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Theoretical investigation on the antioxidative activity of anthocyanidins: A DFT/B3LYP study

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ABSTRACT

Anthocyanidins are an important class of plant pigment, in the present work the antioxidative properties of anthocyanidins have been explored by density functional theory calculations, three main antioxidative mechanisms, which include H atom transfer (HAT), single electron transfer (SET) and sequential proton loss electron transfer (SPLET), have been investigated at B3LYP/6-311G(d,p) level of theory. The O–H bond dissociation enthalpies (BDEs), ionization potentials (IPs), electron affinities (EAs), proton affinities (PAs) and electron transfer enthalpies (ETEs) are investigated in gas phase and aqueous solution. Results show 3-OH and 4′-OH posses lower BDE as compared to other OH groups, the substituents in B-ring influence 4′-OH BDE, but exhibit negligible influence on other OH BDEs. Among all investigated anthocyanidins, pelargonidin has the highest IP and EA in gas phase, substituent in *ortho* position of 4′-OH leads to remarkable decrease in IP and EA. OH substituent in B-ring almost show no influence on PAs and ETEs of 3-, 5-, and 7-phenolate anions, but OCH_3 substituent influence them significantly. For PAs and ETEs of 4′-phenolate anion, two types of substituents both exhibit remarkable influence. Base on the simplest anthocyanidins, pelargonidin, molecular simplification has been performed to explore the necessary pharmacophores responsible for the antioxidative activity of anthocyanidins.

1. Introduction

Natural polyphenolic compounds are considered to be health-promoting phytochemicals as they possess excellent antioxidant activity and exhibit beneficial antibacterial, antiglycemic, antiviral, anticarcinogenic, anti-inflammatory properties [1,2]. Anthocyanidins, derivatives of the 2-phenylbenzopyrylium cation (Fig. 1), are a class of natural polyphenol which are often found in cranberries and many other brilliantly colored fruits and vegetables [3]. Up to now there are reports of 23 anthocyanidins, among which only six are the most common in vascular plants, they are pelargonidin, cyanidin, delphinidin, malvidin, peonidin and petunidin [4] (shown in Fig. 1). The color properties and disease-preventing virtues of anthocyanidins make them particularly attractive candidates to replace artificial food colorants, and furthermore, the color intensity of these compounds can be enhanced by the intermolecular copigmentation between anthocyanidins and other colorless compounds [5].

Antioxidative activity is an important property of anthocyanidins, many experimental works have proved that the antioxidative capacities of pigmented vegetables and fruits highly correspond with the content of anthocyanidins and its glycoside form, i.e. anthocyanins [6–9]. In some reports anthocyanidins have exhibited even more excellent antioxidative activity than vitamins C and E [10], and outstanding radical-scavenging capacity [11]. The antioxidative activities of them are believed to be significant contribution to the other various bioactivities, for instance, prevention of cancer, diabetes, and cardiovascular and neurological disease, as well as anticarcinogenic activity [12–18].

Many theoretical works have been performed to study of the structural and electronic characteristics of anthocyanidins [19–24], the electron distribution and electronic spectra have also been investigated by quantum chemical computations. For many polyphenolic antioxidants, a great deal of works have been published to elucidate their antioxidative mechanism [25–30], but for anthocyanidins, the antioxidative activities and the structure–activity relationship has been seldom investigated [31,32], the mechanism by which the anthocyanidins exhibit excellent antioxidant capacity is still not clear, further discussion should be presented in forthcoming studies. In the present study the antioxidative activities of six anthocyanidins have been investigated at DFT-B3LYP/6-

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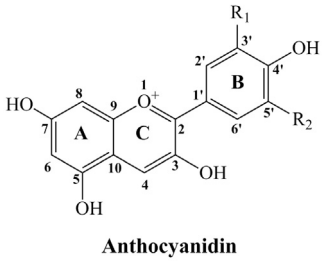
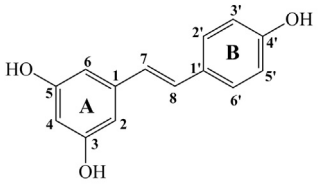
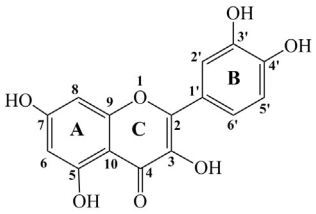


Anthocyanidin

Anthocyanidin	R_1	R_2
Pelargonidin(Pg)	H	H
Cyanidin(Cy)	OH	H
Delphinidin(Dp)	OH	OH
Malvidin(Mv)	OMe	OMe
Peonidin(Pn)	OMe	H
Petunidin(Pt)	OMe	OH



Resveratrol



Quercetin

Fig. 1. Molecular structures of investigated anthocyanidins, resveratrol and quercetin.

