DFT INVESTIGATIONS OF ANTIOXIDANT ACTIVITY OF ALIZARIN RED S

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Abstract: Density functional theory calculations were performed to evaluate the antioxidant activity of alizarin red S molecule. The conformational behavior of molecule were analysed at the M06-2X/6-311++G(d,p) method. The most stable conformer of alizarin red S has two IHBs. The expected antioxidant activity of alizarin red S was justified from ionization potential (IPs) and homolytic O-H bond dissociation enthalpy (BDEs) values. Heterolytic O-H bond cleavages (proton dissociation enthalpies, PDEs) were also computed. Calculated IP, BDE, and PA values suggested that one-step H atom transfer (HAT), rather than SPLET or SET-PT, would be the most favored mechanism for explaining the antioxidant activity of alizarin red S in gas phase. In aqueous solution all of three mechanisms were competitive.

Keywords: alizarin red S, DFT, antioxidativity, BDE, IP

Introduction

The radical scavenging mechanisms of antioxidants depend not only on physicochemical properties of molecules, but also on the properties of the radicals which they form in antioxidative reactions (Estevez et al., 2010). Alizarin red S (ARS) has very good delocalization over the whole molecule, as the whole radical and anion molecule species formed from this molecule in different reaction steps of reactions in which this molecule takes a part. For that reason, and because of its color, there is possibility for using ARS as antioxidants and colorants in food.

ARS molecule has very reactive hydroxyl groups in positions 1 and 2 (Figure 1). Therefore, it is necessary to examine the importance of these molecules in terms of radical scavenging.

There are at least three possible mechanistic paths for antioxidant activity of ARS. Besides the two overall accepted mechanisms (Wright et al., 2001.), knowen as hydrogen atom transfer (HAT):

$$\text{ARS-OH} \rightarrow \text{ARS-O}^{\bullet} + \text{H}^{\bullet} \qquad (1)$$

and single-electron transfer followed by proton transfer (SET-PT),

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$$ARS\text{-}OH \rightarrow ARS\text{-}OH^{+\bullet} + e^{-} \quad (2.1)$$
$$ARS\text{-}OH^{+\bullet} \rightarrow ARS\text{-}O^{\bullet} + H^{+} \quad (2.2)$$

another mechanism has been discovered – sequential proton loss electron transfer (SPLET) mechanism (Musialik and Litwinienko, 2005):

$$ARS\text{-}OH \rightarrow ARS\text{-}O^{-} + H^{+} \quad (3.1)$$
$$ARS\text{-}O^{-} \rightarrow Ars\text{-}O^{\bullet} + e^{-} \quad (3.2)$$

All three of these mechanisms may take place in parellel, but at different rates (Klein et al., 2007).

The HAT mechanism is caracterised by the homolytic bond dissociation entalpy (BDE) of the group OH. SET-PT mechanism can be caracterised by the ionisation potential (IP) in the first step, and by proton dissotiation enthalpy (PDE) in the second step. In SPLET mechanism, the first step corresponds to the proton affinity (PA), and the secont to the electron transfer enthalpy (ETE). All of this values can be calculeted using following equations:

$$BDE = H(ARS\text{-}O^{\bullet}) + H(H^{\bullet}) - H(ARS\text{-}OH) \quad (4)$$
$$IP = H(ARS\text{-}OH^{+\bullet}) + H(e^{-}) - H(ARS\text{-}OH) \quad (5)$$
$$PDE = H(ARS\text{-}O^{\bullet}) + H(H^{+}) - H(ARS\text{-}OH^{+\bullet}) \quad (6)$$
$$PA = H(ARS\text{-}O^{-}) + H(H^{+}) - H(ARS\text{-}OH) \quad (7)$$
$$ETE = H(ARS\text{-}O^{\bullet}) + H(e^{-}) - H(ARS\text{-}O^{-}) \quad (8)$$

In previous equations (4-8), $H(ARS\text{-}OH)$, $H(ARS\text{-}O^{\bullet})$, $H(H^{\bullet})$, $H(ARS\text{-}OH^{+\bullet})$, $H(e^{-})$, $H(H^{+})$ and $H(ARS\text{-}O^{-})$ represent the enthalpies of ARS, ARS-radical, ARS-hydrogen-radical, ARS-radicalcation, electron, proton and ARS-anion respectively.

Enthalpies of proton, electron and hydrogen-radical are already knowen (Marković et al., 2013).

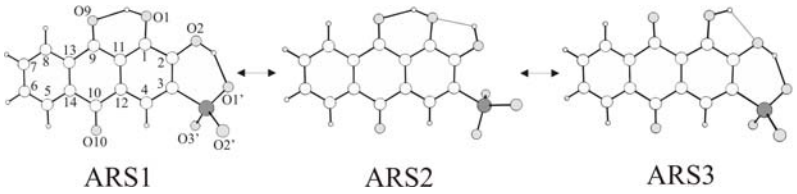
Material and methods

All calculations were performed at density functional level of theory with the hybrid functional M06-2X (Zhao and Truhlar, 2008.), using the Gaussian (G09) program package (Frisch et al., 2009.). Geometrise optimisations were carried out with 6-311++G(d,p) basis set. In all calculations, solvent effects (water) were taken via the polarizable continuum model (PCM/SMD).

