

SCAVENING POTENCY OF ANION OF GALLIC ACID WITH DIFFERENT RADICALS

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Abstract: In this paper antioxidative mechanisms HAT, SPLET and SET-PT of the carboxylate anion of gallic acid (GA^-) with different radicals ($\bullet\text{OO}^-$, $\bullet\text{OH}$, and $\text{CH}_3\text{OO}\bullet$) were investigated. For this reason were examined ΔH_{BDE} , ΔH_{IP} and ΔH_{PA} values of the corresponding reactions in water and pentyl ethanoate as solvents. For this purpose the M052X/6-311++G(d,p) theoretical models was applied.

It was found that SET-PT is not favorable reaction path for all anions of GA (GA^-) in both solvents. On the other hand, HAT and SPLET mechanisms are competitive in all cases. Which of mentioned reaction path is predominant depends on the properties of anions, radicals and solvents.

Key words: gallic acid, anion, DFT, radicals, mechanisms

Introduction

Many diseases in our organism arise as result of radical reactions. As precursors of stabile radicals, which can act as scavengers of free radicals and terminators of radical reactions, polyphenolic acids have important role as nature antioxidants. For that reason, gallic acid (GA) is commonly used in pharmaceutical and chemical industry as well as in food conservation. There is opinion that, besides its conservation ability, GA and its derivatives can be considered as cancer preventors.

Since pK_a value for carboxylic group in GA (3,4,5-trihydroxybenzoic acid) is about 4,2 (Zhang et al., 2009; Erdemgil et al., 2007), it is plausibility to suppose that GA very easy delivers its proton, and it exists as anion of GA (GA^-). Moreover, GA is mostly present as carboxylat monoanion at physiological pH of 7,4. Therefore it is necessary to examine the antioxidant properties of this anion.

GA^- , as GA itself, has three hydroxyl groups, which are potential reaction sites. There are at last thee mechanistic paths for antioxidative action of GA^- in reactions with free radicals ($\text{RO}\bullet$). These are *HAT*, *SET-PT* and *SPLET* mechanisms.

In the HAT mechanism, hydrogen atom is transferred from phenolic compound to a free radical $\text{RO}\bullet$ (Eq. 1). ΔH_{BDE} describes the HAT mechanism, and can be calculated as follows (Eq. 2):



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

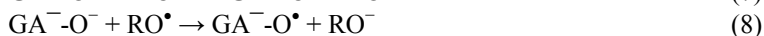
SET-PT reaction mechanism of GA^- with free radical RO^\bullet corresponds to the following reaction (Eq. 2 and 3), and is characterised with ΔH_{IP} and ΔH_{PDE} values (Eq. 4 and 5):



$$\Delta H_{\text{IP}} = H(\text{GA}^- \text{-O}^{\bullet+}) + H(\text{RO}^-) - H(\text{GA}^- \text{-OH}) - H(\text{RO}^\bullet) \quad (5)$$

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

The outcome of the first step in the SPLET mechanism is the formation of the dianion of gallic acid $\text{GA}^- \text{-O}^-$ (Eq. 7). The next step is electron transfer from $\text{GA}^- \text{-O}^-$ to RO^\bullet (Eq. 8). ΔH_{PA} and ΔH_{ETE} are related to the SPLET mechanism, and can be calculated as follows (Eq. 9 and 10):



$$\Delta H_{\text{PA}} = H(\text{GA}^- \text{-O}^-) + H(\text{ROH}) - H(\text{GA}^- \text{-OH}) - H(\text{RO}^-) \quad (9)$$

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

In previous equations, $H(\text{GA}^- \text{-OH})$, $H(\text{GA}^- \text{-O}^\bullet)$, $H(\text{H}^\bullet)$, $H(\text{GA}^- \text{-OH}^{\bullet+})$, $H(\text{e}^-)$, $H(\text{H}^+)$, $H(\text{GA}^- \text{-O}^-)$ and $H(\text{RO}^\bullet)$ represent the enthalpies of GA^- , GA^- -radical, GA^- -hydrogen-radical, GA^- -radicalcation, electron, proton, GA^- -anion and responder free radical respectively.