311G(d,p) level of theory, based on three prevalent radical-scavenging mechanisms, i.e., H atom transfer (HAT) mechanism, single electron transfer (SET) mechanism and sequential proton loss electron transfer (SPLET) mechanism. The phenolic OH bond dissociation enthalpies (BDEs), ionization potentials (IPs) and electron affinities (EAs), proton affinities (PAs) and electron transfer enthalpies (ETEs) were calculated to elucidate the radical-scavenging capacity of anthocyanidins. Though the reaction between antioxidant and free radical is not only determined by the properties of antioxidants but also governed by the characteristics of the scavenged radical, it is feasible to make a valid judgement about the thermodynamic favorability of radical-scavenging reaction according to these calculated quantities of anthocyanidins. Furthermore, the effect of substituent on the antioxidative activity of anthocyanidins, the relationships of molecular structures and antioxidative capacity are also investigated. In addition, the antioxidative properties of two outstanding natural antioxidants, i.e. resveratrol and quercetin (displayed in Fig. 1), have also been comparatively investigated to discuss the antioxidative performance of anthocyanidins.

2. Computational methods

All computational works in this paper are performed with the Gaussian 03 W suite of programs [33]. Geometries of all chemical systems are fully optimized without symmetry constraints by employing Becke's three-parameter hybrid functional B3LYP [34,35] using the standard 6-311G(d,p) basis set, unrestricted formulation is used for radical species. All optimized structures are confirmed to be real minima by frequency calculation at the same level of theory (no imaginary frequency), unscaled zero-point energies (ZPE) are abstracted from frequency analysis to make thermochemical correction to electronic energies and enthalpies of all studied species.

In HAT mechanism the homolytic bond dissociation enthalpies (BDEs) of O–H bond are used as the energetic parameter to evaluate the feasibility of H atom transfer process, relatively low O–H BDE facilitates the H atom transfer reaction between antioxidant and radical to break radical chain reaction. In previous studies SET mechanism is usually understood as single electron transfer from antioxidant to free radical, and it is governed by the adiabatic ionization potential (IP) of antioxidant molecule [36]. Recently, electron transferring from free radical to antioxidant has also been described as SET antioxidative mechanism, and electron affinity (EA) is used to evaluate the electron-accepting capacity of antioxidant [37–39]. In this work, two single electron transfer pathways are both considered to investigate the SET mechanism. In SPLET mechanism heterolytic cleavage of O–H bond firstly happens to lost a proton, and then electron transfer process happens to form phenoxyl radical, proton affinity (PA) and electron transfer enthalpy (ETE) are used to evaluate the two subsequent process, respectively. BDE, IP, EA, PA and ETE were calculated using the procedures as detailed in our previous work [40]. The solvent effects are taken into account using the polarizable continuum model (radii = UFF) by refining the energies of all investigated species on gas-phase equilibrium geometries, the solvent used is water (dielectric constant = 78.3553). Spin density analysis are performed to explore the unpaired electron distribution in all radical species at the same level of theory.

3. Results and discussion

3.1. Optimized molecule geometries of anthocyanidins

The equilibrium geometry of antioxidant molecule is a very important factor which affects its antioxidative efficiency. Extensive researches have been performed to explore molecular structure of anthocyanidins, and contradictory results have been offered by previous theoretical studies. Early semi-empirical studies predicted them planar structure [41], but the HF study predicted nonplanar molecular geometries [42]. A B3LYP/6-31G(d) calculation predicted flavylium planar structure [43], which is in contradiction to the B3LYP/6-31G(d) results in the work of ref [21]. At the B3LYP/D95 level of theory stable planar and nonplanar conformers are both obtained, for all investigated anthocyanidins the planar structures are more stable than the nonplanar structures [20], consistent results have been obtained by Estévez et al. [24] at the B3LYP/6-311++G(d,p) level of theory.

In this work all O–H bond lengths, neighboring C–O bond lengths, C2–C1′ bond length and the planarity of molecules are selected as the structure parameters to characterize the molecule geometries of all investigated anthocyanidins, the planarity is characterized by dihedral angle θ (C3–C2–C1′–C2′). Among six investigated anthocyanidins negligible changes of O–H bond, neighboring C–O bond and C2–C1′ bond lengths are detected, all O–H bond lengths vary less than 0.005 Å, all C–O bond lengths vary less than 0.02 Å, and C2–C1′ bond length varies less than 0.009 Å. As can be seen from the dihedral angles θ (C3–C2–C1′–C2′), all investigated molecules are completely planar, with the AC bicycle and the B-ring sharing the same plane. Moreover, OH groups all locate in the molecular plane completely. Previous work reported that the orientation of 3-OH group controlled the conformation of anthocyanidins, the internal rotations around the C3–O3 result in two conformers, i.e. planarity and nonplanarity. In this work two conformers of pelargonidin are obtained by adjustment of the spatial orientation of 3-OH group (Fig. 2), a distorted 3-OH and a distorted B-ring have been shown in the nonplanar conformation, which can be characterized by dihedral angle β (C2–C3–O3–H3) 27.37° and dihedral angle θ (C3–C2–C1′–C2′) −152.56°. As described in previous paper, the nonplanar conformer is unstable relative to the planar one, the energy difference is about 1.87 kcal/mol at B3LYP/6-311G(d,p) level of theory, which can be interpreted as the internal steric hindrance between 3-OH and 6′ H atom. In the following discussion of this work, only the planar conformations of all anthocyanidins are considered to explore their antioxidative activities.

