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Theoretical exploration of the photosensitive properties of xanthurenic acid, a tryptophan metabolite in cataractous human lenses

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Abstract

Photochemical damage has been considered as an important reason for age-related cataracts. Recently, a photochemically active tryptophan metabolite, xanthurenic acid (Xan), has been isolated from aged cataractous human lenses. Previous studies demonstrated that Xan can promote the photo-oxidation of lens protein in model studies by the photoproduction of singlet oxygen and superoxide anion radicals and could therefore play an important role in the photoinduction of age-related cataracts. Thus, it is of significance to investigate further the photosensitive properties of Xan. The photo-physicochemical properties of Xan, including absorption spectrum, lowest triplet excited-state energy, vertical electron affinity and vertical ionization potential, were explored by means of time-dependent density functional theory (TD-DFT) calculations, as a means of gaining deeper insights into the photosensitive mechanisms of Xan. It was found that Xan can photo-generate various reactive oxygen species through different pathways. For example, Xan can produce singlet oxygen through direct energy transfer pathway, while it can generate the superoxide anion radical through both direct electron transfer and autoionization. Other photosensitive properties of Xan, e.g. its relatively lower singlet oxygen quantum yield, are also discussed.

Keywords: Xanthurenic acid; Photosensitive properties; Reactive oxygen species; Time-dependent density functional theory

1. Introduction

The human lens, which aids in focusing incident light on the retina, is transparent, normally. With age, clouding of the lens occurs leading to the progressive loss of vision, termed as cataract [1]. It has been demonstrated that the cataract results, at least in part, from photochemical damage to the proteins of the lens [2].

The adult human lens absorbs UV radiation transmitted by the cornea in the 295–400 nm band to protect the human retina from photochemical damage and the lenses in children transmit some UV radiation to their retinas [3]. Some tryptophan metabolites, including kynurenine, 3-hydroxykynurenine and 3-hydroxykynurenine glucoside, have been identified as the major absorbing species in both the young and adult lens [4–8]. While these compounds are relatively inert photochemically [5], with age, the chromophores of the human lens change. It has been shown that the photochemically active *N*-formyl kynurenine is formed in the lens [9,10]; *N*-formyl kynurenine can photo-generate reactive oxygen species (ROS) and, thus, has been considered as an important photosensitive species involved in the photochemical induction of cataracts [9,10].

Recently, another new tryptophan metabolite, xanthurenic acid (Xan, Fig. 1), was isolated from cataractous human lenses by Malina and Martin [11]. Previous studies indicated that Xan can photoproduce singlet oxygen ($^1O_2$) and the superoxide anion radical ($O_2^{\bullet -}$) [12] and can promote photo-oxidation of lens protein [13]. Thus, it is of interest to further investigate the photosensitive properties of Xan. In view of the successful

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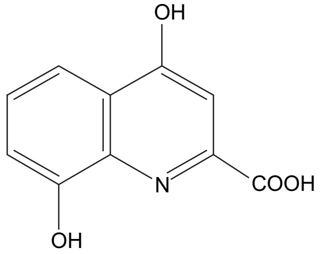

Fig. 1. Molecular structure of xanthurenic acid. The anionic form of Xan generated by proton dissociation from the carbonyl group was taken as the starting point in the present study.

use of time-dependent density functional theory (TD-DFT) in exploring the photo-physicochemical characters of various dyes and pigments [14–18], we attempted to elucidate the photosensitive mechanisms of Xan using theoretical calculations.

2. Methods

Considering the fact that Xan exists in the monoanion form generated by proton dissociation from the carboxyl group in solution, the anionic form was taken as the starting point of the present study. The calculation procedures were as follows: initial structures of Xan and its derived molecules were fully optimized in water by the hybrid B3LYP [19–21] functional with 6-31+G(d,p) Gaussian basis set. The excited-state properties in water were calculated using TD-DFT formalism with the same basis set [22–24]. To ensure accuracy of the calculation, the vertical electron affinities (VEAs) and vertical ionization potentials (VIPs) of Xan were calculated using a combined DFT method labeled as B3LYP/6-31++G(2d,2p)/B3LYP/6-31+G(d,p), which means that B3LYP/6-31++G(2d,2p) was employed to perform a single-point calculation on the basis of B3LYP/6-31+G(d,p)-optimized structures. For molecular oxygen-derived parameters, B3LYP/6-31++G(2d,2p) was employed to undertake full-geometry-optimization calculations. During the calculations, the solvent effects were taken into consideration by employing the self-consistent reaction field (SCRF) method and polarizable continuum model (PCM) [25–27]. All calculations were carried out using the Gaussian 03 package of programs [28].

