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Journal of Photochemistry and Photobiology A: Chemistry 180 (2006) 65–68

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

A TD-DFT study on photo-physicochemical properties of hypocrellin A and its implications for elucidating the photosensitizing mechanisms of the pigment

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Received 17 July 2005; received in revised form 2 September 2005; accepted 21 September 2005

Abstract

As a naturally occurring perylenequinonoid pigment, hypocrellin A (HA) has gained much attention in recent years owing to its excellent photosensitive properties. However, there is little theoretical study on the photo-physicochemical behaviors of HA, which stimulated our interest to perform the study by means of time-dependent density functional theory (TD-DFT) calculations. In this paper, the TD-DFT calculations provide various excited state properties of HA in polar and non-polar solvents, including absorption spectrum, lowest triplet excited state energy, vertical electron affinity and vertical ionization potential, by which we can get some deeper insights into the photosensitive mechanisms of hypocrellin. First, although HA can photo-generate $^1O_2$ through energy transfer in both non-polar and polar solvents, it gives birth to $O_2^{\bullet -}$ only in polar solvents, and it is the HA anion (generated from autoionization) that are responsible for the $O_2^{\bullet -}$-generation. Second, HA cation (also generated from autoionization) is more favored in thermodynamics than triplet excited HA to accept electron from DNA bases. As a result, HA cation likely takes more responsibility than excited HA for the pigment's photo-damage to DNA in oxygen-free media.

Keywords: Perylenequinonoid pigments; Hypocrellin A; Photosensitive mechanisms; Reactive oxygen species; Time-dependent density functional theory

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1. Introduction

Hypocrellin A (HA, Fig. 1) is a naturally occurring perylenequinonoid pigment (PQP), which has gained much attention in recent years owing to its light-induced antitumor, antifungal and antiviral activities [1–10]. HA possesses several advantages over the presently used photodynamic therapy (PDT) agent photofrin II: easy preparation and purification, high yields of reactive oxygen species (ROS), low toxicity and rapid metabolism in vivo, etc. [11]. Thus, HA has great potential to be developed as new generation of PDT medicines.

As known to all, during the photosensitization, pigments in ground state (S_0) are initially excited to the singlet excited state (S_1) and then intersystem cross to the triplet excited state (T_1). Therefore, much experimental effort has been devoted to investigating the S_1 and T_1 state properties of HA to understand its photosensitizing mechanisms [8,12–14]. Nevertheless, there is little theoretical study on this topic. Considering the successful use of time-dependent density functional theory (TD-DFT) in investigating the photo-physicochemical behaviors of dyes and pigments [15–18], we attempt to perform the study by TD-DFT method, which will provide some deeper insights into the photosensitizing mechanisms of HA.

2. Methods

The calculation procedures are as follows. Initial structures of HA and its derived compounds were firstly optimized by semiempirical method AM1 [19]. Then, the molecular structures were fully optimized in solvents (benzene and DMSO)

Abbreviations: AEA, adiabatic electron affinity; HA, hypocrellin A; IPT, intramolecular proton transfer; PCM, polarized continuum model; PDT, photodynamic therapy; PQP, perylenequinonoid pigment; ROS, reactive oxygen species; SCRF, self-consistent reaction field; TD-DFT, time-dependent density functional theory; VEA, vertical electron affinity; VIP, vertical ionization potential

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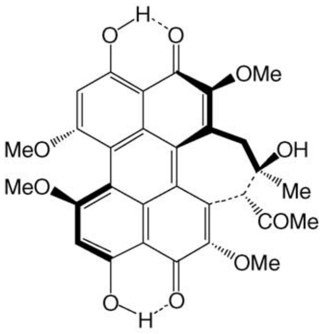
doi:10.1016/j.jphotochem.2005.09.019



Hypocrellin A (I)

Fig. 1. Molecular structure of hypocrellin A.

by the hybrid B3LYP functional with 6-31G Gaussian basis set. Finally, the excited-state properties in benzene and DMSO were calculated by TD-DFT formalism with the same basis set [20], by which absorption spectra and triplet state properties of HA were obtained. The vertical electron affinities (VEAs) and vertical ionization potentials (VIPs) of HA in different environments were calculated by a combined DFT method B3LYP/6-31G(d,p)//B3LYP/6-31G, which means that B3LYP/6-31G(d,p) was employed to perform a single-point calculation on the basis of B3LYP/6-31G-optimized structures. During the calculations, the solvent effects were taken into consideration by employing the self-consistent reaction field (SCRF) method with polarized continuum model (PCM) [21–23]. All of the calculations were performed with Gaussian 03 package of programs [24].