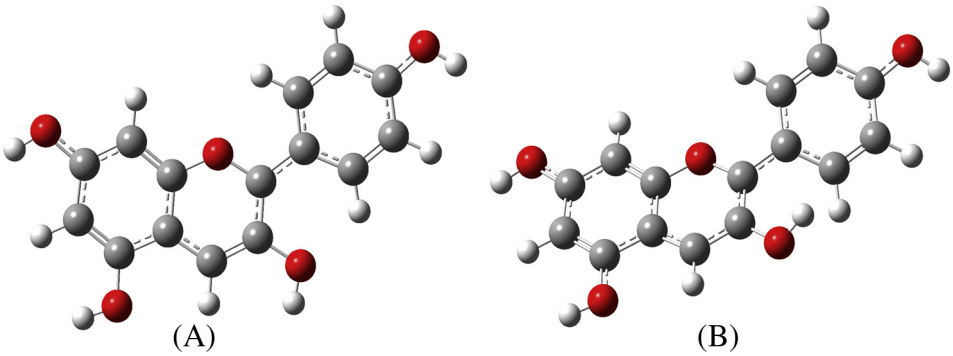


Fig. 2. The B3LYP/6-311G(d,p) optimized geometries of planarity (A) and nonplanarity (B) conformers of pelargonidin.

3.2. O–H bond dissociation enthalpies of anthocyanidins

H atom transfer (HAT) is a major mechanism by which phenolic antioxidant exerts its antioxidative capacity, H atom directly transfers from antioxidant to active free radical to break chain reaction. Six investigated anthocyanidins are differentiated by the number and position of hydroxyl and methoxyl groups in B-ring, pelargonidin is the principal and basic skeleton of anthocyanidins. All OH BDEs of investigated anthocyanidins are calculated and the results are listed in Table 1.

In gas phase, for pelargonidin the BDE sequence for the OH groups is 4′-OH > 7-OH > 5-OH > 3-OH, 3-OH BDE is remarkably lower than BDE of 4′-OH by 11.14 kcal/mol, this indicates that H atom transfer from the 3-OH is easier than from other OH groups, homolytic cleavage of 3-OH happens most possibly to transfer H atom to free radical. In other anthocyanidins which have hydroxyl or methoxyl substituents in *ortho* position of 4′-OH, 4′-OH BDE decrease significantly. The most remarkable case is cyanidin, in which 4′-OH BDE decrease remarkably by 10.95 kcal/mol as compared to that in pelargonidin. For all investigated anthocyanidins 3-OH BDE in B-ring is the lowest one, 3-OH plays very important role in HAT antioxidative mechanism, this is confirmed by previous experimental research that 3-O-glycosylation of anthocyanidins reduces the activity of anthocyanidins against the DPPH radical and ABTS radical [44]. For all investigated anthocyanidins, with the exception of pelargonidin, 4′-OH is another active group in HAT mechanism (Table S1, see Supporting information). As can be concluded from it, OH groups in B-ring and C-ring contribute greatly to the antioxidative activities of these compounds, whereas OH groups in A-ring play relatively little role. An *ortho* hydroxyl or methoxyl substituents in B-ring has remarkable influences on 4′-OH BDE, but influences BDEs of 5-OH and 7-OH negligibly.

Table 1
The homolytic OH BDEs, ionization potentials (IPs) and electron affinities (EAs) of anthocyanidins in aqueous solution, data of resveratrol and quercetin are included for comparison.

	BDEs (kcal/mol)						IPs (kcal/mol)	EAs (kcal/mol)
	3-OH	5-OH	7-OH	3′-OH	4′-OH	5′-OH		
Pelargonidin	78.80	82.63	84.86		85.21		142.17	89.85
Cyanidin	78.81	82.57	84.68	83.81	77.42		140.49	90.13
Delphinidin	78.39	82.50	84.49	83.96	75.59	79.77	139.35	90.37
Malvidin	77.37	81.94	83.42		77.16		139.58	86.73
Peonidin	78.27	82.25	84.07		79.41		141.99	86.28
Petunidin	77.77	82.13	83.76		77.39	78.99	140.87	87.00
Resveratrol	82.29	82.52			77.12		124.31	48.56
Quercetin	77.34	81.70	84.76	75.22	77.00		125.41	55.13

The OH BDEs of anthocyanidins in aqueous solution are calculated and listed in Table 1. As can be seen from it, the solvent effect leads to remarkable decreases in all OH BDEs. Compared to other hydroxyl groups, the solvent effect on 4′-OH BDE is more significant. In all investigated molecules, 3-OH and 4′-OH are the most active, with the exception of 4′-OH in pelargonidin. These indicate that aqueous solution environment is favorable for OH groups homolytic dissociation, consequently enhances the antioxidative activities of these compounds.

The OH BDEs of resveratrol and quercetin are calculated at the same level of theory and listed in Table 1. As can be seen from it, the 4′-OH of resveratrol and the 3′-OH of quercetin have the lowest BDE, and the 3-OH and 4′-OH in quercetin also possess excellent activity. By comparison, it can be seen that the BDE of active OH group of anthocyanidin are comparable to that of resveratrol and quercetin, implying that in HAT mechanism anthocyanidins show comparable antioxidative activity to these two natural antioxidants.

In addition to OH BDEs, the stability of phenoxyl radical is another important factor that impacts their antioxidative activity, the active phenoxyl radical may induce new radical chain reaction, and consequently weakens their antioxidative capacities. Owing to the lowest OH BDE, homolytic dissociation of 3-OH and 4′-OH would happen more favorably to form 3-O-radicals and 4′-O-radicals. The ZPE corrected electronic energies of 3-O-radicals and 4′-O-radicals confirm that these two phenoxyl radicals are more stable than others. The only exception is the 4′-O-radical of pelargonidin, which is more unstable than other radicals.

