

Article

A DFT Study on Deactivation of Triplet Excited State Riboflavin by Polyphenols

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Abstract: The deactivation of triplet excited state riboflavin by polyphenols, *e.g.* rutin and catechin, was studied on the basis of density functional theory calculations. The results show that the H-atom transfer pathway is more feasible on thermodynamic grounds in comparison with the direct energy transfer or direct electron transfer pathways involved in the triplet excited state riboflavin deactivation by rutin/catechin. The findings are helpful to understand the protective effect of polyphenols against the riboflavin induced photosensitizing damage.

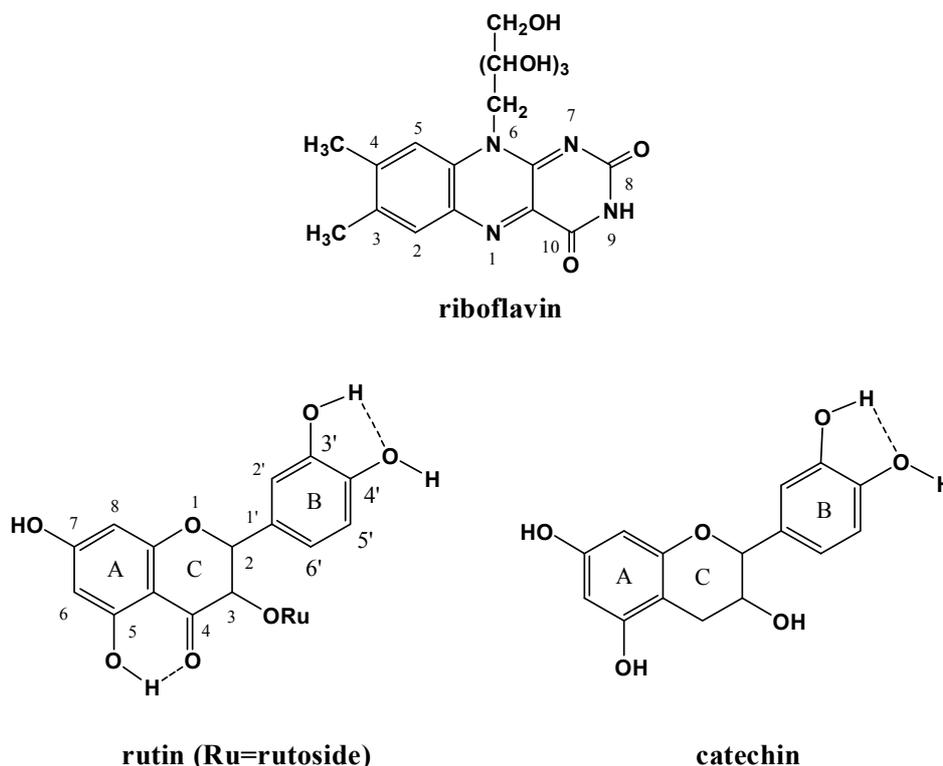
Keywords: Riboflavin, polyphenols, triplet excited state, deactivation, density functional theory.

1. Introduction

Numerous endogenous photosensitizers, among which riboflavin has attracted much attention, can photogenerate various reactive oxygen species (ROS, *e.g.* $^1\text{O}_2$ and $\text{O}_2^{\cdot-}$) [1, 2]. It has been reported that riboflavin is an efficient ROS-generator [1, 3, 4] and can cause photosensitizing DNA damage [5, 6]. Polyphenolic compounds, *e.g.* rutin and catechin (Figure 1), are ideal antioxidants with strong free radical-scavenging ability. Recently, it was reported that rutin and catechin play dual roles in

protecting from the photosensitizing damage caused by riboflavin, that is, as ROS scavengers and triplet excited (T_1) state riboflavin quenchers [7]. The free radical-scavenging mechanisms of rutin and catechin have been investigated before [8], however, more effort is needed to elucidate the deactivating mechanisms of T_1 state riboflavin by rutin/catechin. In recent years, density functional theory (DFT) calculations have been widely used to study both the photosensitization and deactivation mechanisms of excited state photosensitizers [4, 9-12]. Therefore, in the present study, we attempt to explore how T_1 state riboflavin was deactivated by rutin/catechin by means of theoretical calculations.

Figure 1. Molecular structures of riboflavin, rutin and catechin.



