

MECHANISMS OF ANTIOXIDATIVE REACTIONS OF ALIZARIN WITH FREE RADICALS

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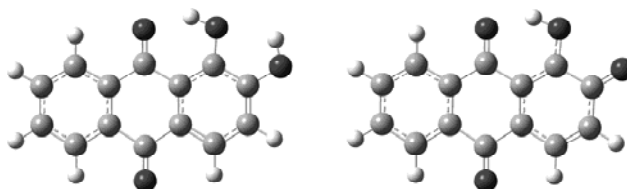
Abstract: Different metabolic processes as nus products form variety free radicals. Those radicals have damaging influence to people health, when they are formed in human bodies, or react as active components in reactions of nutrients decay. In this article are investigated mechanisms of radical scavenging activity of alizarin in reactions with $\bullet\text{OH}$, $\bullet\text{OOH}$ and $\text{CH}_3\text{OO}\bullet$ radicals. All results are obtained using DFT method. For calculations is used M06-2X functional in combination with 6-311++G(d,p) basis set. Lipid and aqueous environment are imitated. All conclusions are based on ΔH values, as thermodynamically indicator for anticipation of reaction pathway.

Key words: radical scavenging, alizarin, free radicals, DFT, enthalpy changes

Introduction

The causes of chemical food spoilage are reactions of free radicals, produced in some metabolic processes of bacteria and microorganisms, with nutrient molecules. The role of antioxidants is to remove free radicals and to terminate their propagation and participation in chain reactions. One of the ways to terminate those reactions using antioxidants is to determine and be able to predict mechanism of antioxidative action of antioxidant with free radicals.

More and more attention is paid to testing and using of natural antioxidants. Among this great class, anthraquinones have great importance. Alizarin is nature anthraquinone. Because of its red color, and because of its applying in traditional medicine (Cai et al., 2006), which support its positive action to the human health, it can be used as colorant and antioxidant in food technology.



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Slika 1. Najstabilnije konformacije molekula alizarina (levo) i radikala alizarina (desno)
 Figure 1. The most stable conformations of molecule (left) and radical (right) of alizarin

Chemical structure of alizarin presents quinone core with two hydroxyl groups, one in position 1 and the other in position 2, as is shown on Figure 1. In reaction of homolytic O-H bond breaking, two possible radical forms can be formed. On Figure 1 is presented the most stable radical conformation of alizarin.

If alizarin reacts with free radical (RO[•]) via mechanism in which homolytic bond dissociation of O-H group of alizarin happens, alizarin radical (A-O[•]) is directly formed (Eq. 1). Mechanism is labeled as HAT (Hydrogen Atom Transfer). Thermal effect of this reaction mechanism can be determined by calculating the changing of enthalpy in reaction process, ΔH_{BDE} (Eq. 2):



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

Dissociation of O-H bond can be heterolytic. In this case two different mechanisms are described, and both of them are two-steps processes. The first one is known as SET-PT, and implies that in first step electron moves from alizarin to the free radical, forming alizarin radical-cation (A-OH^{•+}), and anion of free radical (RO⁻) (Eq. 3). In second step proton goes from alizarin radical-cation to the free-radical-anion, forming radical of alizarin and neutral molecule that accords to the free radical (ROH) (Eq. 4). According to SET-PT mechanism, appropriate thermodynamically values are ionization potential (IP) and proton dissociation enthalpy (PDE). Their pursuant changes of enthalpy are ΔH_{IP} (Eq. 5) and ΔH_{PDE} (Eq. 6):



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

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

The second mechanism in which heterolytic O-H bond dissociation occurs, in first step of reaction proton migrates from alizarin to the free-radical-anion (Eq. 7) and in second step transfers electron, forming alizarin-radical (Eq. 8). This mechanism, known as SPLET, can be described via proton affinity (PA) and electron transfer energy values (ETE), so corresponding ΔH values are ΔH_{PA} (Eq. 9) and ΔH_{ETE} (Eq. 10):



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

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

Reaction enthalpy, as the quantity of the thermal effect of reaction, successfully describes effectiveness and possibilities of process. If newly formed intermediate

moiety is more thermodynamically stable than the initial one, than ΔH is negative, and summary reaction is exothermic. It implies that reaction pathway is favorable. Otherwise, positive value of enthalpy difference indicates that reaction is endothermic and is not favored (Košinova et al., 2011).

Material and methods

The equilibrium geometry of alizarin molecule and all corresponding ionic and radical species obtained in every single reaction step of antioxidative acting of alizarin with free radicals, as the all corresponding derivatives of free radicals, are evaluated using Gaussian 09 program package (Frisch et al., 2009). As one of the recommended functionals for kinetic calculations by its developer, for geometry optimization is used M06-2X functional (Zhao and Thurler, 2008), and the split-valence basis set 6-311++G(d,p). Since the water is the main solvent in body, it is assumed that most of the reactions occur in the aquatic environment. The other important surrounding is lipid, because of its presence in cell membranes. To imitate the lipid environment is used pentylethanoate as solvent. For involving the influence of these two important, and in the polarity opposite environments in calculations, the Polarizable Continuum Model (PCM) is used and combined with SMD, continuum solvation model (Marenich et al., 2009).