3. Results and discussion

Due to the tautomerization reaction, HA may exist in diverse isomers. However, HA(I) (Fig. 1) has been recognized as the most stable isomer [25–28]. Thus, HA(I) was used as starting point of the present study.

3.1. Photo-physicochemical properties of HA

3.1.1. Absorption spectra of HA

Table 1 lists the B3LYP/6-31G-calculated five lowest singlet excitation energies (*E*) and oscillator strengths (*f*) of HA in benzene and DMSO. We can find that there exist three absorption bands for HA, which is consistent with the experimental observations (bands III, II and I) (Table 1). Both in benzene and DMSO, the three absorption bands correspond to the first ($S_0 \rightarrow S_1$), second ($S_0 \rightarrow S_2$) and third ($S_0 \rightarrow S_3$) lowest excitations. However, the theoretical absorptions for HA differ from the experimental data (Table 1) [14]. This maybe results from the flexible seven-membered ring of HA, which has been revealed leading to the complexity of photo-physicochemical behaviors of HA in solution [27,28]. Previous studies proposed that absorption bands III and II for HA result from intramolecular proton transfer (IPT) between the phenolic hydroxyl and the quinonoid carbonyl groups [14,29], while our present study indicates that bands III and II arise from $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ excitations rather than from IPT process.

Table 1
Five lowest singlet excitation energies (*E*) and oscillator strengths (*f*) of hypocrellin A

Compounds	Excited states	*E* (eV)	λ (nm)	*f*	λ_{exp} (nm)[a]
HA[b]	S_1	2.35	528	0.19	583 (band III) [14]
	S_2	2.46	504	0.30	541 (band II) [14]
	S_3	2.74	453	0.23	471 (band I) [14]
	S_4	2.81	441	0.04	
	S_5	3.00	414	0.00	
HA[c]	S_1	2.36	526	0.19	582 (band III) [14]
	S_2	2.48	499	0.25	540 (band II) [14]
	S_3	2.69	461	0.28	472 (band II) [14]
	S_4	2.80	442	0.05	
	S_5	3.08	403	0.00	

[a] Experimental value.
[b] In benzene.
[c] In DMSO.

3.1.2. Lowest triplet excited-state energy of HA

Due to the much longer lifetime of T_1 state than S_1 state, T_1 state is responsible for the photosensitive reaction. Therefore, the lowest T_1 excitation energy (E_{T_1}) of a photosensitizer is crucial to understanding its photosensitizing mechanisms. The TD-DFT-calculated E_{T_1} values of HA are listed in Table 2, from which we can find that the E_{T_1} values are very close in benzene and DMSO, suggesting that the solvent polarity has little influence on the E_{T_1} of HA. Moreover, the theoretical E_{T_1} of HA in benzene (1.53 eV) is comparable to the experimental data (1.78 ± 0.11 eV) [14], suggesting that the theoretical results are applicable to elucidating the photosensitizing behaviors of HA.

3.1.3. Vertical electron affinities and vertical ionization potentials of HA

The photosensitizers in ground and excited states may act as electron-donors or electron-acceptors during the photosensitizing reactions. Thus, the vertical electron affinities and vertical ionization potentials of HA in S_0 and T_1 states were calculated and listed in Table 2. The VEA in S_0 state (VEA_{S_0}) was estimated as −2.70 eV in benzene and −3.39 eV in DMSO. The evident solvent effect on VEA_{S_0} stems from the fact that anion is better stabilized in polar solvents than in non-polar environments. The VEA in T_1 state (VEA_{T_1}) for HA was −4.23 eV in benzene and −4.93 eV in DMSO (Table 2), respectively. As shown in Table 2, the VIP in S_0 state (VIP_{S_0}) for HA is 6.94 eV in benzene and 6.23 eV in DMSO, indicating that HA is more ready to donate an electron in DMSO. In combination with E_{T_1}, VIP in T_1 state (VIP_{T_1}) for HA was estimated to be 5.41 eV in