Generally the unpaired electron delocalization, which is characterized by spin density distribution, has important influence on the stabilities of radicals. Spin density distributions in 3-O-radicals and 4′-O-radicals of all investigated molecules are analyzed and displayed in Fig. 3. In all 3-O-radicals, spin density is found to be evenly distributed over the remaining O atom, C-ring and A-ring, whereas in 4′-O-radicals the spin density is mainly confined to the remaining O atom and B-ring. Hydroxyl or methoxyl substituent in 3′- and 5′-positions has negligible influence on the spin density distribution of 3-O-radical, but it influences the spin density distribution of 4′-O-radical significantly. In 4′-O-radical of pelargonidin, the spin density on remaining O atom, two *orth*-C and *para*-C atoms in B-ring are 0.32, 0.24, 0.27, and 0.29 respectively, in 4′-O-radical of cyanidin the spin density on corresponding atoms decrease to 0.23, 0.20, 0.15 and 0.21, in 4′-O-radical of malvidin the spin density on corresponding atoms further decrease to 0.18, 0.13, 0.17 and 0.19. For malvidin, peonidin and petunidin, the spin density on the remaining O atom in 4′-O-radicals is lower than that in 4′-O-radical of cyanidin and delphinidin, this can be explained by the stronger electron-donating effect of methoxyl group as

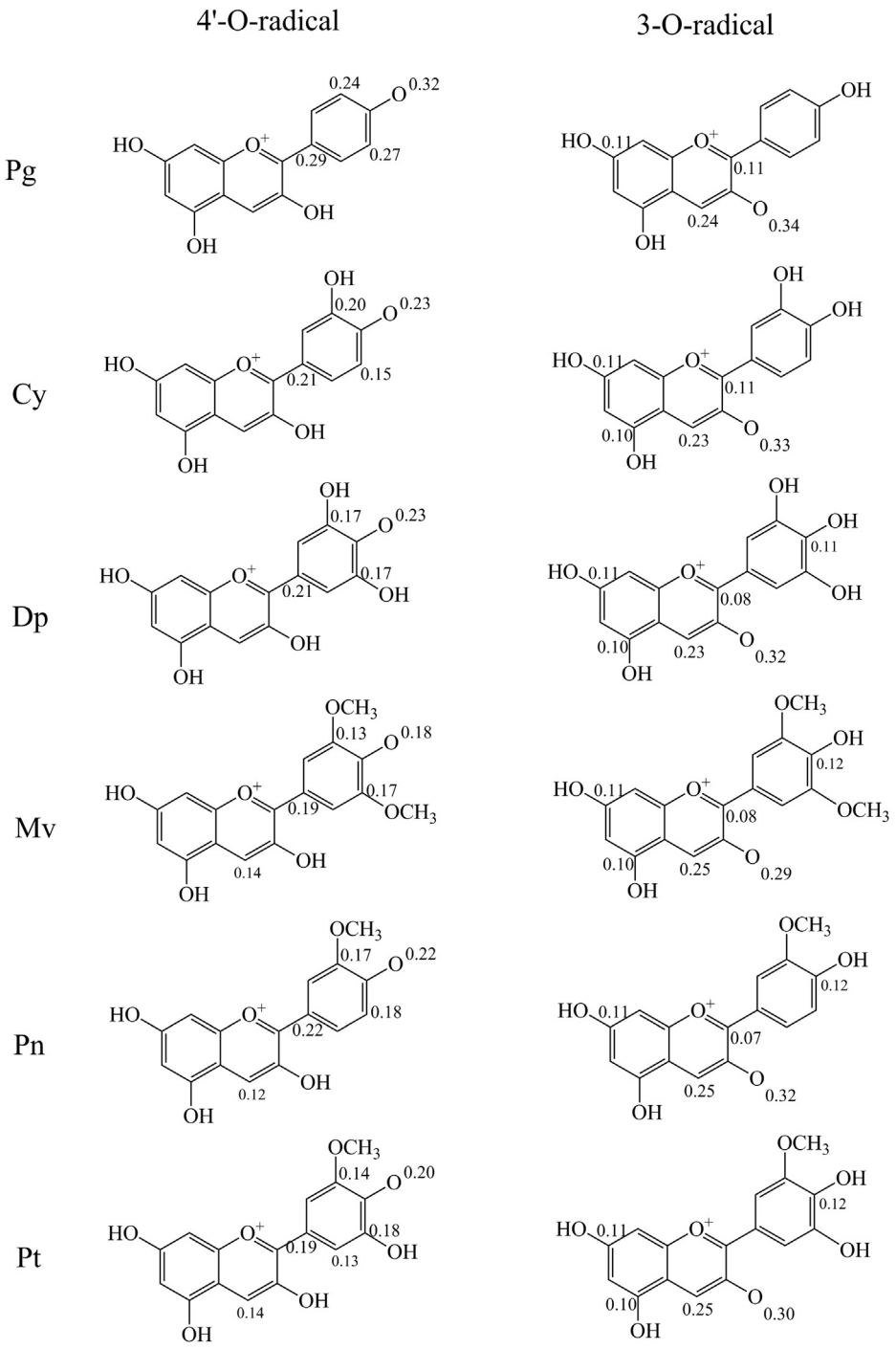


Fig. 3. Spin density distribution in 3-O-radicals and 4′-O-radicals of anthocyanidins.

compared to OH group, which is more favorable for spin density localization.

