THEORETICAL INVESTIGATION OF ANTIRADICAL ACTIVITY OF DELPHINIDIN

Zoran Marković, Dejan Milenković, Jasmina Dimitrić Marković, Miloš Mojović

Abstract: Delphinidin, one of the natural anthocyanin pigments was theoretically (at M05-2X/6-311+G(d,p) level of theory) investigated for its ability scavange potentially highly damaging hydroxyl and superoxide anion radicals. Theoretical calculations point to HAT and SPLET mechanisms as operative for delphinidin in all solvents under investigations.

Key words: delphinidin, hyrdoxyl radical, superoxide anion radical, HAT and SPLET mechanisms

Introduction

Anthocyanins are natural pigments widely distributed in nature (Delgado-Vargas & Paredes-López, 2003). These compounds are flavonoids that belong to the family of polyphenols. Delphinidin (2-(3,4,5-Trihydroxyphenyl)chromenylium-3,5,7-triol) (Fig.1) is an natural anthocyanin pigment that can be found in *cranberries* and *Concord grapes* as well as *pomegranates* (Ribéreau-Gayon & Ribéreau-Gayon, 1958).

The present paper aims to provide quantitative tools to thoroughly and comprehensively determine antiradical mechanisms of delphinidin by calculating the energy requirements of the reactions of delphinidin 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 majority of theoretical investigation of delphinidin is focused on the all rings, where OH groups are located. Geometry optimizations for all species involved in radical scavenging mechanisms have been carried out using density functional method (M05-2X),

Zoran Marković, Department of Chemical-Technological Sciences, State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Republic of Serbia (zmarkovic@kg.ac.rs)

Dejan Milenković, Bioengineering Research and Development Center , Prvoslava Stojanovića 6, 34000 Kragujevac, Republic of Serbia (deki82@kg.ac.rs)

Jelena Đorović, Bioengineering Research and Development Center , Prvoslava Stojanovića 6, 34000 Kragujevac, Republic of Serbia (jelena.djorovic@kg.ac.rs)

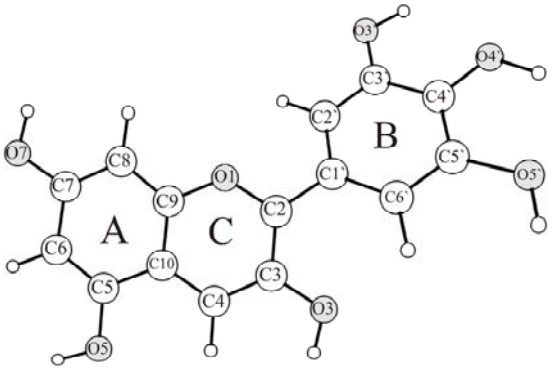
Svetlana Jeremić, Department of Chemical-Technological Sciences, State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Republic of Serbia (jeremics@kg.ac.rs)

Jasmina Dimitrić Marković, Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Republic of Serbia (markovich@ffh.bg.ac.rs)

Miloš Mojović, Faculty of Physical Chemistry, University of Belgrade, Studentskitrg 12-16, 11000 Belgrade, Republic of Serbia (milos@ffh.bg.ac.rs)

developed by the Truhlar group (Zhao and Truhlar, 2008) and 6-311+G(d,p) basis set implemented in the Gaussian 09 package (Frisch et al., 2009). The influence of water, dimethylsulfooxide (DMSO), ethanol and dimethylformamide (DMF) as solvents was approximated by (SMD) solvation model (Marenich et al., 2009).

Geometries were fully optimized without imposing any restriction. Local minima were confirmed by the absence of imaginary frequencies. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies.



Slika 1. Hemijska struktura delfinidina

Figure 1. Chemical structure of delphinidin

Results and discussion

In order to examine the influence of different radicals to an antiradical mechanism of delphinidin (Fig.1), the reactive particles $RO^{\bullet}$ were used. In the present paper $RO^{\bullet}$ particle represents hydroxyl and superoxide anion radicals.

