical ion structures to establish reasonable resonance structures used in the proposed photodecomposition mechanism of folic acid by determining the most reasonable location of the radicals and ions on the aromatic rings. A proposed mechanism of the photolysis of folic acid in the absence of oxygen is then proposed based upon the DFT calculations which agree well with the currently existing data of this process.

1. Introduction

1.1. Background

Folic acid (FA) is a water-soluble essential vitamin that is yellow-orange in color and is known to aid in the metabolism of amino acids and is necessary for the biosynthesis of DNA and RNA [1–3]. Folic acid has been shown to be effective in the prevention of birth defects such as spina bifida and anencephaly and therefore folic acid has received much attention recently in the area of birth defect prevention [4–6]. The electrochemistry has been well studied and has been a focus of research for years due to its biologically relevant redox chemistry [2,7–10]. Folic acid derivatives participate in photochemical processes such as photosynthesis, the light harvesting antennae of some lower organisms, DNA photolyases, and is being investigated for its use as a photosensitizer in some cancer treatments [1,11–20]. Although folic acid participates in many photochemical processes, reports on its photochemistry are rare and some reports that exist which may help describe its photochemistry often provide apparently conflicting results [21–23].

Although it is known that folic acid decomposes when exposed to light to produce 6-carboxypterin [21,24–27], the specific mecha-nism of photodegradation, identity of any intermediate structures, as well as any intramolecular photochemical relationship between the two aromatic portions of folic acid remains to be studied. Although attempts have been made recently to identify the stable intermediates following FA irradiation, surprisingly few reports exist even proposing a photochemical mechanism for this reaction [28,29]. Although the presence of oxygen has been shown to influence FA photodecomposition, no reports exist which demonstrate that molecular oxygen is required for photodegradation to occur. A key step of one mechanism proposed in the literature involves a photo-induced dehydrogenation which is unlikely to occur and no experimental evidence of H_2 production from the irradiation of folic acid has been reported.

1.2. Folic acid photophysics

Folic acid consists of a pterin ring (PT) system, a *p*-aminobenzoic acid (PABA) portion, and the amino acid glutamic acid (Glu) (Fig. 1). Upon irradiation, folic acid decomposes through cleavage of the bond between the PABA and pterin ring system. PT and simple PT derivates proposed here and in previous reports to be intermediates in the photodecomposition process (6-carboxypterin, CPT, and 6-formylpterin, FPT; Fig. 2) have reported fluorescence quantum yields ranging from $\Phi_F = 0.07$–0.33 (350 nm excitation) in aqueous solutions with a pH of 5.5–10.5 [30,31]. In comparison, folic acid has a very low fluorescence quantum yield of $\Phi_F < 0.005$ when excited

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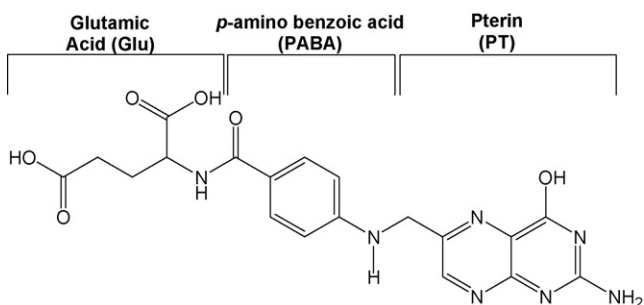 *E-mail address:* christopher.martin@lamar.edu (C.B. Martin).

Fig. 1. Molecular structure of folic acid (FA).

X = CH_3; 6-methylpterin (MPT)
CH_2OH; 6-hydroxymethylpterin (HMPT)
CHO; 6-formylpterin (FPT)
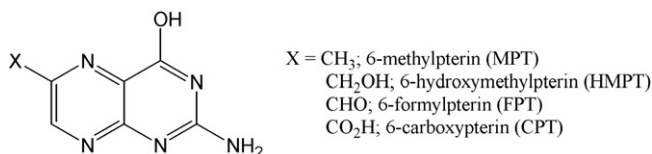CO_2H; 6-carboxypterin (CPT)

Fig. 2. Proposed pterin intermediates of FA photodecomposition.