2. Theoretical Methods

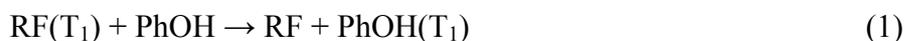
The calculation procedures are as follows. First, the geometries of riboflavin, rutin and catechin were fully optimized by DFT [14, 15] and B3LYP functional [16-18] with 6-31G(d,p) Gaussian basis set *in vacuo*. Then, the lowest T_1 excitation energies (E_{T1}) of the three molecules were estimated by time-dependent DFT (TD-DFT) [19-21] with the same basis set. Moreover, in view of the fact that the diffusion functions are crucial for treatment of anion and cation radicals, the vertical electron affinities (VEA) and vertical ionization potentials (VIP) of riboflavin, rutin and catechin were calculated by 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 using B3LYP/6-31G(d,p)-optimized geometries [10]. The O-H bond dissociation enthalpy (BDE) of rutin/catechin and H-atom affinity (HAA) of riboflavin were obtained by a hybrid method combining DFT and semiempirical method AM1, labeled as (RO)B3LYP/6-311+G(2d,2p)/AM1, which takes advantages of accuracy and economy [8]. The solvent (benzene and water) effects were taken into account by employing the self-

consistent reaction field (SCRF) method with polarizable continuum model (PCM) [22-24] for the single point calculations. All the calculations were accomplished using the Gaussian 03 package of programs [25].

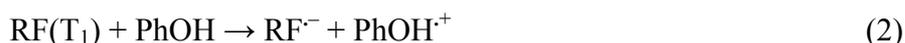
3. Results and Discussion

As we know, the ground state photosensitizer is initially excited to the singlet excited state upon irradiation and then may intersystem cross to the relatively long-lived T_1 state. T_1 state riboflavin can react with molecular oxygen to photogenerate various ROS [1, 3, 4] and at the same time, it can be deactivated by antioxidants through the following possible pathways:

The first deactivating pathway may proceed through the direct energy transfer between T_1 state riboflavin (RF) and polyphenols (PhOH) (Equation 1).



The second deactivating pathway involves the electron transfer between T_1 state riboflavin and polyphenols (Equation 2).



Moreover, as the polyphenolic antioxidants are ideal H-atom donors [8], T_1 state riboflavin may be deactivated by polyphenols through a H-atom transfer process (Equation 3).



Therefore, the corresponding electronic parameters of riboflavin, rutin and catechin, including E_{T_1} , VEA, VIP, O-H BDE and HAA, were estimated and listed in Table 1, according to which, the deactivating reactions of T_1 state riboflavin by rutin/catechin were analyzed.

Table 1. Theoretically estimated lowest triplet excitation energy (E_{T_1} , in eV), vertical electron affinity (VEA, in eV) and vertical ionization potential (VIP, in eV) of polyphenols (rutin and catechin) and riboflavin in benzene and water.

Compounds	Solvents	E_{T_1}	VIP _{S0}	VEA _{S0}	VEA _{T1} ^a
Rutin	benzene	3.12	6.76		
	water	3.13	5.95		
Catechin	benzene	3.62	6.49		
	water	3.63	5.82		
Riboflavin^b	benzene	2.10		-2.52	-4.62
	water	2.09		-3.32	-5.41

^a VEA_{T1} = VEA_{S0} - E_{T_1} ;

^b data from ref [4].

Primarily, the E_{T_1} of riboflavin, rutin and catechin have been calculated using TD-DFT methods, whose accuracy in estimating the T_1 state properties of various photosensitizers has been verified [4, 9-13]. It can be seen that the theoretical E_{T_1} of rutin/catechin is much higher than that of riboflavin

(Table 1), implying that the direct energy transfer-based deactivating pathway (Equation 1) is not feasible on thermodynamic grounds in both solvents.