In HAT mechanism, the hydrogen atom is transferred from phenolic compound to the free radical $RO^{\bullet}$:

$$D{-}OH + RO^{\bullet} \rightarrow D{-}O^{\bullet} + ROH \quad (1)$$

$\Delta \boldsymbol{H}_{BDE}$ for the HAT mechanism can be calculated using the following equation:

$$\Delta \boldsymbol{H}_{BDE} = \boldsymbol{H}(DO^{\bullet}) + \boldsymbol{H}(ROH) - \boldsymbol{H}(D{-}OH) - \boldsymbol{H}(RO^{\bullet}) \quad (2)$$

where the $\boldsymbol{H}(DO^{\bullet})$, $\boldsymbol{H}(ROH)$, $\boldsymbol{H}(D{-}OH)$, and $\boldsymbol{H}(RO^{\bullet})$ 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 $Ph{-}OH^{\bullet+}$ and corresponding anion.

$$D{-}OH + RO^{\bullet} \rightarrow D{-}OH^{\bullet+} + RO^{-} \quad (3)$$

$\Delta \boldsymbol{H}_{IP}$ for the first step of the SET-PT mechanism can be calculated as follows:

$$\Delta \boldsymbol{H}_{IP} = \boldsymbol{H}(D{-}OH^{\bullet+}) + \boldsymbol{H}(RO^{-}) - \boldsymbol{H}(D{-}OH) - \boldsymbol{H}(RO^{\bullet}) \quad (4)$$

where the $\boldsymbol{H}(D{-}OH^{\bullet+})$ and $\boldsymbol{H}(RO^{-})$ 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 $D{-}OH^{\bullet+}$ by RO^{-}:

$$D{-}OH^{\bullet+} + RO^{-} \rightarrow D{-}O^{\bullet} + ROH \quad (5)$$

$\Delta \boldsymbol{H}_{PDE}$ can be calculated using the following equation:

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

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 $D{-}O^{-}$:

$$D{-}OH + RO^{-} \rightarrow D{-}O^{-} + ROH \quad (7)$$

$\Delta \boldsymbol{H}_{PA}$ can be calculated as follows:

$$\Delta \boldsymbol{H}_{PA} = \boldsymbol{H}(D{-}O^{-}) + \boldsymbol{H}(ROH) - \boldsymbol{H}(D{-}OH) - \boldsymbol{H}(RO^{-}) \quad (8)$$

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

$$D{-}O^{-} + RO^{\bullet} \rightarrow D{-}O^{\bullet} + RO^{-} \quad (9)$$

$\Delta \boldsymbol{H}_{ETE}$ can be determined by the equation:

$$\Delta \boldsymbol{H}_{ETE} = \boldsymbol{H}(D{-}O^{\bullet}) + \boldsymbol{H}(RO^{-}) - \boldsymbol{H}(D{-}O^{-}) - \boldsymbol{H}(RO^{\bullet}) \quad (10)$$

The potential antiradical activity of delphinidin, for each reactive site (OH group), is simulated in the reactions with hydroxyl ($^{\bullet}OH$) and superoxide anion ($^{\bullet}OO^{-}$) radicals. They are among the biologically relevant radicals that have been detected by spin trapping techniques using electron paramagnetic resonance (EPR) spectroscopy and synthetic nitrones (Villamena et al., 2005). Reaction enthalpies for the reaction of delphinidin with selected radicals, related to three mechanisms of free radical scavenging activity (HAT, SET-PT and SPLET), were calculated using M05-2X/6-311+G(d,p) model. Calculations were performed in the aqueous phase, DMSO, ethanol and DMF. The preferred mechanism of antiradical activity of delphidin can be estimated from ΔH_{BDE}, ΔH_{IP}, and ΔH_{PA} values. The lowest of these values indicates which mechanism is favorable. The reaction enthalpies of these two radicals with delphidin are presented in Table 1.

Tabela 1. Izračunate reakcione entalpije (kJ/mol) za reakciju delfidina sa hidroksi radikalom i superoksid radikal anjonom

Table 1. Calculated reaction enthalpies (kJ/mol) for the reactions of delphidin with hydroxyl radical and superoxide radical anion

M05-2X/6-311+G(d,p)											
	Voda ε=78.35 *Water ε=78.35*						DMSO ε=46.83 *DMSO ε=46.83*				
delfinidin *delphinidin*	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						125			
DOH-3 + $^{\cdot}OH$	-130		-228	-145	15		-136		-260	-145	10
DOH-3` + $^{\cdot}OH$	-130		-229	-129	-1		-136		-261	-121	-15
DOH-4` + $^{\cdot}OH$	-157		-255	-163	6		-166		-290	-176	11
DOH-5` + $^{\cdot}OH$	-132		-231	-128	-4		-138		-262	-119	-19
DOH-5 + $^{\cdot}OH$	-117		-216	-151	34		-121		-245	-152	31
DOH-7 + $^{\cdot}OH$	-111		-210	-151	40		-113		-237	-153	41
		417						448			
DOH-3 + $^{\cdot}OO^{-}$	93		-325	-16	109		86		-362	-16	103

