

## EXAMINATION OF ELECTRON TRANSFER MECHANISM OF CYANIDIN

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**Abstract:** Cyanidin, as one important plant pigment, was theoretically (at M05-2X/6-311+G(d,p) level of theory) investigated for its ability to scavenge potentially, highly damaging hydroxyl radical. Free radical scavenging of cyanidin was studied through electron transfer mechanism – ET (the second step in SPLET mechanism) in water and ethanol, as solvents. Examination was performed using density functional theory (DFT) and Marcus theory. Based on the thermochemical and kinetic data, it is clear that O–H group of cyanidin in position 3` is the most suitable for reaction with hydroxyl radical through mentioned antioxidant mechanism.

**Key words:** cyanidin, hydroxyl radical, ET 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. Cyanidin (Cy) (2-(3,4-dihydroxyphenyl) chromenylium-3,5,7-triol) is one of the major water-soluble anthocyanidins.

This paper addresses the DFT investigation of the reaction of cyanidin and hydroxyl radical. The reaction is quantified in terms of values of thermodynamic and kinetic parameters.

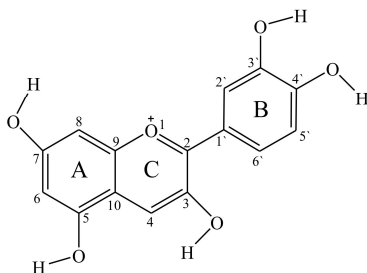
### Material and methods

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

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Slika 1. Hemijska struktura cijanidina  
 Figure 1. Chemical structures of cyanidin.

### Results and discussion

**Thermodynamic parameters:** Scavenging properties of Cy are related to their ability to transfer H atom to a free radical specie. In order to examine the influence of radical specie to an antiradical mechanism of Cy (Fig.1), the reactive particle HO<sup>•</sup> were used. In the present paper, the equations for calculation of the thermodynamic parameters were taken from Dimitrić-Marković and co-workers (2014).

The potential antiradical activity of Cy, for each reactive site (OH group), is simulated in the reactions with hydroxyl (•OH) radical. The reaction enthalpies for the reaction of Cy with selected radical were calculated using M05-2X/6-311+G(d,p) model. Calculations were performed in water and ethanol as solvents. The preferred mechanism of antiradical activity of Cy can be estimated from  $\Delta H_{BDE}$ ,  $\Delta H_{IP}$ , and  $\Delta H_{PA}$  values. The calculated reactions enthalpies are presented in Table 1.

Tabela 1. Izračunate reakcione entalpije (kJ/mol) za reakciju cijanidina sa hidroksi radikalom

Table 1. Calculated reaction enthalpies (kJ/mol) for the reactions of Cy with hydroxyl radical

M05-2X/6-311+G(d,p)										
cyanidin	Water $\epsilon=78.35$					Ethanol $\epsilon=24.85$				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	$\Delta H_{BDE}$	$\Delta H_{IP}$	$\Delta H_{PDE}$	$\Delta H_{PA}$	$\Delta H_{ETE}$	$\Delta H_{BDE}$	$\Delta H_{IP}$	$\Delta H_{PDE}$	$\Delta H_{PA}$	$\Delta H_{ETE}$
		100					120			
CyOH-3+•OH	-131		-231	-145	14	-129		-249	-150	22
CyOH-3'+•OH	-136		-236	-130	-7	-134		-254	-133	-1
CyOH-4'+•OH	-140		-240	-156	16	-138		-259	-163	25
CyOH-5+•OH	-119		-219	-151	32	-115		-236	-157	42
CyOH-7+•OH	-114		-214	-151	37	-110		-230	-158	48

Which of the mechanisms is preferred can be estimated from the lowest value of calculated thermodynamic parameters. On the basis of thermodynamically values from

Table 1, it is clear that only HAT and SPLET are operative radical scavenging mechanisms of Cy in all solvents under investigations. On the basis of obtained  $\Delta H_{BDE}$  values, it is clear that 4'-OH group should be more reactive OH group of Cy. The 4'-OH group has the lowest  $\Delta H_{BDE}$  value in all solvents.  $\Delta H_{PAS}$  values of all present OH groups of Cy indicating proton transfer from C4' group is easier comparing to other OH groups. In all solvents  $\Delta H_{PAS}$  are significantly lower than corresponding  $\Delta H_{BDE}$  values. This indicates that SPLET mechanism thermodynamically represents the more probable reaction pathway in both solvents. Based on these results further was mechanistic investigated the second step of SPLET mechanism.