Results and discussion

Change in reaction enthalpy is good indicator to estimate if products of reaction are thermodynamically more stable than reactants, and to conclude if reaction is possible or not. In order to determine antioxidative reactivity of alizarin in reactions with radical those are frequently formed in different metabolic processes, ΔH values of reactions of alizarin with hydroxyl radical ($\bullet\text{OH}$), hydroperoxy radical ($\bullet\text{OOH}$), and lipid peroxy radical, here represented by methyl peroxy radical ($\text{CH}_3\text{COO}\bullet$), are calculated. The changes in reaction enthalpies are calculated according to equations 2, 5, 6, 9 and 10, listed above. All calculations are done in two different simulated environments. Polar environment is simulated by water, and lipid environment is simulated by pentylethanoate as solvent. These two solvents are characterized by a large difference in the dipole moment ($\epsilon = 78.35$ for water and $\epsilon = 4.73$ for pentylethanoate).

Based on ΔH values introduced in Table 1, it can be concluded that HAT mechanism is favorable and operative mechanism for hydroxyl radical in both environment. For other radicals, irrespective of their radical nature, ΔH values are positive, which indicates that electric charge is not the only factor that influences on reaction pathway. For hydroperoxyl and methyl peroxy radicals obtained values were surprisingly. It points to the fact that greatness of moiety, and the number of oxygen atoms in species, as the positive inductive effect of hydrogen atom in hydroxyl radical, and methyl group in $\text{CH}_3\text{OO}\bullet$ radical, affect the reaction mechanism. Hydroxyl radical is enough small for almost equal charge distribution over the whole moiety, but for the other two mentioned radicals, it is not the case, and charge is unevenly distributed. This is why in water ΔH values are lower, and HAT mechanism is more possible to be followed than in pentylethanoate.

Tabela 1. Reakcione entalpije/kJ mol⁻¹ za reakcije alizarina sa hidroksi radikalom, hidroperoksi radikalom i metilperoksi radikalom
 Table 1. Reaction enthalpies/kJ mol⁻¹ for the reactions of alizarin with hydroxyl radical, hydroperoxyl radical and methyl peroxy radical.

	HAT	SET-PT		SPLET	
	ΔH_{BDE}	ΔH_{IP}	ΔH_{PDE}	ΔH_{PA}	ΔH_{ETE}
Voda, $\epsilon = 78.35$ Water, $\epsilon = 78.35$					
A1 + •OH	-130.46	71.55	-202.01	-90.63	-39.83
A1 + •OOH	0.45	164.31	-163.86	-52.48	52.93
A1 + CH ₃ OO•	6.57	170.09	-163.52	-52.14	58.71
Pentiletanoat, $\epsilon = 4.73$ Pentylethanoate, $\epsilon = 4.73$					
A1 + •OH	-123.07	268.07	-391.14	-168.55	45.48
A1 + •OOH	9.27	349.20	-339.93	-117.34	126.61
A1 + CH ₃ OO•	18.06	352.17	-334.12	-111.52	129.58

SET-PT mechanism is not favorable in water, nor in pentylethanoate, based on ΔH_{IP} values. Electron transfer from alizarin to the free radical specie destabilizes reactants in both solvents, which results high positive values of ΔH_{IP} .

Comparing ΔH_{PA} values, it can be concluded that all of them are negative. It implies that SPLET mechanism is favorable for all inspected types of radicals, and in both investigated solvent conditions. Lower values are for nonpolar than for polar solvent. The explanation lies in fact that active moieties in first step of SPLET mechanism are anionic species, which are additionally stabilized by surrounding with molecules of solvent. In nonpolar solvent with small values of dielectric constant, stabilizing effect is negligible, and in pentylethanoate free-radical-anion accept proton from alizarin molecule easier.

Conclusion

In this work reaction mechanisms of alizarin true scavenging reactions with hydroxyl, hydroperoxyl and methyl peroxy radical were investigated. In order to estimate which reaction pathway is followed in every single reaction, enthalpy changes are calculated. All results are obtained using M06-2X method, combined with 6-311++G(d,p) basis set. Water and lipid environment are simulated by using PCM/SMD solvation model.

ΔH values indicate that any of investigated radicals doesn't react with alizarin via SET-PT mechanism in any of examined solvent conditions. For hydroperoxyl and methyl peroxy radicals the greatness of moiety, and the number of oxygen atoms in species influence that HAT is not predominant mechanism. These two types of radicals follow SPLET mechanism, as consequence the fact that active moieties in first step of SPLET mechanism are anionic species, which are additionally stabilized by surrounding with molecules of solvent. For hydroxyl radical reaction can obey HAT and SPLET mechanisms with almost the same possibility.

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MEHANIZMI ANTIOKSIDATIVNIH REAKCIJA ALIZARINA SA SLOBODNIM RADIKALIMA

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Izvod: Tokom različitih metaboličkih procesa kao nus-produkti nastaju mnoštvo slobodnih radikala . Ovi radikali imaju štetan uticaj na zdravlje ljudi, ukoliko nastaju u čovekovom organizmu, ili reaguju kao aktivne komponente u reakcijama kvarenja hrane. U ovom radu su ispitani mehanizmi antiradikalske aktivnosti alizarina u reakcijama sa •OH, •OOH i CH₃OO• radikalom. Svi rezultati dobijeni su primenom DFT metode. Za izračunavanja je korišćen M06-2X funkcional u kombinaciji sa 6-311++G(d,p) bazisnim skupom. Simulirano je lipidno i vodeno okruženje. Svi zaključci bazirani su na ΔH vrednostima, kao termodinamičkom indikatoru za predviđanje reakcionog puta.

Ključne reči: uklanjanje radikala, alizarin, slobodni radikali, DFT, promena entalpije