at the same wavelength as the other pterins [30]. The decreased fluorescence has been attributed to the long chain substituent (PABA-Glu) in FA acting as an "internal quencher" and therefore enhancing radiationless decay [30]. This explanation, however, fails to address whether the decreased Φ_F in FA is a consequence of intramolecular collisional deactivation, intramolecular electron transfer, intramolecular energy transfer, or other processes which originate from the singlet excited state. These distinctions are very important for understanding of the mechanism of photodecomposition in addition to any further photochemistry that occurs when FA is exposed to UV radiation that initially excites the PT ring. Hirakawa has proposed that the decreased fluorescence of FA is due to the intramolecular photo-induced electron-transfer reaction between the pterin and PABA rings [32]. Unfortunately, no further reports investigating the electron-transfer reaction have been reported and this process has not been used in proposing a possible mechanism of the photodegradation reaction of FA.

1.3. Similarities between folic acid and riboflavin

Riboflavin (RB) is another photosensitive vitamin with many similarities to FA. It has similar redox properties, is also photosensitive, and shares some important structural features with pterins and FA [33] (Fig. 3). Riboflavin has a high quantum yield of fluorescence ($\Phi_F = 0.26$ at neutral pH) [34]. When RB is present in flavin adenine dinucleotide (FAD), the fluorescence diminishes greatly and the compound is relatively photostable [35,36]. The reason for the increased photostability is the rapid (through space) photo-induced electron-transfer reaction between adenine and flavin followed by the (through space) back-electron-transfer reaction which dissipates the energy from the absorbed photon [36].

The similarities in the structural, electrochemical, and quenched fluorescence properties between pterin and riboflavin (and thus
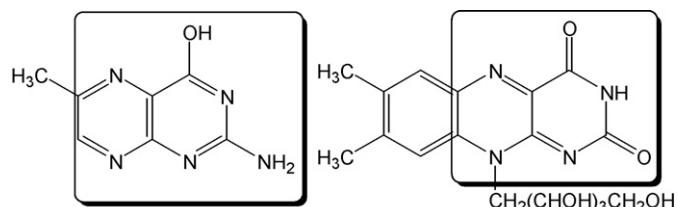

Fig. 3. Structural similarities between a pterin (FA) and isoalloxazine (FAD) rings.

folic acid and FAD) support Hirakawa's report that that decreased fluorescence of the pterin in folic acid is due to a photo-induced intramolecular electron-transfer reaction between the PABA and pterin rings. When FA is irradiated in the presence of RB or free pterins (both of which are known electron acceptors and photosensitizers of singlet oxygen), the rate of photodegradation of FA is greatly increased. It has been suggested that singlet oxygen is responsible for the photodecomposition of FA in aerobic conditions, which is a faster photodecomposition process compared to the anaerobic conditions [37]. The reduced fluorescence of FA compared to free pterins suggests that a photo-induced electron-transfer product serves as a reactive intermediate which may result in an oxygen-independent photodecomposition. It is therefore proposed that the initial step in the photodegradation of folic acid proceeds through a photo-induced electron-transfer reaction.

The purpose of this study is to use density functional theory calculations [38–47] towards understanding the initial steps of the oxygen-independent photodecomposition of folic acid and to propose possible mechanisms which explain this chemistry. Some simple spectroscopic experiments have been used to complement the computational studies. More complicated mechanisms involving the participation of molecular oxygen and reaction with solvents are currently underway. This study will investigate the thermodynamic feasibility of a minimalistic intramolecular mechanism of photodegradation of folic acid.