Table 2
Lowest triplet excitation energies (E_{T_1} in eV), vertical electron affinities (VEAs in eV) and vertical ionization potentials (VIPs in eV) of hypocrellin A in benzene and DMSO

	E_{T_1}	VEA_{S_0}	VEA_{T_1}[a]	VIP_{S_0}	VIP_{T_1}[b]
Benzene	1.53	−2.70	−4.23	6.94	5.41
DMSO	1.54	−3.39	−4.93	5.98	4.44

[a] $VEA_{T_1} = VEA_{S_0} - E_{T_1}$.
[b] $VIP_{T_1} = VIP_{S_0} - E_{T_1}$.

benzene and 4.44 eV in DMSO, respectively. On the basis of these values, we can investigate the photosensitive mechanisms of HA.

3.2. Elucidation of photosensitizing mechanisms of HA

It is well known that photosensitization involves two mechanisms, namely, reacting directly with substrates (e.g. DNA, RNA and proteins) (mechanism I) or damaging through intermediate of oxygen (through energy transfer or electron transfer to generate toxic ROS) (mechanism II).

3.2.1. Mechanism I of HA photosensitization

Previous studies suggest that HA and its derivatives can damage DNA upon excitation both in oxygen and oxygen-free media [30,31]. In the oxygen-free media, mechanism I should be responsible for the photo-damage and two possible pathways may be involved. First, T_1 state HA can abstract an electron directly from DNA bases (Eq. (1)). Second, cation radical of HA ($HA^{\bullet+}$) can be generated by autoionization reaction between T_1 and S_0 states (Eq. (2)) or both T_1 states (Eq. (3)), which may accept an electron from DNA (Eq. (4)).

$$HA(T_1) + D \rightarrow HA^{\bullet-} + D^{\bullet+} \quad (1)$$

$$HA(T_1) + HA(S_0) \rightarrow HA^{\bullet+} + HA^{\bullet-} \quad (2)$$

$$HA(T_1) + HA(T_1) \rightarrow HA^{\bullet+} + HA^{\bullet-} \quad (3)$$

$$HA^{\bullet+} + D \rightarrow HA(S_0) + D^{\bullet+} \quad (4)$$

Reaction (1) is governed by the VEA_{T_1} of HA and the VIPs of bases. If the summation of both parameters is negative, the reaction is permitted. The VIPs of DNA or RNA bases were calculated as: A, 6.79 eV; G, 6.38 eV; T, 7.44 eV; C, 7.14 eV and U, 7.75 eV in benzene and A, 5.90 eV; G, 5.50 eV; T, 6.38 eV; C, 6.21 eV and U, 6.61 eV in DMSO. Thus, the summation of VEA_{T_1} of HA (Table 2) and VIPs of DNA or RNA bases are positive in benzene (>2.15 eV) and DMSO (>0.57 eV), suggesting that the electron transfer between T_1 state HA and bases are not thermodynamically favorable.

According to the theoretical parameters listed in Table 2, the total energies of reactions (2) and (3) (for reaction (2), $VEA_{T_1} + VIP_{S_0}$ or $VIP_{T_1} + VEA_{S_0}$; for reaction (3), $VEA_{T_1} + VIP_{T_1}$) for HA in benzene is positive, and therefore, both reactions are not allowed. Thus, $HA^{\bullet+}$ cannot be generated and reaction (4) has no chance to occur in benzene. A similar analysis indicates that in DMSO, reaction (2) is still forbidden. However, reaction (3) is permitted owing to its negative reaction energy (−0.49 eV). As the VEA for $HA^{\bullet+}$ is calculated to be −5.71 eV in DMSO, once $HA^{\bullet+}$ is formed through reaction (3), it can abstract an electron from guanine (whose VIP is 5.50 eV).

In conclusion, according to the present calculations, HA cannot bring direct damage to DNA in oxygen-free non-polar solvents. While in oxygen-free polar solvents, the photo-damage to DNA by HA more likely results from the electron transfer between DNA bases and $HA^{\bullet+}$ rather than from the reaction between bases and T_1 state HA. However, as the generation of $HA^{\bullet+}$ depends largely on the concentration of T_1 state HA, the DNA damage by $HA^{\bullet+}$ may be trivial compared with that by ROS, which is supported by the experimental observation that the photo-damage on DNA by HA in oxygen-free media is much weaker than that in oxygen media [31].

