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LETTER TO THE EDITOR

Quenching Reactions of Triplet State Riboflavin by Vitamin C: A Theoretical Investigation

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Sir,

Vitamin C (ascorbic acid), the water-soluble, small-molecule antioxidant, is of great biological significance [1, 2]. Previous studies [3, 4] demonstrated that vitamin C can inhibit the photo-oxidative damage caused by riboflavin which can induce Type-I and II photosensitized oxidation mechanisms [5–7]. Considering the fact that many antioxidants can inhibit the riboflavin's photosensitizing damage through deactivating its triplet state [7–9], it is of significance to investigate the reactions of vitamin C with triplet state riboflavin, which may contribute to the protective effect of vitamin C. In the present study, the quenching mechanisms of triplet state riboflavin by vitamin C were explored by means of density functional theory (DFT) calculations, which have been widely used in investigating both the antioxidant and photosensitization mechanisms of various bioactive compounds [7, 11–14].

The calculation methods are as follows. Firstly, molecular structures of vitamin C (monoanion form in neutral solution) and riboflavin were fully optimized by hybrid functional B3LYP with 6-31G(d,p) Gaussian basis set in vacuo. Then, the lowest triplet excitation energy (E_{T1}) of the two molecules was estimated by time-dependent (TD)-DFT with the same basis set in solvent (water). Moreover, as the diffuse functions are essential for treatment of anion and cation radicals, the vertical electron affinities (VEA) and vertical ionization potentials (VIP) of vitamin C and riboflavin were estimated using a combined DFT method labeled as B3LYP/6-31+G(d,p)//B3LYP/6-31G(d,p), which means that B3LYP/6-31+G(d,p) was used to perform a single-point calculation on the basis of B3LYP/6-31G(d,p)-optimized structures. The O-H bond dissociation enthalpy (BDE) of vitamin C and H-atom affinity (HAA) of riboflavin were calculated by (RO)B3LYP/6-311+G(2d,2p)//AM1 method, which takes advantages of accuracy and economy [11]. During the single point calculations, the solvent (water) effect was taken into consideration by employing the self-consistent reaction field (SCRF) method with the polarizable continuum model (PCM) of Tomasi and coworkers [15–17]. All the calculations were accomplished with the Gaussian 03 package of programs [18].

During the photosensitization, riboflavin is initially excited to the singlet state upon irradiation and then may intersystem cross to the relatively long-lived triplet state. Triplet state riboflavin can react with molecular oxygen to photogenerate various reactive oxygen species [5–7] and at the same time, it can be deactivated by vitamin C, theoretically through the following possible pathways.

Firstly, triplet state riboflavin (RF) may react with vitamin C (VC) through direct energy transfer (Eq. 1).

$$\mathrm{RF(T_1)} \rightarrow \mathrm{VC} \rightarrow \mathrm{RF} + \mathrm{VC(T_1)} \quad (1)$$

To explore the feasibility of this pathway, the E_{T1} of riboflavin and vitamin C have been calculated using the TD-DFT method, whose accuracy in estimating the T_1 state properties of various photosensitizers has been verified [7, 12–14]. It can be seen that the theoretical E_{T1} of vitamin C (71.52 kcal/mol) is much higher than that of riboflavin (48.20 kcal/mol) (Table 1), indicating that the direct energy transfer quenching pathway (Eq. 1) is not favorable on thermodynamic grounds.

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Table 1 Theoretically estimated lowest triplet excitation energy (E_{T1}), vertical electron affinity (VEA), vertical ionization potential (VIP), bond dissociation enthalpy (BDE) and H-atom affinity (HAA) of vitamin C and riboflavin in ground (S_0) and triplet (T_1) state (in kcal/mol)

	E_{T1}	VEA_{T1}[a]	VIP	BDE[b]	HAA^{c}_{T1}
Vitamin C	71.52		122.14	68.71	
Riboflavin[d]	48.20	−124.75			−106.19

[a] $VEA_{T1} = VEA_{S0} - E_{T1}$

[b] H-atom abstraction from hydroxyl at position 2

[c] $HAA_{T1} = HAA_{S0} - E_{T1}$

[d] Ref [10]

Secondly, triplet state riboflavin may react with vitamin C through direct electron transfer (Eq. 2).

