

INVESTIGATION OF ANTIOXIDATIVE MECHANISMS OF KAEMPFEROL WITH HYDROXYL RADICAL AND SUPEROXIDE RADICAL ANION

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Abstract: Kaempferol, one of the most bioactive plant flavonoids was theoretically (at M05-2X/6-311G(d,p) level of theory) investigated for its ability scavenge potentially highly damaging hydroxyl and superoxide anion radicals. Theoretical calculations point to HAT and SPLET mechanisms as operative for kaempferol in all solvents under investigations.

Key words: kaempferol, hydroxyl radical, superoxide anion radical, HAT and SPLET mechanisms

Introduction

Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) (Fig.1) is a natural flavonoid that can be found mainly in broccoli, tea, kale, ginko, cabbage, endive, leek, beans, tomato, strawberries, grapes and many medical herbs used in traditional medicine (Calderón-Montaño et al., 2011).

The present paper aims to provide quantitative tools to thoroughly and comprehensively determine antiradical mechanisms of kaempferol by calculating the energy requirements of the reactions of kaempferol and selected radical species in different media. Calculated energy requirements may indicate which radical scavenging mechanism is thermodynamically preferred and point out active sites for radical inactivation.

Material and methods

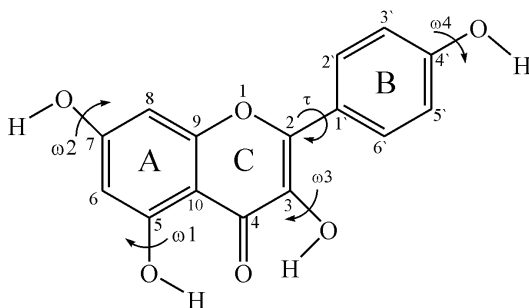
The conformations of different kaempferol forms (neutral, radical, radical-cation, and anion) are fully optimized by the new local density functional method (M05-2X), developed by the Truhlar group (Zhao and Truhlar, 2008) and 6-311G(d,p) basis set (Shakila et al, 2011; Taşal and Kumalar, 2013) implemented in the Gaussian 09 package (Frisch et al., 2009).

Potential energy surfaces are obtained in relation to the torsion angle τ between the rings B and C, defined by the O1–C2–C1'–C2' atoms (Figure 1). The torsion angle τ was scanned in steps of 10° without constraints on all other geometrical parameters.

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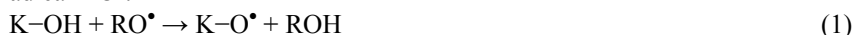
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Slika 1. Hemijska struktura kempferola
 Figure 1. Chemical structure of kaempferol

In order to examine the influence of different radicals to an antiradical mechanism of the most stable kaempferol conformer (conformer I) (Fig. 2), the reactive particle RO[•] was introduced. In the present paper this particle represents superoxide anion and hydroxyl radicals.

In HAT mechanism, the hydrogen atom is transferred from phenolic compound to the free radical RO[•]:



ΔH_{BDE} for the HAT mechanism can be calculated using the following equation:

$$\Delta H_{BDE} = H(KO^{\bullet}) + H(ROH) - H(K-OH) - H(RO^{\bullet}) \quad (2)$$

where the $H(KO^{\bullet})$, $H(ROH)$, $H(K-OH)$, and $H(RO^{\bullet})$ are the enthalpies of the phenolic radical, molecule obtained after hydrogen atom abstraction from the phenolic compound, starting phenolic compound, and reactive radical species, respectively.

The first step in the SET-PT mechanism is transfer of an electron from phenolic compound to free radical species, yielding the phenolic radical cation Ph-OH^{•+} and corresponding anion.

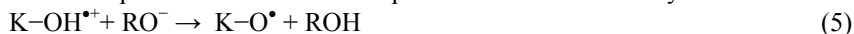


ΔH_{IP} for the first step of the SET-PT mechanism can be calculated as follows:

$$\Delta H_{IP} = H(K-OH^{\bullet+}) + H(RO^{-}) - H(K-OH) - H(RO^{\bullet}) \quad (4)$$

where the $H(K-OH^{\bullet+})$ and $H(RO^{-})$ are the enthalpies of the radical cation of initial phenolic compound and anion generated from the corresponding initial radical.