3.2.2. Mechanism II of HA photosensitization

As to the ROS-associated pathway, first, T_1 state HA may react with ground state oxygen ($^3O_2$) through energy transfer to generate singlet excited oxygen ($^1O_2$) (Eq. (5)).

$$HA(T_1) + {}^3O_2 \rightarrow HA(S_0) + {}^1O_2 \quad (5)$$

It can be seen from Table 2 that the E_{T_1} of HA in benzene and DMSO are higher than the excited-state energy of $^1O_2$, 1.06 eV [15], indicating that the energy transfer between T_1 state of HA and $^3O_2$ in both solvents is permitted. This agrees well with the fact that HA can efficiently give birth to $^1O_2$ with high yields (0.83 in benzene) when irradiated by light [14].

Second, T_1 state HA may react with $^3O_2$ through electron transfer to generate superoxide anion radical ($O_2^{\bullet-}$) (Eq. (6)). The prerequisite of the reaction is that the summation of VIP_{T_1} of HA and the adiabatic electron affinity of $^3O_2$ (AEA_{O_2}) is negative.

$$HA(T_1) + {}^3O_2 \rightarrow HA^{\bullet+} + O_2^{\bullet-} \quad (6)$$

However, the summation of VIP_{T_1} for HA (Table 2) and AEA_{O_2} (−2.33 eV in benzene and −3.65 eV in DMSO [17]) are positive either in benzene or DMSO, suggesting that $O_2^{\bullet-}$ could not be generated through this pathway in both solvents. This is consistent with the fact that $O_2^{\bullet-}$-generation by irradiating HA in benzene has not been reported. Nevertheless, $O_2^{\bullet-}$ was indeed observed during the photosensitization of HA in DMSO [12]. Thus, it is speculated that there exists an alternative $O_2^{\bullet-}$-generating pathway for HA in DMSO, i.e. the electron transfer reaction between $HA^{\bullet-}$ (generated from autoionization, Eq. (3)) and $^3O_2$ (Eq. (7)).

$$HA^{\bullet-} + {}^3O_2 \rightarrow HA(S_0) + O_2^{\bullet-} \quad (7)$$

In benzene, reaction (7) is forbidden, because of the positive total reaction energies ($AEA_{O_2} - VEA_{S_0} = 0.37$ eV). However, a similar analysis indicates that reaction (7) is permitted in DMSO owing to its negative reaction energy (−0.26 eV). Therefore, $O_2^{\bullet-}$ can be generated in DMSO by photo-irradiation of HA, manifesting the positive effect of polar solvents to facilitate electron transfer reaction. Nevertheless, it should be stressed that it is $HA^{\bullet-}$ that is responsible for the $O_2^{\bullet-}$-generation in DMSO. This provides a deeper insight into the $O_2^{\bullet-}$-generating mechanism of HA in polar solvents [12]. Once $O_2^{\bullet-}$ is given, other ROS, such as H_2O_2 and $^{\bullet}OH$, can be produced through Fenton reaction [32] or Haber–Weiss reaction [33], which will efficiently amplify the photosensitizing activity of HA.

4. Conclusion

The photo-physicochemical properties of HA in polar and non-polar solvents, including absorption spectrum, lowest triplet excited-state energy, vertical electron affinity and vertical ionization potential, were examined by TD-DFT method. On the basis of the calculated results, the photosensitive mechanisms of HA were discussed and some deeper insights were gained. First, we indicated that the experimentally observed HA's photo-damage to DNA in oxygen-free media is more likely arises from $HA^{\bullet +}$ rather than from T_1 state HA. Second, we revealed that it is the anion of HA that is responsible for the $O_2^{\bullet -}$-generation in polar solvents. Therefore, theoretical methods are useful to investigate the photosensitive behaviors of HA and can be used to explore photo-physicochemical properties of other perylenequinonoid pigments.

Acknowledgments

This work was supported by the National Basic Research Program of China (2003CB114400) and the National Natural Science Foundation of China (Grant No. 30100035).

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