3.3. Ionization potentials (IPs) and electron affinities (EAs) of anthocyanidins

Another mechanism by which antioxidant trap free radicals is single electron transfer (SET). Electron can transfer from antioxidant to free radical, or transfer from free radical to antioxidant, to break the radical chain reaction. Ionization potential (IP) and electron affinity (EA) of antioxidant are often used to predict the antioxidative capacity through SET pathway. Lower ionization potentials imply that the antioxidant is a better electron donor, whereas higher electron affinity corresponds to a better electron acceptor. The calculated IPs and EAs of all anthocyanidins in aqueous solution are listed in Table 1.

The highest IP value in gas phase is 244.37 kcal/mol for pelargonidin, hydroxyl or methoxyl substituent in 3′-position leads to remarkable decrease in IP, the IPs of cyanidin and peonidin are lower than that of pelargonidin by 4.39 kcal/mol and 7.90 kcal/mol, respectively. Additional hydroxyl or methoxyl substituent in 5′-position leads to a further decrease, the IP of delphinidin is lower

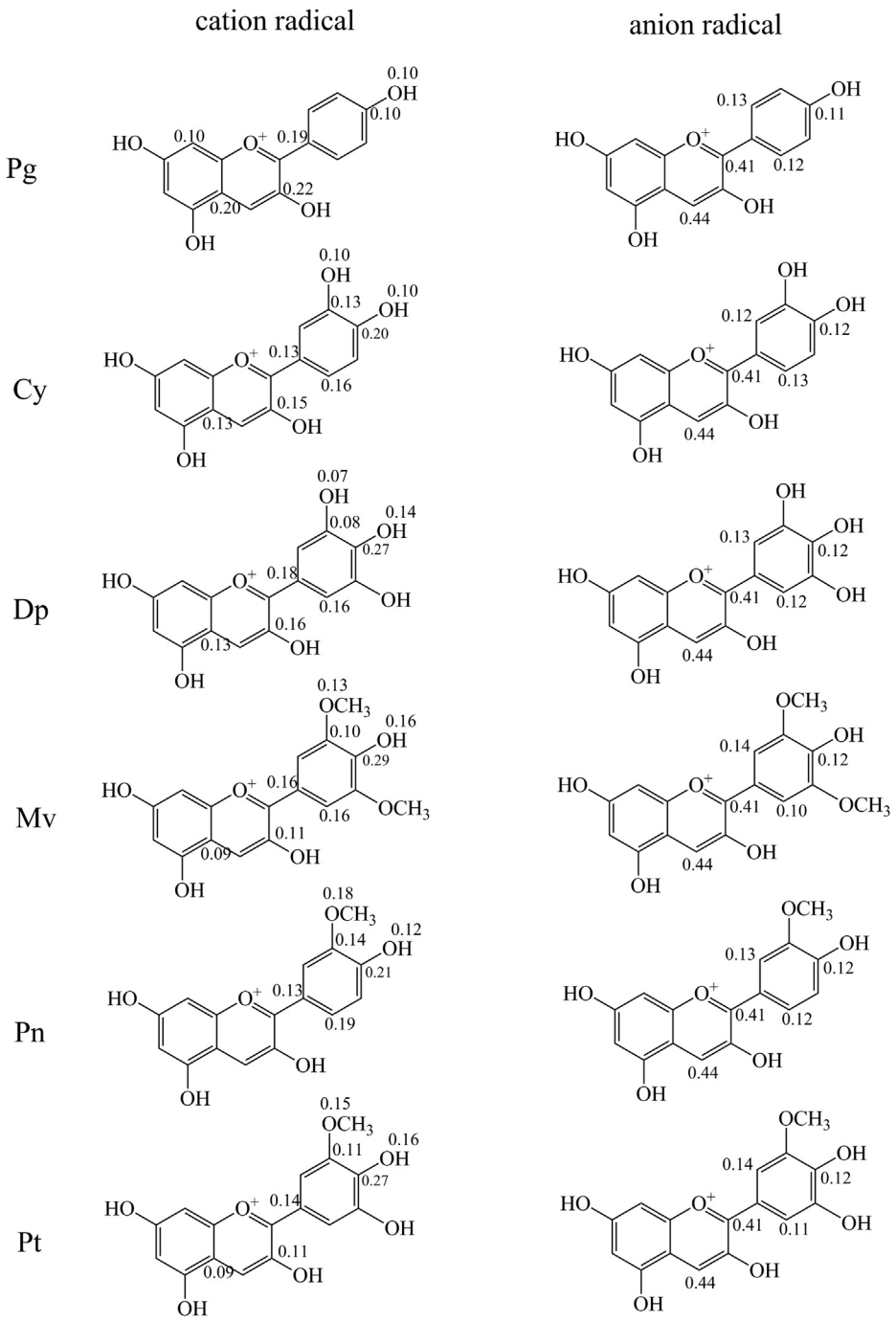


Fig. 4. Spin density distribution in cation radicals and anion radicals of anthocyanidins.

than that of cyanidin by 2.08 kcal/mol, the IP of malvidin decreases by 5.07 kcal/mol as compare to that of peonidin (Table S1, see Supporting information). As can be seen from it, the substituent effect of methoxyl group is more significant than that of hydroxyl group, this could be attribute to the stronger electron-donating capacity of methoxyl, which enhances the electron density on the molecular skeleton to favor electron transferring to free radicals. This is also confirmed by IP of petunidin, 234.33 kcal/mol, which locates between delphinidin and malvidin.