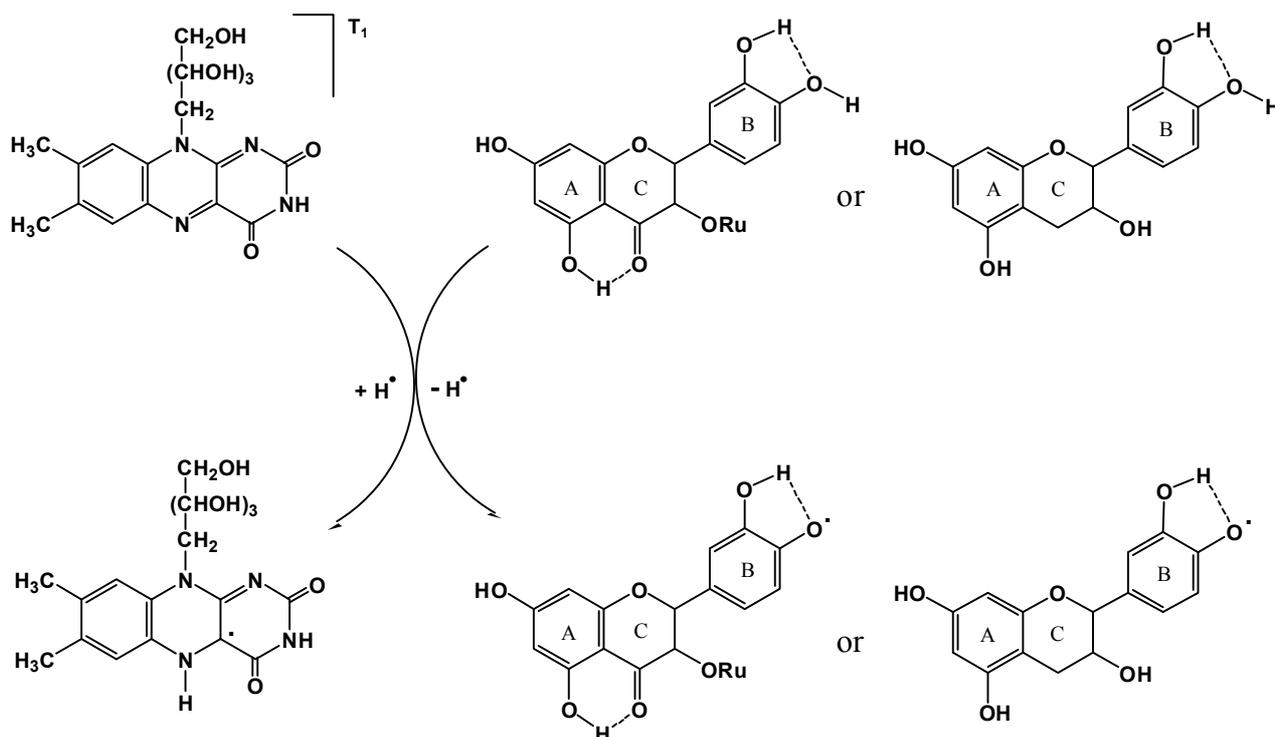
As to the direct electron transfer-based deactivating pathway (Equation 2), its feasibility depends on the VEA of T_1 state riboflavin (VEA_{T_1}) and VIP of rutin/catechin. According to the theoretical results, the summation of VEA_{T_1} of riboflavin and VIP of rutin/catechin is positive both in benzene and water, implying that the electron transfer-based deactivating pathway is also not favorable from the thermodynamic point of view.

Table 2. Theoretically estimated O-H bond dissociation enthalpy (BDE, in kcal/mol) of the phenolic compounds (rutin and catechin) and T_1 state H-atom affinity (HAA_{T_1} , in kcal/mol) of riboflavin in benzene and water.

Compounds	Solvents	O-H BDE	$HAA_{T_1}^a$
Rutin	benzene	78.18	
	water	79.97	
Catechin	benzene	78.97	
	water	80.73	
Riboflavin	benzene		-97.24
	water		-106.19

$$^a HAA_{T_1} = HAA_{S_0} + E_{T_1}.$$

Figure 2. Theoretically postulated H-atom transfer-based triplet excited state riboflavin deactivating pathway by rutin/catechin.



Thirdly, rutin and catechin are excellent H-atom donating substrates [8]. To explore whether the H-atom transfer reaction from rutin/catechin to T₁ state riboflavin (Equation 3) can occur or not, the O-H BDE of rutin and catechin, which has been successfully used to measure the molecular H-atom-donating ability [8], and the HAA of riboflavin, an appropriate theoretical parameter to characterize the molecular H-atom-abstracting ability [8], have been calculated. Despite the fact that rutin and catechin possess several phenolic hydroxyls that may donate H-atoms, previous studies demonstrated that the hydroxyl at position 4' (Figure 1) is the most active one [26] and the corresponding O-H BDE in benzene and water is listed in Table 2. The theoretically estimated HAA of T₁ state riboflavin at N1 (Figure 1), which has been reported to be the thermodynamically favorable position to accept a H-atom [27], is -97.24 kcal/mol in benzene and -106.19 kcal/mol in water (Table 2). As the summation of HAA_{T₁} of riboflavin and the O-H BDE of rutin/catechin is negative in both solvents, the H-atom transfer-based quenching pathway is thermodynamically feasible. Therefore, the H-atom transfer-based T₁ state riboflavin deactivating mechanism by rutin/catechin is proposed as illustrated in Figure 2.

4. Conclusions

In summary, through comparing the electronic parameters of riboflavin, rutin and catechin, including E_{T1}, VEA, VIP, BDE and HAA, it can be inferred that the H-atom transfer pathway is more feasible on thermodynamic grounds relative to the direct energy transfer or direct electron transfer pathways responsible for the T₁ state riboflavin deactivation by rutin/catechin. The results have important implications to design/screen better polyphenolic antioxidants as protectors against the photo-oxidative damage induced by riboflavin.

Acknowledgements

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