2. Materials and methods

2.1. Theoretical methods

Theoretical calculations were performed using Gaussian 03 on a seven (2.0 GHz) node Linux Beowulf cluster (CACLIUM) at Lamar University. Calculations were performed at the B3LYP/6-31+G*//B3LYP/6−31G* [38–47] levels using the PCM (Polarizable Continuum Model) using a water solvent dielectric field [48]. The calculated sum of the electronic and thermal free energies (ΔG, kcal/mol) was used for each structure and results were not scaled. Vibrational analyses were performed to confirm that all structures minimized to local minima by the absence of any imaginary vibrational frequencies. Atomic charges and spin densities were calculated by performing a natural population analysis (NPA)
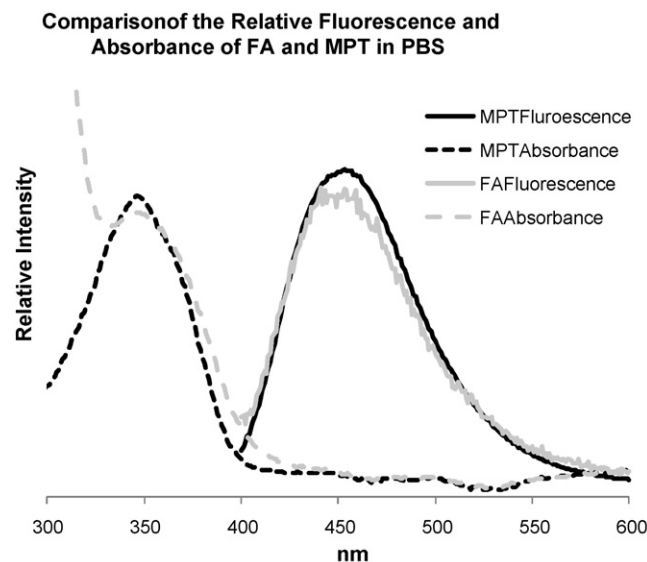

Fig. 4. Absorbance and fluorescence of folic acid and 6-methylpterin. Intersection of the absorbance and emission spectra is an estimation of the excited-state singlet energy (70 kcal/mol).

on the optimized structures [49]. All radical species contained a low amount of spin contamination indicated by the value of $0.76 \leq S^2 \leq 0.79$.

2.2. Experimental methods

Absorbance measurements were performed on a Varian Cary 50 Bio and fluorescence measurements were performed on a Varian Eclipse. Folic acid was purchased from Aldrich and 6-methylpterin was purchased from Schircks and both compounds were used without further purification. All spectroscopic measurements were performed in neutral phosphate buffered saline (PBS) which was prepared by dissolving 8.5 g of sodium chloride, 0.2 g of monobasic potassium phosphate, and 2.9 g of disodium hydrogen phosphate dihydrate to 1000 mL water. When all the solids were dissolved, the pH of the solution was adjusted to 7.3 using 1 M hydrochloric acid. The energy of the excited-state singlet was estimated by plotting the absorbance and emission on the same graph and scaling the emission maximum and absorbance at the excitation wavelength to relatively equal heights. The point of the emission and absorbance intersection was then considered to be an estimation of the singlet excited state energy.

3. Results

3.1. Thermodynamics

Density functional theory (DFT) calculations were performed on models for the PABA and pterin portions of FA to further
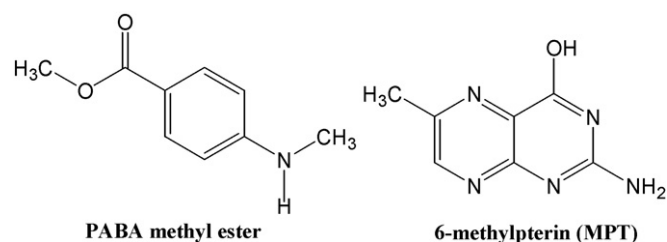
Fig. 5. Computational models for the electron-transfer reaction between PABA and pterin.

