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A theoretical study of the different radical-scavenging activities of catechin, quercetin, and a rationally designed planar catechin

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Abstract

To improve the radical-scavenging activity of catechin, a planar catechin analogue was designed and synthesized by Fukuhara [J. Am. Chem. Soc. 124 (2002) 5952]. Although the planar catechin is less active than quercetin, it is much more active than catechin in its ability to scavenge galvinoxyl radical, suggesting that the rational design was successful. However, an interesting question remains: what is the basis for the enhanced radical-scavenging activity of the planar catechin? By DFT calculations, we determined that the galvinoxyl radical is scavenged through an electron-transfer mechanism rather than a hydrogen-atom-transfer mechanism. Moreover, the antioxidant anion, derived from proton dissociation, plays a key role in the radical-scavenging process. Hence, the different radical-scavenging activities of the three antioxidants may result from the different ionization potentials of their anions.
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Keywords: Antioxidant; Catechin; Density functional theory; Hydroxyl bond dissociation enthalpy; Ionization potential; Hydroxyl proton dissociation enthalpy; Solvent effect

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1. Introduction

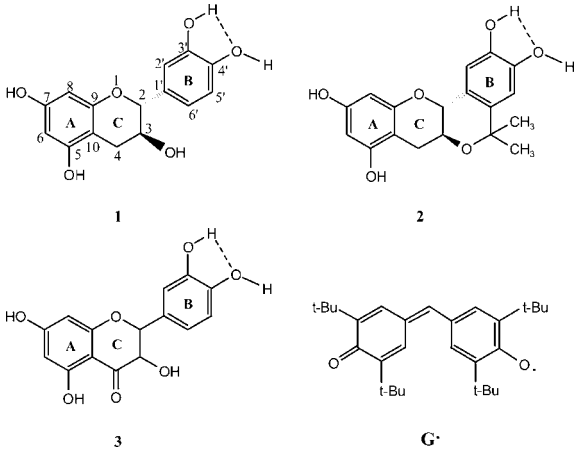
Catechin (**1**, Scheme 1), the main component of green tea polyphenols, has received much attention due to its high antioxidant activity and potential application in preventing radical-induced diseases [1–3]. To further enhance its radical-scavenging activity and reduce its prooxidant toxicity, a planar catechin analogue (**2**, Scheme 1) was designed and synthesized [4,5], based on the well-known structural feature of quercetin (**3**, Scheme 1) where the 2,3-double bond and the planarity of the ring system stabilize the radical generated after a H-atom-donating reaction [6,7]. It was postulated that the **2**-derived radical (at position 4′) would be stabilized through hyperconjugation between the π electrons on ring B and the σ electrons on C2 of ring C [4].

The rational design was successful, because although **2** is less active than **3**, it is much more active than **1** in its ability to scavenge the galvinoxyl radical (G·) in deaerated acetonitrile and much safer than **1** with respect to its prooxidant toxicity [4,5]. However, an interesting question remains: what is the basis for the enhanced radical-scavenging activity of **2**? The answer to the question will give insight into the radical-scavenging mechanisms of phenolic antioxidants and will assist in the design of better phenolic antioxidants. In view of the successful use of theoretical methods in clarifying the radical-scavenging mechanisms and elucidating the structure–activity relationships (SAR) for various antioxidants [8–16], we explored the different activities of the three phenolic antioxidants by theoretical calculations. Our results are reported herein.

2. Methods

2.1. Theoretical models

At least two mechanisms are involved in the radical-scavenging properties of phenolic antioxidants (ArOH). One involves a direct H-atom transfer



Scheme 1. Structures of catechin (1), planar catechin (2), quercetin (3), and the galvinoxyl radical (G·).