3. Results and discussion

3.1. Photophysical properties of Xan

As we know, the photosensitizer is first raised from the ground state (S_0) to the first singlet excited state (S_1) upon irradiation, which may then come to reside in the first triplet excited state (T_1) through intersystem crossing. Hence, the S_1 and T_1 state properties of Xan were explored. Table 1 shows the main excitation energies, oscillator strengths and transition coefficients of Xan. It is evident that the theoretically predicted maximum absorption wavelength ($\lambda_{\max}$) (327 nm) of Xan mainly involves electronic transition from the HOMO to the LUMO (82.7%); as shown in Fig. 2, this can be assigned to $\pi \rightarrow \pi^*$ transition. Moreover, the theoretically estimated maximum absorption wavelength of Xan is close to the experimental value (334 nm) [12], which verifies the methodology employed. In addition, the second excitation energy results mainly from transition of the HOMO − 1 to the LUMO with negligible intensity (Table 1), so it does not contribute greatly to the spectral band appearance. In comparison, the third excitation energy arises mainly from the HOMO → LUMO + 1 transition.

Table 1
The main excitation energies (E), oscillator strengths (f) and transition coefficients of Xan in water

Excited state	Transition character	Weight	E (eV)	λ (nm)	f
S_1	HOMO → LUMO	0.643	3.79	327	0.093
	HOMO → LUMO + 1	−0.119			
S_2	HOMO − 1 → LUMO	0.673	3.93	316	0.000
	HOMO − 1 → LUMO + 1	−0.152			
S_3	HOMO − 4 → LUMO	−0.258	4.21	295	0.037
	HOMO − 3 → LUMO	−0.259			
	HOMO → LUMO	0.114			
	HOMO → LUMO + 1	0.581			

The lowest T_1 excitation energy (E_{T_1}) of a photosensitizer is crucial to understanding its photosensitive mechanisms, as the T_1 state is mainly responsible for the photosensitive reaction. As shown in Table 2, the E_{T_1} of Xan was calculated to be 2.59 eV. As there is no experimental value of the E_{T_1} of Xan, the present theoretically predicted data can be used in the following discussion, as the effectiveness and accuracy of the TD-DFT method in estimating the E_{T_1} of various photosensitizers have been proven [14–18].

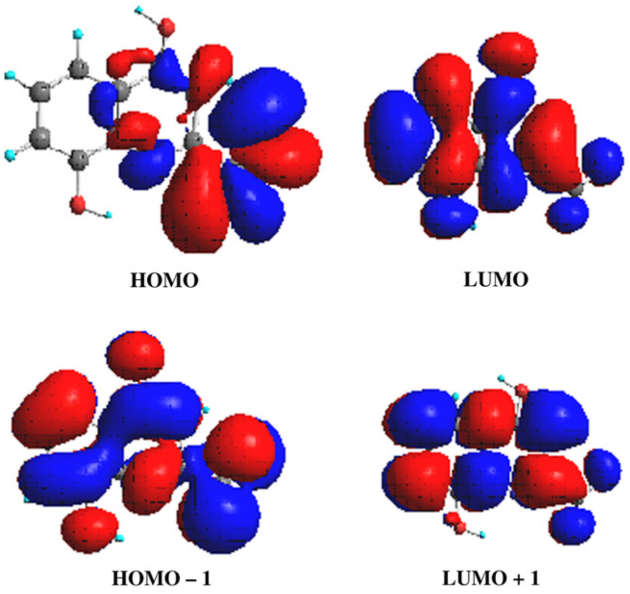

Fig. 2. Plots of the HOMO, LUMO, HOMO − 1, and LUMO + 1 molecular orbitals of xanthurenic acid in water.

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 xanthurenic acid in water

E_{T_1}	VEA_{S_0}	VEA_{T_1} [a]	VIP_{S_0}	VIP_{T_1} [b]
2.59	−1.79	−4.38	5.72	3.13

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

3.2. Photochemical properties of Xan

As mentioned above, residing in the long-lived T_1 state, Xan may undergo a series of phototoxic reactions with molecular oxygen to give birth to various ROS.

3.2.1. Direct energy transfer pathway

The first important ROS-generating pathway involves direct energy transfer between the T_1 state Xan and the ground state oxygen ($^3O_2$) to generate $^1O_2$ (Eq. (1)), one of the most important ROS.

$$\mathrm{Xan(T_1)} + {}^3\mathrm{O_2} \rightarrow \mathrm{Xan(S_0)} + {}^1\mathrm{O_2} \tag{1}$$

It can be seen from Table 2 that the E_{T_1} of Xan (2.59 eV) is higher than the excited-state energy of $^1O_2$ (1.05 eV for $^1\Delta_g$ state and 1.65 eV for $^1\Sigma_g^+$ state) [29], suggesting that Xan can photo-generate $^1O_2$ through direct energy transfer pathway. This is consistent with the experimental result that Xan can produce $^1O_2$ with a quantum yield of 0.17 when irradiated with UV light [12].