understand the proposed photo-induced electron-transfer reaction and subsequent bond fragmentation since DFT has been reported to accurately predict the electronic structure of heteroaromatic systems. Irradiation of FA may excite either the PABA or pterin chromophores depending on the excitation wavelength. To simplify the resulting photochemistry, irradiation at longer wavelengths (330–400 nm) will allow for selective excitation of the pterin system only. Based upon fluorescence studies of 6-methylpterin and folic acid (25 μM, PBS) performed with excitation at 350 nm (82 kcal/mol) and the emission at 550 nm (52 kcal/mol), it is estimated that the excited-state singlet of the pterin is approximately 70 kcal/mol above the ground state (absorbance and emission overlap approximately 400 nm), which is consistent with data in the literature [50,51] (Fig. 4). Based on this experimental result, it can be concluded that the electronics of the pterin portion of folic acid ($S_0 \rightarrow S_1$ gap) is very similar to the unsubstituted pterin and
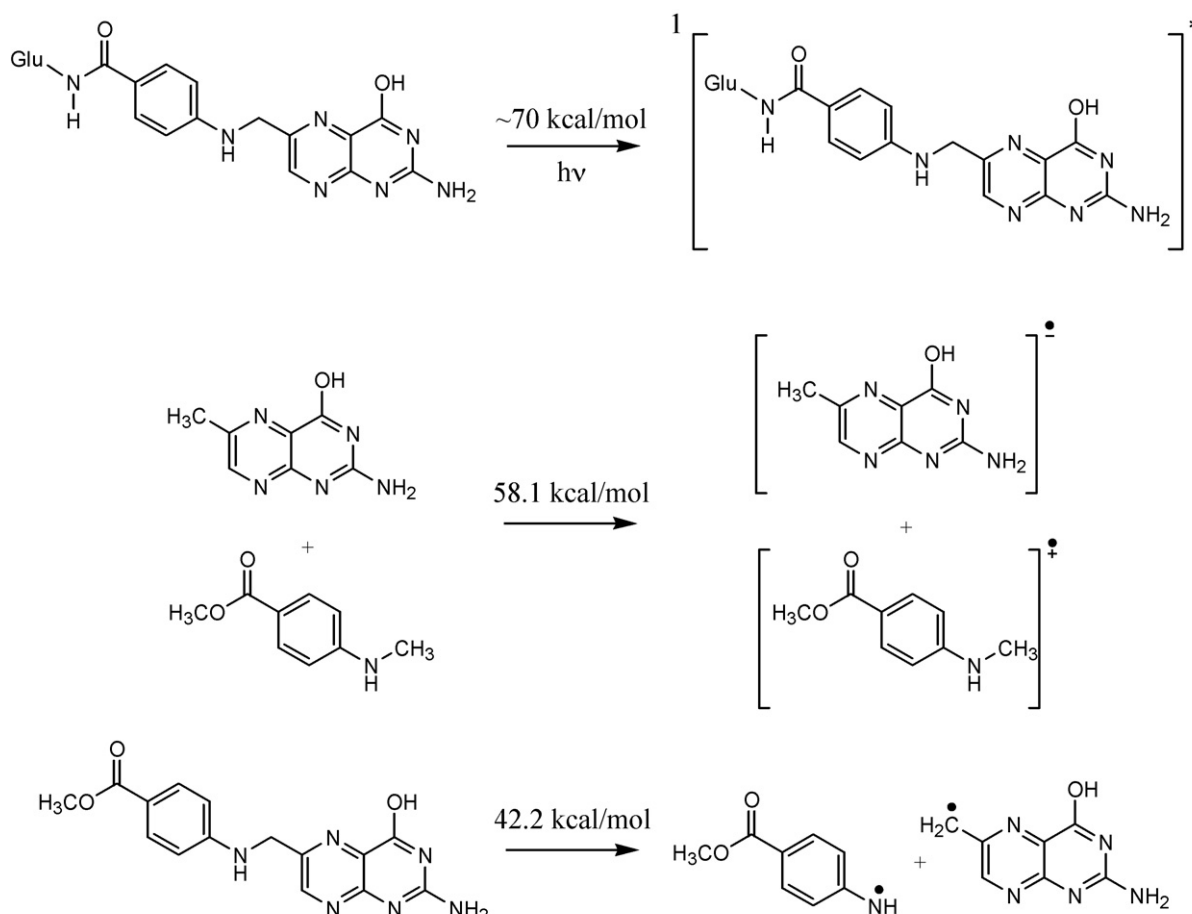
Fig. 6. Thermodynamics of the reaction of FA to the excited-state singlet (*vida infra*), electron transfer resulting the radical ion pair, and radical products after bond cleavage (B3LYP/6-31+G*//B3LYP/6−31G* using PCM dielectric).
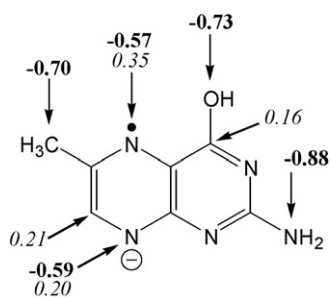
Fig. 7. Natural population analysis (NPA) calculated atomic charge (bold) and spin density (italics) of the MPT radical anion (B3LYP/6−31G* PCM water). The structure represents the resonance form most consistent with the theoretical data.