Enthalpies of proton, electron and hydrogen-radical in water as in pentyl ethanoate as a medium are already known (Marković et al., 2013). Which mechanism is predominant depends on reaction conditions, at first on solvent influence, as on the nature of present free radicals (Klein et al., 2007).

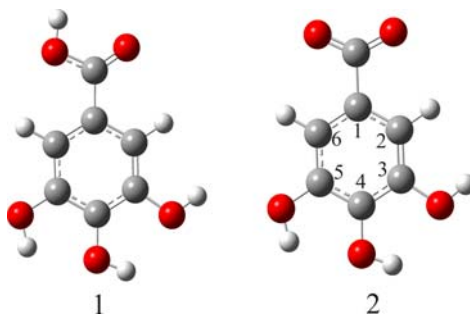
Material and methods

All calculations were performed at density functional level of theory with the hybrid functional M052X (Zhao and Truhlar, 2008.), using the Gaussian (G09) program package (Frisch et al., 2009.). Geometrisation optimisations were carried out with 6-311++G(d,p) basis set. In all calculations, solvent effects (water and pentyl ethanoate) were taken via the polarizable continuum model (PCM/SMD).

Results and discussion

Due to investigate antioxidative activity of GA^- , conformation analyze of GA^- is first done. It is found that the conformer with the lowest free energy is conformer

labeled as 1 on the Figure 1. Abstracting proton from carboxyl group of this conformer is obtained the most stable GA^- , labeled as 2 on the Figure 1. All obtained values of thermodynamic parameters are calculated for this anion conformer.



Slika 1. Najstabilniji konformer galne kiseline (1), i najstabilniji anjon galne kiseline (2), dobijen deprotonovanjem karboksilne grupe najstabilnijeg konformera galne kiseline

Figure 1. The most stable conformer of the gallic acid (1), and the most stable conformer of the anion of the gallic acid (2), obtained by deprotonating of carboxyl group of most stable conformer of the gallic acid

Shown anion moiety can take part in antioxidative reactions by forming radical species at three possible sites of molecule, actually, by leaving hydrogen atom from one of three present hydroxyl groups. Since molecule is symmetric, it will be examined possibility of forming radical species only at positions 3 and 4 (atoms of carbon are labeled on Figure 1). Based on calculated BDE values for both reactive positions in GA^- , it is found that position 4 is more favorable for antioxidative reactions, and all following results appertain to position 4 of GA^- .

Calculations are done in water as solvent, because about $\frac{3}{4}$ of our body is made of water. Pentyl ethanoate, on the other hand, simulate lipid environment. Reaction enthalpies of GA^- and three radicals: superoxide radical anion ($^-\bullet O_2$), hydroxyl radical ($^{\bullet}OH$), and lipid peroxy radical, here represented by $MeOO^{\bullet}$, related to three mechanisms of free radical scavenging activity (*HAT*, *SET-PT* and *SPLET*) are calculated by DFT methods. We selected these three radicals because they are among the most important radicals in food chemistry. The enthalpies of the reactions of these three radicals with GA^- are presented in Table 1. All values of thermodynamic properties are calculated in accordance with former reactions (Eq. 2, 5, 6, 9 and 10).

Tabela 1. Izračunate vrednosti termodinamičkih parametara za mehanizme antoksidativnih reakcija GA^- sa $\cdot O_2$, $\cdot OH$ i $MeOO\cdot$ radikalima u $kJ\ mol^{-1}$
 Table 1. Calculated values of thermodynamic parameters of antioxidant reactions for GA^- with $\cdot O_2$, $\cdot OH$ and $MeOO\cdot$ radicals in $kJ\ mol^{-1}$