3.2.2. Direct electron transfer pathway

Secondly, through direct electron transfer pathway, T_1 state Xan can react with $^3O_2$ to generate superoxide anion radical ($O_2^{\bullet-}$) (Eq. (2)) with the prerequisite that the summation of VIP_{T_1} of Xan and the adiabatic electron affinity of $^3O_2$ (AEA_{O_2}) is negative.

$$\mathrm{Xan(T_1)} + {}^3\mathrm{O_2} \rightarrow \mathrm{Xan}^{\bullet+} + \mathrm{O_2^{\bullet-}} \tag{2}$$

According to the calculated results, the summation of VIP_{T_1} (3.13 eV, Table 2) and AEA_{O_2} (−3.81 eV) is negative, suggesting that $O_2^{\bullet-}$ can be generated through this pathway in water. This is in good agreement with the experimental observation that $O_2^{\bullet-}$ can be photo-generated by Xan in polar solvents [12]. The feasibility of this pathway benefits from the strong electron-donating group of the T_1 state Xan in its anionic form and similar photosensitive mechanisms of hypericin have also been observed in a previous study [18]. Moreover, considering the fact that the direct energy transfer and electron transfer pathways between the T_1 state Xan and $^3O_2$ are competitive and electron transfer is favoured in polar solvents, reaction (2) will predominate in comparison with reaction (1) for Xan, which accounts, at least in part, for the experimentally observed relatively lower $^1O_2$-yield of Xan in polar solvent [12].

3.2.3. Autoionization pathway

Besides the possible reactions with molecular oxygen, the Xan T_1 state may also react with the surrounding S_0 state (Eq. (3)) or T_1 state (Eq. (4)) Xan to generate the anion–cation radical couples of Xan, termed as autoionization reactions. Once the anion radical is formed, it could then pass one electron to $^3O_2$ and generate $O_2^{\bullet-}$ (Eq. (5)).

$$\mathrm{Xan(T_1)} + \mathrm{Xan} \rightarrow \mathrm{Xan}^{\bullet+} + \mathrm{Xan}^{\bullet-} \tag{3}$$

$$\mathrm{Xan(T_1)} + \mathrm{Xan(T_1)} \rightarrow \mathrm{Xan}^{\bullet+} + \mathrm{Xan}^{\bullet-} \tag{4}$$

$$\mathrm{Xan}^{\bullet-} + {}^3\mathrm{O_2} \rightarrow \mathrm{Xan} + \mathrm{O_2^{\bullet-}} \tag{5}$$

The VEA and VIP of Xan in S_0 and T_1 states have been calculated (Table 2), according to which, the probability of each reaction is estimated. For reaction (3), the summation of the VEA_{T_1} of Xan (−4.38 eV) and the VIP_{S_0} (5.72 eV) is positive and thus, the reaction is forbidden. In contrast, reaction (4) is permitted to give rise to $\mathrm{Xan}^{\bullet-}$ due to its negative reaction energy (VEA_{T_1}(−4.38 eV) + VIP_{T_1}(3.13 eV) = −1.25 eV). Subsequently, $\mathrm{Xan}^{\bullet-}$ can pass one electron to molecular oxygen to generate $O_2^{\bullet-}$ according to the present results. This accounts for the second $O_2^{\bullet-}$-generating pathway of Xan besides the direct electron transfer between the T_1 state Xan and $^3O_2$ pathway as discussed above [12]. As we know, once $O_2^{\bullet-}$ is generated, it can be converted into H_2O_2 and subsequently the highly reactive $^\bullet OH$ through the Fenton [30] or the Haber–Weiss reaction [31], in the presence of transition-metal ions, will amplify the photosensitizing activity of Xan.

4. Conclusions

Xan is a new photochemically active tryptophan metabolite isolated from cataractous human lenses, which has been assumed to be involved in photochemical damage of the human lens. Considering the successful application of theoretical methods in investigating the photo-physicochemical properties of various photosensitizers, we attempted to explore the photosensitive properties of Xan using the TD-DFT method. The results indicate that Xan can photo-generate singlet oxygen through direct energy transfer pathway, while it generates $O_2^{\bullet-}$ through both direct electron transfer and autoionization. Other photosensitive characters of Xan, e.g. its low singlet oxygen quantum yield, were also discussed. This offers some deeper insights into the photosensitive mechanisms of Xan.

Acknowledgments

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