Fig. 8. Natural population analysis (NPA) calculated atomic charge (bold) and spin density (italics) of the NMA radical cation (B3LYP/6−31G* PCM water). The structure represents the resonance form most consistent with the theoretical data.

is not significantly affected by the PABA moiety. Therefore, 6-methylpterin can serve as a reasonable model for the pterin portion of folic acid without the need to fully calculate the entire folic acid molecule, which will be computationally very difficult. The com-
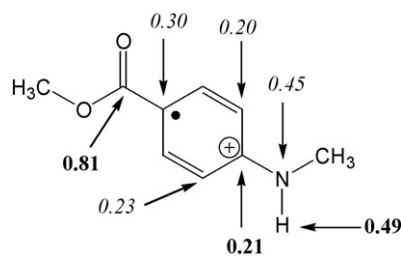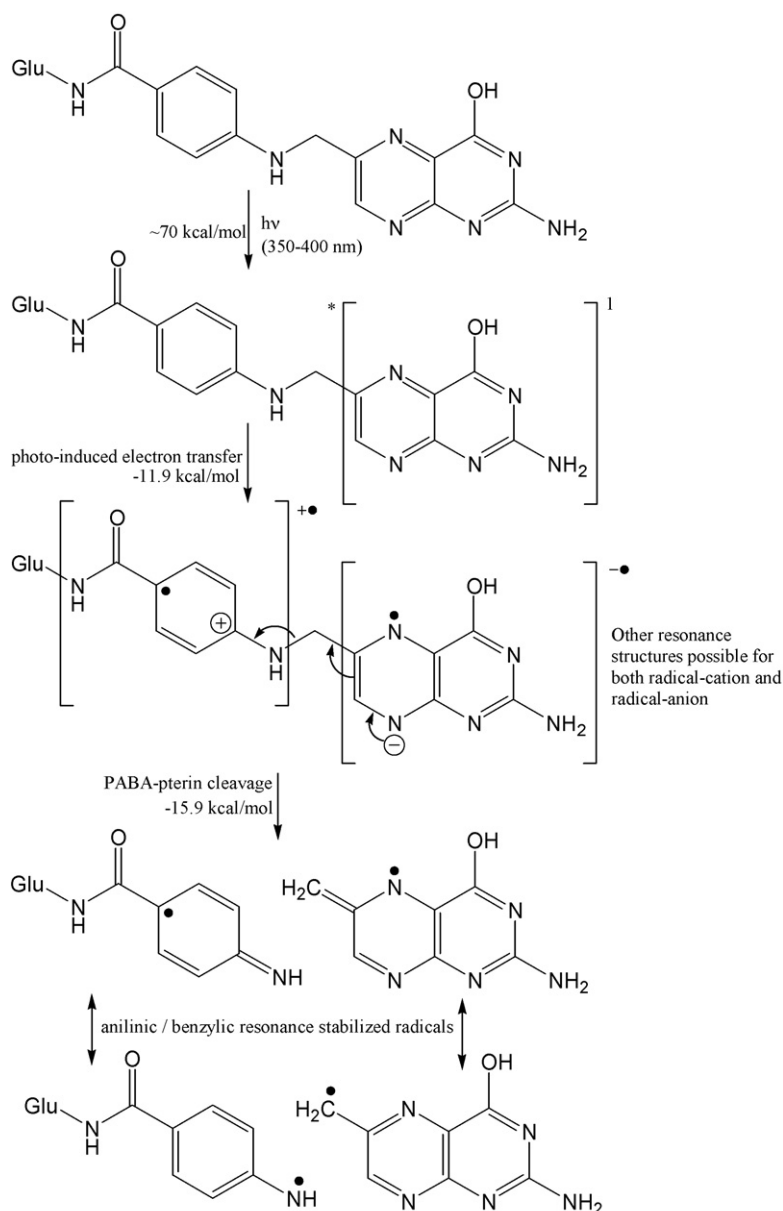
putational models for FA replaced the glutamic acid portion with a methyl ester. This substitution allows for the inclusion of the chromophoric systems within the calculations without the additional computational resources needed to handle the additional atoms (Glu) which would extend computational times significantly. The