Eq. (1) and the other involves a proton-coupled electron-transfer pathway Eq. (2).

$$G^{\bullet} + ArOH \rightarrow GH + ArO^{\bullet} \quad (1)$$

$$G^{\bullet} + ArOH \rightarrow G^{-} + ArOH^{\bullet+} \rightarrow GH + ArO^{\bullet} \quad (2)$$

Both mechanisms are solvent-dependent [17] and may co-exist in a certain chemical or biological systems [18]. The first one is preferred in non-polar solvents [19–22], and the reaction is governed by the bond dissociation enthalpies (BDEs) of the phenolic antioxidants and GH [23–28]. To a first approximation, if the BDE of the former is less than that of the latter, the reaction is permitted. The relative ratio of $G^{\bullet}$ and ArOH also influences the reaction. In polar solvents, an intermolecular hydrogen bond can form between the phenolic hydroxyl, especially a catechol group, and the solvent molecule. This hydrogen bond will hamper the H-atom-donating process [29–32] and facilitate the second pathway [33–35]. In this case, the electron-transfer reaction is the rate-controlling step, and the corresponding theoretical parameters are the ionization potentials (IPs) for the phenolic antioxidants and G^{-} [28]. The prerequisite for the reaction is that the IP of the phenolic antioxidants is lower than that of G^{-}. For both pathways, the lower these parameters are, the more active the phenolic antioxidants.

2.2. Calculation methods

In this paper, BDEs and IPs were calculated by two kinds of combined density functional theory (DFT) methods, labeled as (RO)B3LYP/6–311+G(2d,2p)//AM1/AM1 [28,36] and B3LYP/6–31G(d)//AM1/AM1 [37,38], respectively, which take advantage of accuracy and economy. The detailed calculation procedures are as follows. During the calculation of BDEs, the geometry optimization and the determination of vibrational frequencies were performed using the semiempirical AM1 method [39]. Then, single-point electronic energies (SPEs) were obtained by DFT methods using (RO)B3LYP functional [40,41] at 6–311+G(2d,2p) level. Employing the molecular enthalpy in gas-phase at 298.15 K, which consists of (RO)-B3LYP/6–311+G(2d,2p)-calculated SPE, AM1-calculated zero point vibrational energy (ZPVE, scaled by a factor of 0.973) [28,36], vibrational contribution to energy (scaled by a factor of 0.973) [28,36], translational, rotational, and PV-work terms, hydroxyl BDE equals $H_r + H_h - H_p$, in which, H_r is the enthalpy of radical generated after H-abstraction reaction, H_h is the enthalpy of hydrogen-atom, −0.49765 hartree, and H_p is the enthalpy of parent molecule. During the calculation of IPs, B3LYP functional at 6–31G(d) level was used to calculate SPE on the basis of AM1-optimized structure. Thus, the molecular energy (E) in gas-phase consists of B3LYP/6–31G(d)-calculated SPE and AM1-calculated ZPVE (scaled by a factor of 0.973) [37,38]. The ionization potential $= E_c - E_p$, where E_c is the energy for cation radical and E_p is the energy for parent molecule. DFT methods failed to give accurate hydroxyl BDEs for *ortho*-*tert*-butyl-substituted phenols [28,42], so for the calculation of $G^{\bullet}$-related parameters four *tert*-butyl groups were replaced by four

methyl groups. Since the electronic effect of methyl group is very similar to that of *tert*-butyl group [43], the substitution does not change the result. During the calculation, intramolecular hydrogen bond (IHB) was considered to give the most stable conformation of each molecule. The solvent (acetonitrile) effect was taken into consideration in all of the calculations by employing the self-consistent reaction field (SCRF) method with a polarized continuum model (PCM). The effectiveness of the model has been verified in previous studies [44,45]. All of the quantum chemical calculations were accomplished by the Gaussian 98 program [46].