Among six investigated anthocyanidins, the highest EA value in gas phase is 121.50 kcal/mol for pelargonidin, thus indicating it possess the best electron-accepting capacity. Substituents in 3′- and 5′-positions lead to a decrease in electron affinity, and methoxyl group exhibits more remarkable influence than hydroxyl group. Malvidin, peonidin and petunidin, which include methoxyl group in *ortho* position of 4′-OH, have lower electron affinity than other anthocyanidins.

The influence of aqueous solution environment on IPs and EAs of anthocyanidins are also investigated, a remarkable decrease in IPs and EAs of antioxidant indicates that cation radicals produce more smoothly in aqueous solution but the formation of anion radicals (in fact this radical is neutral because all anthocyanidins are cation species) is unfavorable in aqueous solution. The solvent effect would promote the electron-donating capacity but reduce the electron-accepting capacity of anthocyanidins.

For comparison, the IPs and EAs of resveratrol and quercetin are also calculated and listed in Table 1. As can be seen from it, the IP of anthocyanidins is higher than that of resveratrol and quercetin by more than 10 kcal/mol, and the EA is also higher than that of resveratrol and quercetin by more than 30 kcal/mol. This indicates that anthocyanidins have weaker electron-donating capacity but better electron-accepting capacity, as compared to resveratrol and quercetin, electron transfers reaction from free radicals to anthocyanidins is more thermodynamically favorable than that in reverse direction (from anthocyanidins to free radicals) in single electron transfer mechanism, this can be explained as the flavylium ion characteristics of anthocyanidins, which have better electrophilicity than the neutral molecules of resveratrol and quercetin.

For all molecules, the cation radicals and anion radicals have planar geometries. Spin density distribution analysis shows that in cation radicals spin density mainly distributes over B-ring and the substituent groups in B-ring, the contribution of A-ring and C-ring to unpaired electron delocalization is negligible. While in anion radicals spin density distributes evenly over B-ring and C-ring, A-ring moiety hardly contributes to the unpaired electron delocalization. The substituent effect on spin density distribution in cation radicals is more remarkable than that in anion radicals, the detailed spin density distribution of cation radicals and anion radicals are displayed in Fig. 4.

3.4. Proton affinities (PAs) and electron transfer enthalpies (ETEs) of phenolate anions

Sequential proton loss electron transfer (SPLET) is the third important antioxidative mechanism. The first proton loss process, which is governed by the acid strength of phenolic OH, is the crucial step of this mechanism. The obtained phenolate anion (ArO^-) is a much better electron donor which facilitates the following electron transfer process. Basic solvent and highly ionizing solvents favor the proton loss process, while it is suppressed by acidic media [45].

Previous experimental research showed the radical-scavenging activity of anthocyanidins was strongly pH-dependent [46], this validates that SPLET mechanism plays an important role in antioxidative behavior of anthocyanidins. In present work, the proton affinities (PAs) and electron transfer enthalpies (ETEs) of all phenolate ions in gas phase and aqueous solution are calculated at B3LYP/6-311G(d, p) level of theory to explore the most likely deprotonation site of anthocyanidins and to evaluate the feasibility of following electron transfer process, the detailed results are listed in Table 2.

For all anthocyanidins, with the exception of cyanidin, 5-OH provides the most acidic hydrogen, and deprotonation of 5-OH give the most stable phenolate anion, the PAs of 5-phenolate anions of pelargonidin, delphinidin, malvidin, peonidin and petunidin are 247.20, 247.60, 249.69, 261.10 and 261.88 kcal/mol, respectively. While for cyanidin 4′-OH is the most acidic, and 4′-phenolate anion is more stable than other phenolate anions, the PA of 4′-phenolate anion is 245.29 kcal/mol (Table S2, see Supporting information). It can be derived from comparisons between PAs of different anthocyanidins that OH substituent in 3′- or 5′-position has slight influence on PAs of 3-, 5- and 7-phenolate anions, but has remarkable influence on PA of 4′-phenolate anion. The PA of 4′-phenolate anion decreases from 252.10 kcal/mol for pelargonidin to 245.29 kcal/mol for cyanidin, which has an OH substituent in 3′-position. Additional OH substituent in 5′-position makes PA increase to 248.98 kcal/mol for delphinidin. OCH_3 substituent in 3′-position shows remarkable influence on PAs of all phenolate anions, an increase of more than 10.0 kcal/mol in PAs of all phenolate anion between pelargonidin and peonidin can be observed. Additional OH substituent in 5′-position has negligible influence on PAs of 3-, 5- and 7-phenolate anions, but results in an increase of 4.08 kcal/mol in PA of 4′-phenolate ion. However, additional OCH_3 substituent in 5′-position reduces PAs of all phenolate anions significantly, the PAs of corresponding phenolate anions for malvidin is close to that for pelargonidin.