Scheme 1. Proposed photo-induced electron-transfer mechanism precluding the oxygen-independent photodecomposition of folic acid.

intramolecular electron-transfer reaction was modeled using the *para* substituted methyl ester of *N*-methyl aniline (NMA) for the PABA portion and 6-methylpterin (MPT) for the pterin (Fig. 5). Inclusion of the methyl groups on each structure simulates the methylene in FA which isolates the PABA and pterin rings. These structures were selected because they allow the charges to be completely isolated on each of the pi-systems to calculate the energy of the charge-separated species immediately following electron transfer. Since gas-phase calculations lack solvent parameters critical for ion stability, all structures were calculated using the PCM model with water as the solvent dielectric. The sums of the calculated energies of the radical cation of the NMA model and the radical anion of the pterin were compared to the energy of the respective ground-state singlets, also calculated using the PCM solvation model. The pair of radical ions was found to be 58.1 kcal/mol higher in energy than the calculated energy of the ground-state structures (Fig. 6).

After the electron-transfer reaction, the C–N bond between the PABA and pterin rings is proposed to cleave resulting in a pair of neutral radicals. The calculations indicate that the NMA anilinic and pterin benzylic radical pair is 42.2 kcal/mol higher in energy than the ground state of the FA model (Fig. 6).

3.2. Charge and spin densities

A natural population analysis (NPA) was performed on the radicals and radical ions to determine the charge and spin density of these intermediates. Spin density is defined as the total alpha electron spins on a certain atom minus the total beta electron spins on that atom. In radicals, an alpha spin is assigned to the unpaired electron and therefore an excess in the alpha spin is an indication of a high population of the unpaired electron (alpha–beta). The pterin radical anion contained the largest anion density on the exocyclic oxygen (−0.73) and nitrogen (−0.88) atoms. Within the ring system, the greatest anionic charge was found on the ring nitrogen *meta* to the benzylic methyl group (−0.59) (Fig. 7). It is therefore reasonable to assign a significant amount of negative charge density on this nitrogen atom for purposes of resonance. The largest unpaired electron density in the pterin radical anion was found to be on the two ring atoms *ortho* to the benzylic methyl group (N = 0.35; C = 0.21) and the nitrogen atom *meta* to the methyl group (0.20). When the ring atom containing the most negative charge density and the ring atom containing most spin density are considered, the most reasonable resonance structure for the pterin radical anion following the photo-induced electron-transfer reaction with NMA is determined.

Analysis of the calculated charge density of the NMA methyl ester radical cation shows that the most positive charge is on the carbonyl carbon (0.81) and the anilinic hydrogen (0.49). When the charges on the carbons within the ring are considered, the most positive charge density is located on the carbon atom (0.21) adjacent to the nitrogen. The atoms which contained the most radical character by NPA analysis included the anilinic nitrogen (0.45) and the three ring carbons which may be used for resonance delocalization (*ortho* 0.20, 0.23 and *para* 0.30) (Fig. 8). When the ring carbons which may contain the radical or the cationic charge are considered, the most reasonable resonance contribution for the radical cation of the PABA portion of FA after photo-induced electron transfer contains the charge at the nitrogen-containing carbon and the radical at the carbonyl containing carbon.

After electron transfer and upon cleavage of the C–N bond, two neutral radical species may be produced. The highest unpaired electron density in the NMA radical was found on the anilinic nitrogen (spin density of 0.60) although it was resonance stabilized throughout the aromatic system on the predicted three ring carbons (spin density of 0.24–0.26). The pterin radical contained a high amount

of spin density on the methylene of the pterin ring (0.82) and the *ortho* ring nitrogen atom (0.20).