The second step of this mechanism is deprotonation of K-OH^{•+} by RO⁻:



ΔH_{PDE} can be calculated using the following equation:

$$\Delta H_{PDE} = H(K-O^{\bullet}) + H(ROH) - H(K-OH^{\bullet+}) - H(RO^{-}) \quad (6)$$

The first step in the SPLET mechanism is deprotonation of phenolic compound by RO⁻ or other base. The outcome of this reaction is the formation of the phenoxide anion K-O⁻:



ΔH_{pA} can be calculated as follows:

$$\Delta H_{pA} = H(K-O^{-}) + H(ROH) - H(K-OH) - H(RO^{-}) \quad (8)$$

In the next step electron transfer from $K-O^-$ to RO^\bullet takes place:



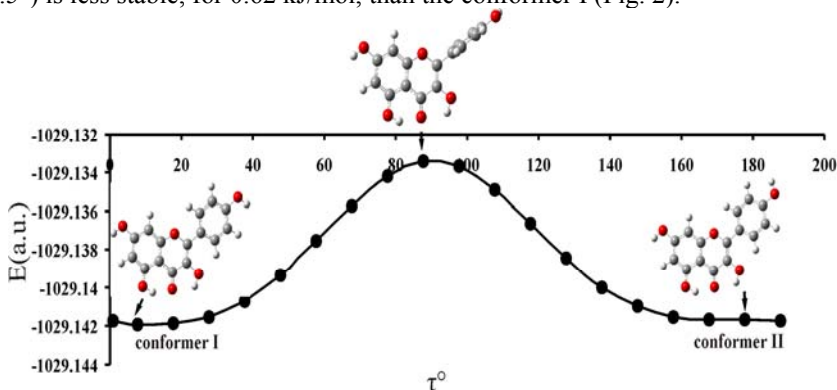
ΔH_{ETE} can be determined by the equation:

$$\Delta H_{ETE} = H(K-O^\bullet) + H(RO^-) - H(K-O^-) - H(RO^\bullet) \quad (10)$$

The potential antiradical activity of kaempferol, for each reactive site (OH group), is simulated in the reactions with hydroxyl ($^\bullet OH$) and superoxide anion ($^\bullet OO^-$) radicals, the radical species among the most important in biological and food chemistry. Reaction enthalpies for the reaction of kaempferol with selected radicals, related to three mechanisms of free radical scavenging activity (HAT, SET-PT and SPLET), are calculated using M05-2X/6-311G (d, p) model.

Results and discussion

To determine the preferred relative positions of the rings B and C, conformational space of kaempferol structure is investigated as a function of torsional angle $\tau(O1-C2-C1'-C2')$ between those rings, in gas-phase (Fig. 2). The lowest energy structure (conformer I) is characterized by a torsional $C3-C2-C1'-C2'$ angle of 7.8° which indicates that rings B and C are almost planar. Another conformer II (dihedral angle of 172.5°) is less stable, for 0.62 kJ/mol, than the conformer I (Fig. 2).



Slika 2. Energetski profil za rotacionu barijeru oko $C2-C1'$ za strukturu kempferola u gasovitoj fazi

Figure 2. Energy profile for rotation barrier around the inner bond $C2-C1'$ for structure kaempferol in gas phase

The preferred mechanism of antiradical activity of kaempferol can be estimated from ΔH_{BDE} , ΔH_{IP} , and ΔH_{PA} values. Namely, the lowest of these values indicates which mechanism is favorable.