For electron transfer enthalpies of phenolate anions, OH substituent and OCH_3 substituent in C-ring exhibit different influence. OH substituents have negligible influence on ETEs of 3-, 5- and 7-phenolate anions, but OCH_3 substituents lead to slight decrease. Whereas for ETE of 4′-phenolate anion two types of substituents both result in remarkable decrease, and the OCH_3 substituent effect is more significant. The number of substituents shows negligible influence on ETEs of 3-, 5- and 7-phenolate anions, but the decrease extent of ETE of 4′-phenolate anion increases with the number of substituents. Another point should be noted that 3′-OH and 5′-OH in cyanidin, delphinidin, and petunidin all exhibits weak acidity, deprotonation from these sites is unfavorable in comparison with other OH sites.

The influence of aqueous solution environment on proton affinities and electron transfer enthalpies of all phenolate anions are investigated and the results are listed in Table 2. As can be seen from comparison with data in gas phase, the solvent effect induces significant increase in PAs, but remarkable decrease in ETEs of all phenolate anions, this indicates that aqueous solution environment is unfavorable for deprotonation process, but favorable for the following electron transfer process.

For comparison, the proton affinities and electron transfer enthalpies of all phenolate anions of resveratrol and querctetin are calculated at the same level of theory. The proton affinities of phenolate anions of anthocyanidins is remarkably lower than that of resveratrol and quercetin, implying that anthocyanidins have stronger acidic OH group, which is favorable for first proton lost process and facilitates the following electron transfer process. Therefore, in SPLET mechanism anthocyanidins show more excellent antioxidative activity than resveratrol and quercetin.

Table 2
Proton affinities (PAs) and electron transfer enthalpies (ETEs) of phenolate anions of anthocyanidins in aqueous solution, data of resveratrol and quercetin are included for comparison.

	3-O-phenolate anion		5-O-phenolate anion		7-O-phenolate anion		3′-O-phenolate anion		4′-O-phenolate anion		5′-O-phenolate anion	
	PAs (kcal/mol)	ETEs (kcal/mol)	PAs (kcal/mol)	ETEs (kcal/mol)	PAs (kcal/mol)	ETEs (kcal/mol)	PAs (kcal/mol)	ETEs (kcal/mol)	PAs (kcal/mol)	ETEs (kcal/mol)	PAs (kcal/mol)	ETEs (kcal/mol)
Pelargonidin	278.56	113.99	277.69	118.60	278.25	120.11			279.66	119.11		
Cyanidin	278.56	113.96	277.66	118.53	278.21	119.92	292.74	104.67	274.57	116.63		
Delphinidin	278.29	113.89	277.60	118.53	278.14	119.81	292.46	105.01	275.90	113.50	285.71	107.92
Malvidin	278.47	112.68	277.98	117.64	278.58	118.37			279.31	111.46		
Peonidin	278.75	113.27	277.92	118.02	278.48	119.13			277.85	115.17		
Petunidin	278.43	113.15	277.82	117.98	278.37	118.89			279.19	111.87	285.54	107.33
Resveratrol	298.83	96.66	298.59	97.13					295.66	94.67		
Quercetin	299.08	91.66	290.18	105.34	290.09	108.53	291.67	97.55	295.42	95.28		

3.5. Structrue-activity relationship for the antioxidative activity of anthocyanidins

The excellent antioxidative activities of anthocyanidins have been investigated through experimental and theoretical methods, but the relationship between the antioxidative activity and the chemical structure is not yet fully understood. Molecular simplification method is proved to be a useful and economical way to investigate antioxidative mechanism of antioxidants [47]. In this work, the molecular structure of pelargonidin, which is the basic sketelon of anthocyanidins, is systematically simplified by dividing it into several simplified models (SMs) to determine the necessary pharmacophores responsible for antioxidative activity (Fig. 5). The OH BDEs, IPs and EAs of simplified models, the PAs and ETEs of the corresponding phenolate anions are investigated at the same level of theory in gas phase. A comparison with the corresponding properties of pelargonidin has been made to elucidate their respective contribution to the antioxidative activity of anthocyanidins, the results are shown in Table 3. For ease of comparison, the atomic number is consistent with that in pelargonidin (shown in Fig. 5).

As can be seen from Table 3, the 3-OH BDE in SM1 (3-hydroxyl pyrylium ion) is 101.09 kcal/mol, which is significantly higher than the corresponding 3-OH BDE in pelargonidin by 20.95 kcal/mol. In SM2 the 3-OH BDE decreases remarkably to 89.58 kcal/mol, and in SM3 it further decrease to 84.45 kcal/mol. The 3-OH BDEs in all simplified models are significantly higher than the corresponding 3-OH BDE in pelargonidin, this indicates that individual pyrylium, benzopyrylium and 2-phenyl pyrylium moiety is unfavorable for homolytic cleavage of 3-OH, it is synergy effect of them that leads to the lower BDE of 3-OH in pelargonidin. In anthocyanidins 4′-OH is another active phenolic OH group, the calculated 4′-OH BDE in SM3 is 93.64 kcal/mol, which is higher than 4′-OH BDE in pelargonidin by 2.36 kcal/mol, this indicates 4′-OH BDEs of anthocyanidins are somewhat, but not strongly, dependent on the A-ring moiety. Similarly, the BDEs of 5-OH and 7-OH in SM2 are slightly higher than that of pelargonidin.