DOH-3` + $^{\cdot}OO^{-}$	92		-326	0	92	86		-362	8	78
DOH-4` + $^{\cdot}OO^{-}$	66		-352	-34	99	57		-391	-47	104
DOH-5` + $^{\cdot}OO^{-}$	90		-327	1	89	85		-363	11	74
DOH-5 + $^{\cdot}OO^{-}$	105		-312	-22	127	102		-346	-23	124
DOH-7 + $^{\cdot}OO^{-}$	111		-306	-22	133	110		-338	-24	134
	Etanol ε=24.85 *Ethanol ε=24.85*					DMF ε=37.22 *DMF ε=37.22*				
		118					129			
DOH-3 + $^{\cdot}OH$	-129		-247	-153	24	-135		-264	-148	12
DOH-3` + $^{\cdot}OH$	-129		-247	-133	4	-136		-265	-123	-13
DOH-4` + $^{\cdot}OH$	-156		-274	-171	16	-165		-294	-176	11
DOH-5` + $^{\cdot}OH$	-131		-249	-131	0	-137		-266	-121	-17
DOH-5 + $^{\cdot}OH$	-115		-233	-159	44	-120		-249	-154	34
DOH-7 + $^{\cdot}OH$	-109		-227	-159	50	-112		-241	-156	43
		452					455			
DOH-3 + $^{\cdot}OO^{-}$	94		-358	-23	117	87		-368	-18	105
DOH-3` + $^{\cdot}OO^{-}$	93		-358	-3	96	86		-369	7	80
DOH-4` + $^{\cdot}OO^{-}$	67		-385	-41	108	57		-398	-46	104
DOH-5` + $^{\cdot}OO^{-}$	92		-360	-1	93	85		-370	9	76
DOH-5 + $^{\cdot}OO^{-}$	108		-344	-28	136	102		-353	-25	127
DOH-7 + $^{\cdot}OO^{-}$	114		-338	-28	142	110		-345	-26	136

On the basis of the thermodynamically values (ΔH_{BDE}, and ΔH_{PA}) from Table 1, it is clear that delphinidin reacts with hydroxyl radical via both, HAT and SPLET mechanisms in all solvents under investigations. As can be seen from Table 1, the C4'−OH group of delphinidin is the most favoured site for homolytic and heterolytic O−H breaking in all solvents.

In the case of the superoxide anion radical the reaction representing HAT mechanisam, for delphidin, is endothermic in all solvents see Table 1. On the other hand the small negative values of ΔH_{PA} indicate SPLET mechanisam as possible, but not much probable one. This could explain no antiradical activity of Dp towards superoxide anion radical.

Conclusion

The ΔH_{BDE}, ΔH_{IP}, and ΔH_{PA} values were used to investigate antioxidative capacity of delphinidin in the presence of $^{\bullet}OH$, and $^{\bullet}OO^{-}$.

SET-PT is not a plausible mechanism for delphinidin, due to high ΔH_{IP} values for the reactions with all radicals in all solvents.

Reaction enthalpies (ΔH_{BDE} and ΔH_{PA}), calculated using the M052X/6-311+G(d,p) level of theory, are exothermic in all solvents. The obtained resultas shows that delphinidin reacts with hydroxyl radical via both, HAT and SPLET mechanisms in all solvents under investigations. The C4'−OH group of delphinidin is the most favoured site for homolytic and heterolytic O−H breaking in all solvents.

Obtained results also show that delphinidin reacts with $^{\bullet}OO^{-}$ via the SPLET mechanism (reaction is egzothermic – $\Delta H_{PA} < 0$), while HAT is no mechanism suitable

for the reaction of delphinidin with superoxide anion radical, because the reaction is endothermic in all solvents ($\Delta H_{BDE} > 0$).

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TEORIJSKO ISPITIVANJE ANTIRADIKALSKE AKTIVNOSTI DELFINIDINA

Zoran Marković, Dejan Milenković, Jelena Đorović, Svetlana Jeremić, Jasmina Dimitrić Marković

Izvod: Teoretski (na M05-2X/6-311+G(d,p) nivou teorije) je ispitivana sposobnost delfinidina, prirodnog antocijaninskog pigmenta, da reaguje sa potencijalno veoma štetnim hidroksi i superoksid anjon radikalima. Proračuni su pokazali da su HAT i SPLET mogući mehanizmi u svim rastvaračima.

Ključne reči: delfinidin, hidroksi radikal, superoksid radikal anjon, HAT i SPLET mehanizmi.