**Kinetic parameters:** One of the viable mechanisms to scavenge free radicals is electron transfer (ET), the second step in SPLET mechanism (Burton & Ingold,1984):



In reaction (1) cyanidin anion acts as the free radical scavenger. Transition states are necessary for calculating the  $\Delta G^\ddagger$  term in Eq. (6) for HAT reactions. However, for electron transfer reaction, transition state cannot be located using electronic structure methods, as it is not possible to describe mechanistic pathway of electron motion. To estimate the reaction barrier (the  $\Delta G^\ddagger$  term) in such cases, the Marcus theory was used (Marcus, 1997). Within this transition-state formalism, the SPLET activation barrier ( $\Delta G_{\text{SPLET}}^\ddagger$ ) is defined in terms of the free energy of reaction ( $\Delta G_{\text{SPLET}}^0$ ) and the nuclear reorganization energy ( $\lambda$ ):

$$\Delta G_{\text{SPLET}}^\ddagger = \frac{\lambda}{4} \left( 1 + \frac{\Delta G_{\text{SPLET}}^0}{\lambda} \right)^2 \quad (2)$$

$\lambda$  is the energy associated with the nuclear rearrangement involved in the formation of products in an ET reaction, which implies not only the nuclei of the reacting species but also those of the surrounding solvent. For  $\lambda$  calculation, a very simple approximation was used:

$$\lambda \approx \Delta E - \Delta G_{\text{SPLET}}^0 \quad (3)$$

$\Delta E$  is the nonadiabatic energy difference between reactants and vertical products, that is,  $\text{Cy-O}^\bullet$  and  $\text{HO}^-$  in geometries of  $\text{Cy-O}^-$  and  $\text{HO}^\bullet$ :

$$\Delta E_{\text{SPLET}} = E(\text{Cy-O}^\bullet) + E(\text{HO}^-) - E(\text{Cy-O}^-) - E(\text{HO}^\bullet) \quad (4)$$

The adiabatic Gibbs free energies of reaction were calculated as:

$$\Delta G_{\text{SPLET}}^0 = [G(\text{Cy-O}^\bullet) + G(\text{HO}^-)] - [G(\text{Cy-O}^-) + G(\text{HO}^\bullet)] \quad (5)$$

This approach is similar to that which was used by Nelsen and co-workers (1987) for a large set of self-exchange reactions.

The rate constants were calculated using transition state theory (TST) as implemented in The Rate program (Duncan et al.,1998) and 1 M standard state using equation (6):

$$k = \sigma \kappa \frac{k_B T}{h} \exp\left(\frac{-\Delta G^\ddagger}{RT}\right) \quad (6)$$

where  $k_B$  and  $h$  stand for the Boltzman and Planck constants. In this equation  $\Delta G^\ddagger$  is the free activation energy, which is calculated as the energy difference between transition state and reactants.  $\sigma$  represents degeneracy accounting for the number of equivalent reaction paths, and  $\kappa$  accounts for tunneling corrections (Marcus, 1997). If calculated rate constant is close to the diffusion limit, appropriate corrections are considered (the Collins–Kimball theory) as proposed by Galano and Alvarez-Idaboy (2013).

The apparent rate constant ( $k_{app}$ ) cannot be directly obtained from TST calculations. The Collins–Kimball (1949) theory is used to correct the rate constant, and  $k_{app}$  is calculated as:

$$k_{app} = \frac{k_d k}{k_d + k} \tag{7}$$

where  $k$  is the thermal rate constant, obtained from TST calculations. This constant,  $k_d$ , for an irreversible bimolecular diffusion-controlled reaction, can be calculated with following equation:

$$k_d = 4\pi R D_{AB} N_A \tag{8}$$

where  $R$  denotes the reaction distance,  $N_A$  is the Avogadro number, and  $D_{AB}$  is the mutual diffusion coefficient of the reactants A (free radical (HO $\cdot$ ) and B (Cy–O $\cdot$ )).  $D_{AB}$  were calculated from  $D_A$  and  $D_B$  according to Truhlar (1985).  $D_A$  and  $D_B$  were estimated from the Stokes–Einstein approach (1903):

$$D = \frac{k_B T}{6\pi\eta\alpha} \tag{9}$$

where  $\eta$  denotes the viscosity of the solvents, in our case water ( $\eta = 8.9 \times 10^{-4}$  Pa s) and ethanol ( $\eta = 1.1 \times 10^{-3}$  Pa s) and  $\alpha$  is the radius of the solute.

On the basis of the obtained the  $\Delta H_{ETE}$  values (Table 1), the reaction between HO $\cdot$  and Cy–O $\cdot$  were studied only in positions C3, C3' and C4'. The kinetic parameters of the reactions were analyzed in terms of their Gibbs free energies (Table 2).