3. Results and discussion

3.1. H-atom-transfer pathway

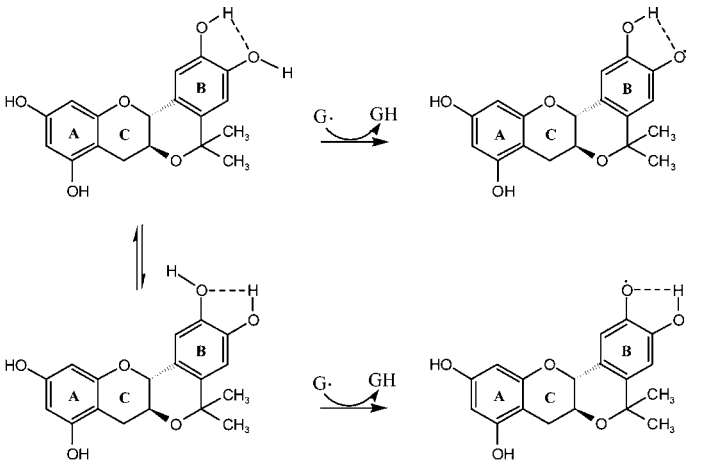
The hydroxyl BDEs for each hydroxyl group of three phenolic antioxidants were calculated and are listed in Table 1. It can be seen that for **2** and **3**, the hydroxyl groups of ring B have the lowest BDEs, which results from the fact that the radical derived from H-atom abstraction can be stabilized by the electron-donating property of the *ortho* hydroxyl and the intramolecular hydrogen bonds (Scheme 2) [9,10,28,47]. However, the BDE of hydroxyl group of ring B of catechin is comparable to that of the 5-hydroxyl group, which is in disagreement with previous in-vacuo results [48] and suggests that the unexpected high BDEs stem from the solvent effect. Due to the steric repulsion between rings B and C, ring B of **1** is twisted out of the plane by 70°, in contrast with the planar structure of **2** and **3**, which likely leads to the different solvent effects on the BDE of the phenolic antioxidants. As the intermolecular hydrogen bonds between three flavonoids' hydroxyls and MeCN are estimated to be ~4 kcal/mol, the actual hydroxyl BDEs for the three flavonoids in acetonitrile will increase ~4 kcal/mol. The hydroxyl BDE of GH was calculated to be 78.34 kcal/mol, which is several kcal/mol lower than those of **1**, **2**, and **3**. Although the concentrations of the three phenolic antioxidants were ~100 times higher than that of G$^{\cdot}$ during the experiments [4], the H-atom-transfer reactions between **1**–**3** and G$^{\cdot}$ are not thermodynamically favorable. Therefore, it is unlikely that

Table 1
Hydroxyl BDEs (in kcal/mol) for catechin, planar catcehin, and quercetin in acetonitrile

	Catechin	Planar catechin	Quercetin
O-H BDE (3′-OH)[a]	85.15	79.85	81.14
O-H BDE (4′-OH)[a]	85.78	79.69	79.64
O-H BDE (5-OH)[a]	85.01	82.82	—[b]
O-H BDE (7-OH)[a]	87.25	87.73	90.88

[a] The hydroxyl group likely to donate a H-atom is indicated in parenthesis.

[b] Not calculated, because the 5-hydroxyl group forms an intramolecular hydrogen bond with 4-oxo, which results in a higher BDE for the 5-hydroxyl group (15 kcal/mol higher than that of the 4′-hydroxyl group) [9,26]. Thus, the 5-hydroxyl group can be neglected with respect to the H-atom-donating reaction.



Scheme 2. Possible H-atom-abstracting mechanism of planar catechin.

the G·-scavenging reaction for the three phenolic antioxidants occurred through H-atom-transfer pathway. Hence, other pathways were considered to explain the difference in activities of the three phenolic antioxidants.

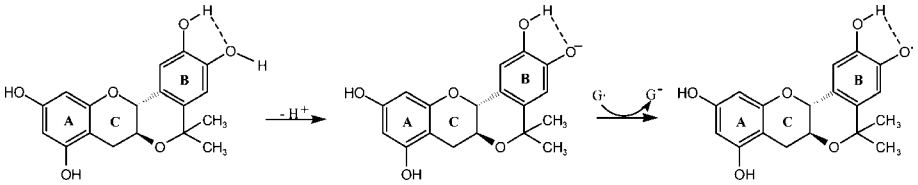
3.2. Electron-transfer pathway

An electron-transfer rather than a H-atom-transfer reaction has been demonstrated for G·-scavenging process of catechin in acetonitrile or propionitrile [35]. To compare the electron-transfer ability of the three phenolic antioxidants, their IPs were calculated. As shown in Table 2, the IPs for the three parent phenolic antioxidants are ~30 kcal/mol greater than that of G^-, 99.87 kcal/mol, implying that the proposed electron-transfer pathway is still forbidden in acetonitrile. Nevertheless, Litwinienko and Ingold [49] recently proposed that phenolic anions play a crucial role in scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in alcohols, due