$$RF(T_1) \rightarrow VC \rightarrow RF^{\cdot-} + VC^{\cdot+} \qquad (2)$$

The precondition is that the total reaction energy is negative. According to the theoretical results, the summation of VEA_{T1} riboflavin and VIP of vitamin C is negative, implying that the electron transfer quenching pathway (Eq. 2) is permitted from the thermodynamic point of view. The feasibility of the direct electron transfer quenching pathway benefits to a large extent from the high electron-donating ability of vitamin C in anion form in solution, while our previous study revealed that this pathway is thermodynamically unfeasible during the triplet state riboflavin quenching by xanthone derivatives [10]. Moreover, through this electron transfer quenching pathway (Eq. 2), $RF^{\cdot-}$ is formed in solution. $RF^{\cdot-}$ will readily react with $^3O_2$ to generate $O_2^{\cdot-}$ [7], which can then be scavenged by vitamin C.

Thirdly, as the hydroxyls of vitamin C are potential H-atom donating groups and thus, T_1 state riboflavin may be quenched by vitamin C through H-atom transfer reaction. The O-H BDE, which has been successfully used to measure the molecular H-atom-donating ability [11], of the three hydroxyls of vitamin C have been calculated and it can be inferred that the hydroxyl at position 2 (Fig. 1) is the one which will most readily donate a H-atom for its lowest BDE (68.71 kcal/mol). At the same time, HAA is an appropriate theoretical parameter to characterize the molecular H-atom-abstracting ability [11]. The HAA of T_1 state riboflavin at N_1 (Fig. 1), which has been reported to be the thermodynamically favorable position to accept a H-atom [8], is estimated to be −106.19 kcal/mol. As the summation of the HAA_{T1} of riboflavin and the O-H BDE of vitamin C is negative, the H-atom transfer reaction from vitamin C to T_1 state riboflavin can occur thermodynamically as illustrated in Fig. 1. In addition, the present finding may also be helpful to understand the riboflavin-sensitized photooxidation of vitamin C to its radical form (Fig. 1) by electron spin resonance (ESR) spectroscopy [19].

In summary, the present theoretical analysis indicates that vitamin C can quench triplet state riboflavin through direct electron transfer and H-atom transfer pathways, which may be involved in the protective effect of vitamin C against the riboflavin induced photo-oxidative damage. Moreover, according to the theoretical results, the H-atom transfer pathway seems more favorable compared with the direct electron transfer pathway. However, the reaction kinetics might be strongly influenced by the solvent effects (e.g., solvent polarity, intermolecular hydrogen bond) and thus, these factors should be taken into account when

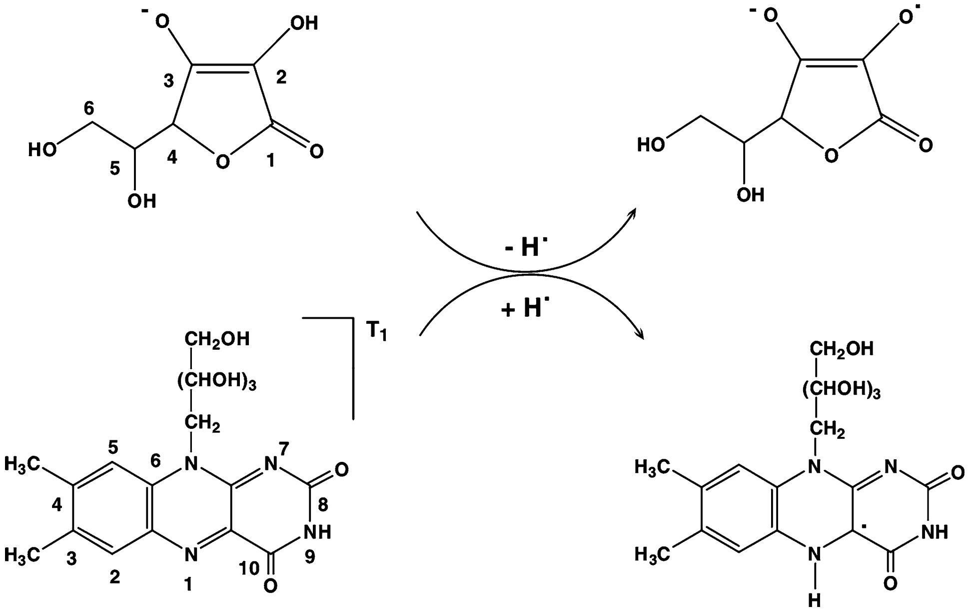

Fig. 1 H-atom transfer-based quenching pathway of triplet state riboflavin by vitamin C

exploring the role of vitamin C as a quencher of triplet state riboflavin.

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