The IP and EA are responsible for the electron-donating and electron-accepting capacity of antioxidant respectively, which determine single electron transfer between antioxidants and free radicals. As showed in Table 3, the IPs and EAs of SM1, SM2 and SM3 are significantly higher than that of pelargonidin, thus indicating that all SMs are better electron acceptor, but poorer electron donator, as compared to pelargonidin. For SM1 the IP is higher than that of pelargonidin by 94.96 kcal/mol, and the EA is higher than that of pelargonidin by 20.14 kcal/mol. For SM2 and SM3, the IP and EA both decrease significantly as compared to SM1, but they are still higher than the corresponding value of pelargonidin. Based on these results, we can infer that in pelargonidin the pyrylium (C-ring) is unfavorable to its electron-donating capacity, but contributes importantly to its electron-accepting capacity. A-ring and B-ring moieties improve the electron-donating capacity, but on the other hand the electron-accepting capacity is remarkably weakened.

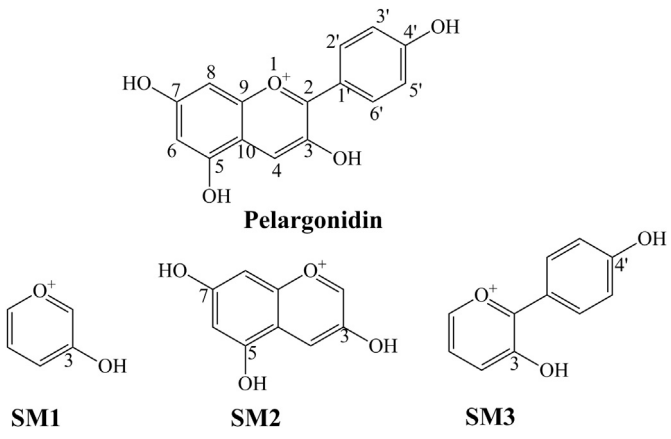


Fig. 5. The structures of pelargonidin and its simplified models.

Table 3
The OH BDEs, IPs and EAs of pelargonidin and simplified models, the PAs and ETEs of the corresponding phenolate anions in gas phase.

		BDEs (kcal/mol)	IPs (kcal/mol)	EAs (kcal/mol)	PAs (kcal/mol)	ETEs (kcal/mol)
SM1	3-OH	101.09	339.33	141.64	228.21	186.61
SM 2	3-OH	89.58	277.31	132.38	245.12	158.16
	5-OH	87.57			236.34	164.87
	7-OH	94.13			238.01	169.50
SM3	4′-OH	93.64	266.18	126.71	247.65	159.59
	3-OH	84.45			237.64	160.59
Pelargonidin	3-OH	80.14	244.37	121.50	249.60	144.27
	5-OH	85.19			247.20	151.64
	7-OH	89.27			249.01	153.74
	4′-OH	91.28			252.10	152.73

The calculated PAs and ETEs of phenolate anions of three SMs are listed in Table 3. Among all investigated species, 3-phenolate anion of SM1 has the lowest PA and the highest ETE, the existence of A-ring and B-ring moieties leads to remarkable increase in PA, but decrease in ETE of 3-phenolate anion. For other phenolate anions the same pattern is consistently observed, these indicate that the coexistence of A-, B- and C-rings reduces the acid strength of phenolic OH, but favors the following electron transfer process.

4. Conclusion

In this work the antioxidative activities of six common anthocyanidins in gas phase and aqueous solution have been investigated by density functional theory calculation at B3LYP/6-311G(d,p) level of theory through HAT, SET and SPLET mechanisms, respectively.

Our results manifest that for all anthocyanidins 3-OH has the lowest BDE, 4′-OH is another active OH group, with the exception of pelargonidin, in which 4′-OH has the highest BDE. Hydroxyl or methoxyl substituents in *ortho* position of 4′-OH leads to significant decrease in 4′-OH BDE, but has slight influence on 3-, 5- and 7-OH BDEs. In aqueous solution all OH BDEs are lower than that in gas phase, thereinto, the solvent effect on 4′-OH BDE is the most significant. Among all investigated compounds, pelargonidin has the highest IP and EA, substituent in *ortho* position of 4′-OH leads to remarkable decrease in IP and EA, and methoxyl substituent group has more significant influence on them. Likewise, a more remarkable decrease in IP and EA can be induced by aqueous solution environment. PAs and ETEs of phenolate anions of all investigated anthocyanidins have also been investigated, and comparison between them has been made to explore the substituent effect. OH substituents in B-ring show negligible influence on PAs of 3-, 5- and 7-phenolate anions, but leads to remarkable decrease in PA of 4′-phenolate anion. OCH_3 substituents in B-ring lead significant increase in PAs of all penolate anions. OH substituent also shows negligible influence on ETEs of 3-, 5- and 7-phenolate anions, but OCH_3 substituent results in remarkable decrease. For the ETEs of 4′-phenolate anion two types of substituents both lead to remarkable decrease. Molecular simplification method has been employed to explore the relationship between molecular skeletons and the antioxidative activity of anthocyanidins.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2013.12.015.

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