	HAT	SET-PT		SPLET	
	ΔH_{BDE}	ΔH_{IP}	ΔH_{PDE}	ΔH_{PA}	ΔH_{ETE}
Voda / Water $\epsilon=78.35$					
$GA^- -4 + \cdot OH$	-171	29	-200	-84	-86
$GA^- -4 + CH_3OO\cdot$	-30	133	-163	-47	17
$GA^- -4 + \cdot OO^-$	36	284	-247	26	11
Pentiletanoat / Pentyl ethanoate $\epsilon=4.73$					
$GA^- -4 + \cdot OH$	-186	152	-337	-109	-77
$GA^- -4 + CH_3OO\cdot$	-40	242	-282	-54	13
$GA^- -4 + \cdot OO^-$	48	619	-571	39	9

The preferred mechanism of antiradical activity of GA^- can be estimated from ΔH_{BDE} , ΔH_{IP} , and ΔH_{PA} values. The lowest of these values indicates which mechanism is favorable. In reaction of GA^- with $\cdot OO^-$, reaction is endothermic and less possible in both solvents, so forming radical is less stable than starting one. It can be concluded that reaction of GA^- with $\cdot OH$ and with $MeOO\cdot$ in water and in pentyl ethanoate is exothermic, which indicates that formed radical is more stable than the starting one, wheter reaction takes place following *HAT* or *SPLET* mechanism. For $\cdot OH$ radical, reaction is easier in pentyl ethanoate than in water, and it traces *HAT* mechanistic path. Reaction with $CH_3OO\cdot$ is easier in pentyl ethanoate, and it can trace both mechanistic paths paralel, but *SPLET* mechanism is predominant (energy of ΔH_{BDE} is for about $15\ kJ\ mol^{-1}$ higher than for ΔH_{PA}).

Conclusion

In this work is examined antioxidant capacity of carboxylate anion of gallic acid in aqueous and in lipid environment (simulated by pentyl ethanoate). Antioxidant activity of GA^- is investigated in reaction with the most frequently free radicals in food.

Reaction capacity is estimated by calculating ΔH_{BDE} , ΔH_{IP} , ΔH_{PDE} , ΔH_{PA} , and ΔH_{ETE} values. All results are obtained using M05-2X method, combined with 6-311++G(d,p) basis set. For mimic the environment influence, it is used PCM/SMD polarizable continuum model.

It is found that in GA^- moiety, position 4 is predominant position for antioxidative reactions. It is concluded that superoxide radical anion does not produce stable radical in reaction with GA^- in any case. $\cdot OH$ radical reacts with GA^- in water as in pentyl ethanoate following *HAT* and *SPLET* mechanistic paths, but *HAT* mechanism is predominant in both solvents. $CH_3OO\cdot$ reacts easier following *SPLET* mechanism. For

both radicals reaction in pentylethanoate is easier than in water. *SET-PT* mechanism is not favorable in any case.

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ANTIOKSIDATIVNI POTENCIJAL ANJONA GALNE KISELINE U REAKCIJI SA RAZLIČITIM RADIKALIMA*S. Jeremić¹, Z. Marković¹, D. Milenković², J. Đorović², G. Jovanović³***Izvod**

U ovom radu su ispitani *HAT*, *SPLET* i *SET-PT* mehanizmi antioksidativnog delovanja karboksilatnog anjona galne kiseline (GA^-) sa različitim radikalima ($\bullet\text{OO}^-$, $\bullet\text{OH}$ i $\text{CH}_3\text{OO}\bullet$). U tom cilju ispitane su ΔH_{BDE} , ΔH_{IP} i ΔH_{PA} vrednosti odgovarajućih reakcija u vodi i pentiletanoatu kao rastvaračima. Za to je korišćen M052X/6-311++G(d,p) teorijski model.

Nađeno je da *SET-PT* mehanizam nije povoljan reakcioni put niti za jedan anjon galne kiseline (GA^-) u oba rastvarača. S druge strane, *HAT* i *SPLET* mehanizmi su konkurentni u svim slučajevima. Koji od reakcionih puteva je predominantan zavisi od osobina anjona, radikala i rastvarača.

Ključne reči: galna kiselina, anjoni, DFT, radikali, mehanizmi.

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