4. Discussion

The DFT calculations support the charge-transfer and subsequent fragmentation mechanism proposed in this paper when the pterin ring of FA is irradiated. First, the pterin ring absorbs a photon of approximately 350 nm forming the excited-state singlet which is approximately 70 kcal/mol above the ground state. This energy represents a vertical excitation and some energy may be dissipated by adiabatic relaxation, internal conversion, and vibrational relaxation on the S_1 surface. An electron is then exothermically transferred from the PABA ring to the pterin ring resulting in a charge-separated radical ion pair calculated to be 58.1 kcal/mol above the ground state. Since this step is predicted to be exothermic by about 12 kcal/mol (or slightly less based on S_1 relaxation pathways), it is reasonable to conclude that this is a relatively competitive process from the pterin excited-state singlet compared to other processes. Finally, a back-electron transfer may occur through space, resulting in the original ground-state singlet, or through the sigma bonding system which results in fragmentation of the C–N bond of the pterin and PABA systems. Calculations indicate that the radical pair produced is 42.2 kcal/mol above the ground-state singlet and is therefore exothermic by approximately 16 kcal/mol compared to the preceding charge-separated species. It is predicted that FA undergoes a photo-induced electron-transfer reaction followed by a "through-bond" back-electron transfer in the photodegradation reaction. This step is proposed to be essentially a heterolytic bond cleavage in which the back-electron-transfer proceeds through the pi- and sigma- bonding system as opposed to the "through space" back electron transfer where the electron bypasses the bonding system and is transferred back to the PABA radical cation. Each step in the proposed process is exothermic and thermodynamically favored according to DFT calculations. Other mechanisms are possible which contain participation of molecular oxygen or solvent and they are currently under investigation and will not be considered here.

Considering the resonance structures of the radical ions, a full mechanism of the photolysis of the pterin-PABA bond can be
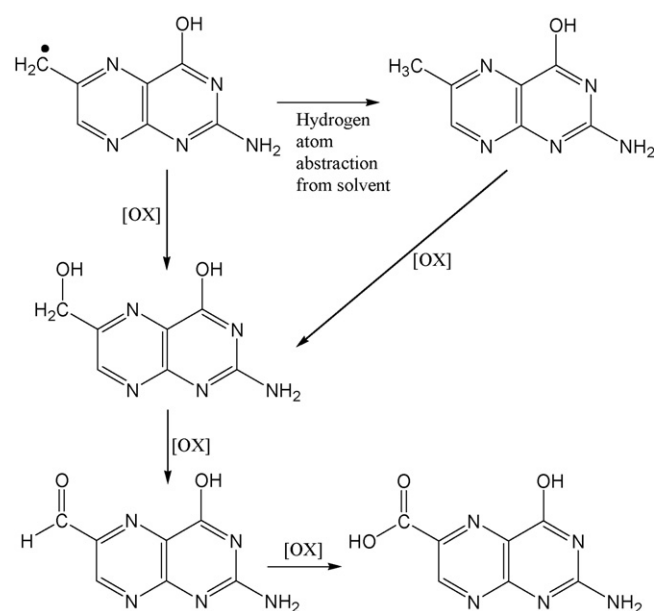
Scheme 2. Proposed reaction pathway of the benzylic radical of 6-methylpterin (MPT) producing 6-carboxypterin (CPT).

proposed (Scheme 1). The anion on the pterin ring results in an exocyclic alkene on the pterin and subsequent bond fragmentation producing in an imine-like PABA species. Both of these radicals are resonance structures of the simple benzylic or anilinic radicals. The resulting pterin radical may either be oxidized directly to the hydroxymethylpterin (HMPT) or it may participate in a hydrogen-atom abstraction resulting in 6-methylpterin (MPT). The benzylic position of either of these pterins is then oxidized (through FPT) to the final product of 6-carboxypterin (CPT), the known photoproduct of FA irradiation (Scheme 2).

5. Conclusion

DFT calculations have been used to support the proposed mechanism of the folic acid photodecomposition. The reaction proceeds from the initial excitation of the pterin ring through a photo-induced electron-transfer reaction followed by a through-bond back electron transfer which results in bond cleavage. Each step was calculated to be energetically favored.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2009.07.025.

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