Tabela 2. DFT izračunate konstante brzine povezane sa drugim korakom SPLET mehanizma

Table 2. DFT calculations of rate constants related to second step of SPLET mechanisms

Cyanidin	$\Delta G_{SPLET}^\ddagger$ (kJ/mol)	$\Delta G_{SPLET}^0$ (kJ/mol)	$\lambda$ (kJ/mol)	$k_d$ (M $^{-1}$ s $^{-1}$ )	$k_{app}$ (M $^{-1}$ s $^{-1}$ )
Water $\epsilon=78.35$					
3+ $\cdot$ OH	21.1	15.0	50.0	4.2x10 $^9$	9.5x10 $^8$
3'+ $\cdot$ OH	9.8	-5.7	49.8	8.3x10 $^9$	7.8 x10 $^9$
4'+ $\cdot$ OH	24.0	18.3	52.9	4.0x10 $^8$	3.4x10 $^8$
Ethanol $\epsilon=24.85$					
3+ $\cdot$ OH	22.0	21.3	30.5	3.4x10 $^9$	6.9x10 $^8$
3'+ $\cdot$ OH	8.1	-0.2	30.2	6.8x10 $^9$	6.6x10 $^9$
4'+ $\cdot$ OH	27.9	27.4	35.4	6.9x10 $^9$	8.0x10 $^7$

As can be seen from Table 2, the 3'-OH group of Cy-O<sup>-</sup> has the lowest values of activation energy (corresponding values of rate constants are higher and possible reaction is faster) in reaction with hydroxyl radical in both solvents, implying that is most favorable position for the reaction via electron transfer mechanism (ET). These results are in accordance with the results presented in Table 1.

### Conclusion

Reaction enthalpies ( $\Delta H_{\text{BDE}}$  and  $\Delta H_{\text{PA}}$ ) are calculated using the M052X/6-311+G(d,p) level of theory. The obtained results show that cyanidin reacts with hydroxyl radical via both, HAT and SPLET mechanisms, in water and ethanol. The C4'-OH group of Cy is the most favored site for homolytic and heterolytic O-H breaking in all solvents. The  $\Delta H_{\text{ETE}}$  values show that 3'-OH group of Cy-O<sup>-</sup> is favored for reaction with <sup>•</sup>OH via ET mechanism.

Mechanistic investigations of antioxidative action of Cy were studied via second step of SPLET mechanism, electron transfer. Simulation of the reaction of anion of cyanidin with the hydroxyl radical confirmed that position 3' of Cy-O<sup>-</sup> is the most suitable for reaction with <sup>•</sup>OH through electron transfer mechanism (ET).

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### References

- Delgado-Vargas F., Paredes-López O. (2003). Natural Colorants for Food and Nutraceutical Uses, CRC Press, Boca Raton, FL.
- Frisch M. J., Trucks G. W., Schlegel H. B., et al. (2010). Gaussian 09, Revision C.01, Gaussian, Inc., Wallingford, CT, USA.
- Marenich A.V., Cramer C.J., Truhlar D.G. (2009). Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. *Journal of Physical Chemistry B*. 113: 6378-6396.
- Dimitrić Marković J.M., Milenković D., Amić D., Mojović M., Pašti I., Marković Z.S. (2014). The preferred radical scavenging mechanisms of fisetin and baicalein towards oxygen-centred radicals in polar protic and polar aprotic solvents. *RSC Advances*, 4: 32228-32236.
- Burton G. W., Ingold K. U. (1984). Beta-Carotene: an usual type of lipid antioxidant. *Science*, 224: 569-573.
- Marcus R. A. (1997). Electron transfer reactions in chemistry. Theory and experiment. *Pure & Appl. Chem*. 69: 13-29.

- Nelsen S. F., Blackstock S. C., Kim Y. (1987). Estimation of inner shell Marcus terms for amino nitrogen compounds by molecular orbital calculations. *J. Am. Chem. Soc.* 109: 677-682.
- Duncan W.T., Bell R.L., Truong T.N. (1998). TheRate: Program for ab initio direct dynamics calculations of thermal and vibrational-state-selected rate constants. *J. Comp. Chem.* 9:1039-1052.
- Galano A., Alvarez-Idaboy J.R. (2013). A computational methodology for accurate predictions of rate constants in solution: Application to the assessment of primary antioxidant activity. *J. Comput. Chem.* 34: 2430-2445.
- Collins F.C., Kimball G.E. (1949). Diffusion-controlled reaction rates. *J. Colloid. Sci.* 4:425– 437.
- Truhlar D. G. (1985). Nearly encounter-controlled reactions: The equivalence of the steady-state and diffusional viewpoints. *J. Chem. Educ.* 62: 104-106.
- Stokes G. G. (1903). *Mathematical and Physical Papers*, Cambridge University Press, Cambridge 1903, 3: 55.