Tabela 1. Izračunate reakcione entalpije (kJ/mol) za reakciju kempferola sa hidroksi radikalom i superoksid radikal anjonom
 Table 1. Calculated reaction enthalpies (kJ/mol) for the reactions of kaempferol with hydroxyl radical and superoxide radical anion

M05-2X/6-311G(d,p)										
Kaempferol	Water ($\epsilon=78.35$)					DMSO ($\epsilon=46.83$)				
	HAT	SET-PT	SPLET			HAT	SET-PT	SPLET		
	ΔH_{BDE}	ΔH_{IP}	ΔH_{PDE}	ΔH_{PA}	ΔH_{ETE}	ΔH_{BDE}	ΔH_{IP}	ΔH_{PDE}	ΔH_{PA}	ΔH_{ETE}
		99					242			
KOH-3 + ·OH	-148		-247	-127	-21	-139		-380	-219	80
KOH-4' + ·OH	-125		-223	-127	3	-119		-361	-230	111
KOH-5 + ·OH	-95		-194	-123	28	-76		-317	-207	131
KOH-7 + ·OH	-95		-194	-141	46	-91		-333	-245	153
		373					692			
KOH-3 + ·OO ⁻	66		-307	19	46	103		-588	-24	128
KOH-4' + ·OO ⁻	89		-284	19	70	123		-569	-36	158
KOH-5 + ·OO ⁻	118		-254	23	96	166		-526	-12	179
KOH-7 + ·OO ⁻	118		-254	5	114	151		-541	-50	201
Ethanol ($\epsilon=24.85$)										
		135					244			
KOH-3 + ·OH	-149		-284	-149	-1	-138		-383	-222	83
KOH-4' + ·OH	-128		-263	-151	23	-119		-363	-231	112
KOH-5 + ·OH	-95		-230	-144	49	-76		-320	-207	131
KOH-7 + ·OH	-97		-232	-165	68	-91		-335	-245	154
		454					697			
KOH-3 + ·OO ⁻	74		-379	12	62	103		-594	-28	131
KOH-4' + ·OO ⁻	96		-358	10	86	122		-575	-37	159
KOH-5 + ·OO ⁻	129		-325	17	112	166		-531	-13	179
KOH-7 + ·OO ⁻	127		-327	-5	131	150		-547	-51	201

On the basis of the values in Table 1, it is clear that kaempferol reacts with hydroxyl radical via both, HAT and SPLET mechanisms in all solvents. For protic polar solvents (water and ethanol), HAT and SPLET are competitive mechanisms, while in other two aprotic polar solvents (DMSO and DMF), SPLET is the prevailing mechanism. It should be also noted that hydrogen atom is abstracted mainly from 3-OH group on the C ring when reaction takes place via HAT mechanism. The 7-OH group in the ring A is dominant position for reaction via SPLET mechanism in all solvents.

In the reaction with superoxide anion radical kaempferol (Table 1) undergoes SPLET mechanism which is dominant in all solvents while the reaction by HAT mechanism is endothermic in all solvents. Moreover, the aprotic polar solvents (DMF and DMSO) are more suitable medium for reactions of kaempferol with superoxide anion radical.

Conclusion

Reaction enthalpies are calculated using the M052X/6-311G(d,p) level of theory. In protic polar solvents (water and ethanol) kaempferol reacts with hydroxyl radical via competitive HAT and SPLET mechanisms, while in aprotic polar solvents (DMSO and DMF) SPLET prevails. Preferred sites of action are C3-OH (via HAT) and C7-OH (via SPLET) functionals. Aprotic polar solvents (DMF and DMSO) are more favorable for the interaction with superoxide anion radical. In those solvents kaempferol undergoes SPLET mechanism.

Acknowledgment

The authors acknowledge financial support of the Ministry of Science of the Republic of Serbia, grants No. 172015 and 174028.

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ISPITIVANJE ANTIOKSIDATIVNIH MEHANIZAMA KEMPFEROLA SA HIDROKSI RADIKALOM I SUPEROKSID RADIKAL ANJONOM

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Izvod: Teoretski (na M05-2X/6-311G(d,p) nivou teorije) je ispitivana sposobnost kempferola, važnog bioaktivnog biljnog jedinjenja flavonoida, da reaguje sa potencijalno veoma štetnim hidroksi radikalom i superoksid radikal anjonom. Proračuni su pokazali da su HAT i SPLET mogući mehanizmi u svim rastvaračima.

Ključne reči: kempferol; hidroksi radikal, superoksid radikal anjon, HAT i SPLET mehanizmi

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