Table 2
Ionization potentials (in kcal/mol) for catechin, planar catcehin, quercetin, and their anions in acetonitrile

	Catechin	Planar catechin	Quercetin
IP (parent)	132.19	134.45	131.66
IP (3′-O^-)[a]	106.58	99.94	97.85
IP (4′-O^-)[a]	105.68	100.22	97.86
IP (3-O^-)[a]	—[b]		96.88
IP (5-O^-)[a]	101.46	101.52	—[c]
IP (7-O^-)[a]	101.89	100.80	109.20

[a] The hydroxyl group indicated in parenthesis loses a proton.
[b] Not calculated, because the 3-hydroxyl group is not a phenolic hydroxyl and thus cannot lose a proton.
[c] Not calculated, because the 5-hydroxyl group forms an intramolecular hydrogen bond with 4-oxo, which makes dissociation negligible. Thus, it can be neglected with respect to the deprotonation reaction.



Scheme 3. Proton-dissociation coupled with electron transfer mechanism of planar catechin.

to their extremely high electron-donating ability. In addition, our study showed that the anions of phenolic antioxidants, derived from proton dissociation, had to be considered to determine the difference in the radical-scavenging activity of phenolic antioxidants in water [45]. As a polar and hydrogen-accepting solvent, acetonitrile will facilitate proton dissociation of the three phenolic antioxidants, so that the phenolic antioxidant-derived anions are perhaps responsible for the electron-transfer mechanism (Scheme 3). As expected, the lowest IPs for the three phenolic antioxidant-derived anions are comparable with that of G^- (Table 2), and decline in the order: $\mathbf{1}^- > \mathbf{2}^- > \mathbf{3}^-$, consistent with the G•-scavenging activity sequence: $\mathbf{3} > \mathbf{2} > \mathbf{1}$. In addition, the first p*K*a value of **1** and **3** was determined to be 9.02 and 7.03, respectively [50,51]. The lower p*K*a of **3** likely contributes to its higher activity.

It is interesting to note that the lowest IP in $\mathbf{3}^-$ is for 3-O^-, suggesting that the 3-hydroxyl group plays an important role in enhancing quercetin's antioxidant activity in polar solvents, which is in accord with the structure–activity relationships for flavonoid antioxidants, in which the 3-hydroxyl group and the 1,4-pyrone enhance activity [1,2,6]. Previous studies of flavonoids focused on BDE and non-polar-solvent experiments [9,10,47,48], where the effect of the 3-hydroxyl and the 1,4-pyrone could not be understood. The present finding indicates that the 3-hydroxyl group and the 1,4-pyrone exert some effects in polar solvents and especially in proton-associated electron-transfer pathway to scavenge radicals.

4. Conclusion

The DFT calculations show that the three flavonoids (**1**, **2**, and **3**) scavenge G• in acetonitrile through an electron-transfer mechanism rather than a H-atom-transfer mechanism. Moreover, it is the flavonoid-derived anions that are responsible for the G•-scavenging process. As a result, the different radical-scavenging ability of the flavonoids ($\mathbf{3} > \mathbf{2} > \mathbf{1}$) are related to the IPs of the anions. However, it should be pointed out that in non-polar solvents, **2** may not be more active than **1**, because proton dissociation is not possible and the in-vacuo hydroxyl BDEs for **1** and **2** are very similar to each other (77.29 kcal/mol vs 77.48 kcal/mol).

As the anion-based radical-scavenging mechanism was neglected in most previous studies, our finding is important in understanding the structure–activity relationships of antioxidants and in the rational design of novel phenolic antioxidants. The anion-based mechanism may be ubiquitous in polar solvents, because compounds other

than catechol-containing flavonoids, such as phenolic acids [45] and monophenols [49], have been shown to have such a mechanism in polar environments.

Acknowledgments

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