Results and discussion

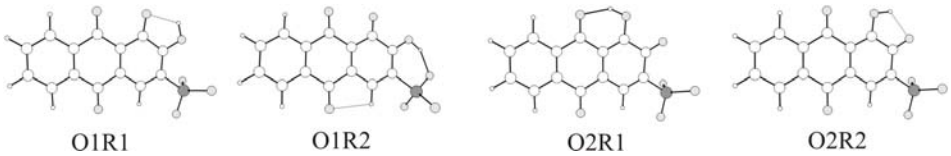
Molecule of ARS has three possible rotamers. In the most stable structure of ARS (labeled as ARS1 in Fig.1), there are two internal hydrogen bonds (IHB), which produce

stabilizing effect. Other conformations (labeled as ARS2 and ARS3), with only one internal hydrogen bond, are less stable with respect to the absolute minimum by 18.63 and 20.58 kJ mol^{-1} respectively.



Slika 1. Najstabilnija konformacija ARS-a je konformacija ARS1. ARS2 i ARS3 su manje stabilni rotameri.
Figure 1. The most stable conformation of ARS is conformation ARS1. ARS2 and ARS3 are less stable rotamers.

In the ARS molecule there are two possible sites for radical scavening (O1 and O2), implying that two different ARS-radicals can be formed. Each radical can adopt two conformations. It is summary four radical conformations, labeled as O1R1, O1R2, O2R1 and O2R2, as is showen on the Fig. 2.



Slika 2. Optimizovane geometrije četiri konformacije radikala ARS molekula
Figure 2. The optimized geometries of the four conformations for ARS-radicals

The BDE, IP, and PDE values, as the PA and ETE values, computed at the M06-2X/6-311++G(d,p) level of theory, in the gas and water, are presented in the Table 1. The species necessary to perform these calculations were generated from the most stable conformation of ARS (ARS1 in the Fig.1).

It can be concluded, on the basis of the BDE values, that between two present OH groups of ARS, the 2-OH has greater ability to donate H-atom. The 2-OH group in the radical conformation labeled as O2R1 in the Fig. 2, has the lowest BDE value in both phases, so it represents the first site that can donate its H-atom. The IP values of ARS are significantly lower in the aqueous than in the gaseous phase, which is a consequence of the stabilization of charged species in polar solvents. The PA values for the aqueous solution are several times lower than the corresponding values in the gaseous phase (Table 1), which is a consequence of the interactions of the species with the solvent molecules. This implies that the reaction which conforms to the SPLET mechanism is thermodynamically preferred in the aqueous solution.

Tabela 1. Parametri antioksidativnih mehanizama izračunati primenom DFT-metode za ARS-radikale (u kJ mol^{-1}) u različitim sredinama

Table 1. DFT calculated parameters of antioxidant mechanisms for ARS-radicals (in kJ mol^{-1}) in different environments

	Gasna faza *Gas-phase*					Voda *Water*				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
		756					506			
O1R1	409		964	1388	332	389		-20	80	407
O1R2	405		960	1404	313	395		-14	91	401
O2R1	396		951	1386	321	371		-37	65	404
O2R2	406		961	1382	335	382		-27	70	409

Table 1 reveals that the HAT mechanism is dominant in the gaseous phase, because the BDE values are significantly lower than the corresponding IP and PA values. In the aqueous solution the PDEs are not only significantly lower than the corresponding BDE values, but those are negative. This indicates that the SET-PT mechanism represents thermodynamically most probable reaction pathway in polar solvents. On the other hand, if we compare BDE to the sums IP + PDE (SET-PT) and PA + ETE (SPLET), it is clear that these values are mutually very similar. One can conclude, on the basis of these facts, that all three mechanisms are competitive and concurrent in the aqueous solution.

Conclusion

In this work conformation analysis of ARS molecule was performed. Furthermore, dissociation enthalpies, proton affinities, and ionization potential, related to HAT, SET-PT and SPLET mechanisms were studded.

The results obtained using M06-2X method, combined with 6-311++G(d,p) basis set, indicate planar structure of molecule, and conformation with two internal hydrogen bond as the most stable, while the other conformations with only one IHB bond are less stable.

The 2-OH group is the most favoured for homolytic and heterolytic O−H breaking, in the gaseous, as in the aqueous phase. Concerned with it, the most stable radical conformation is the one with IHB between C2-OH hydroxyl group and C9-O carbonil group, labeled as R2O1 at Fig. 2.

Based on the obtained results, it can be predicted that the HAT mechanism is dominant in the gas-phase. On the other hand, in water as solvent, all three reaction pathways are competitive and concurrent.

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DFT ISPITIVANJE ANTIOKSIDATIVNE AKTIVNOSTI ALIZARINA RED S

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Izvod

Upotrebom DFT metode vršena su izračunavanja u cilju određivanja antioksidativne aktivnosti alizarina red S. Konformaciona priroda molekula analizirana ispitana je primenom M06-2X/6-311++G(d,p) metoda. Najstabilniji konformer alizarina red S ima dve unutrašnje vodonične veze. Očekivana antioksidativna aktivnost alizarina red S određena je izračunavanjem vrednosti jonizacionih potencijala (IP) i i vrednosti entalpija homolitičkog raskidanja O-H veza (BDE). Vrednosti entalpije heterolitičkog raskidanja O-H veza (entalpije disocijacije protona, PDE), su takođe izračunate. Izračunate IP, BDE i PA vrednosti ukazuju na to da je jednostepeni transfer H-atoma (HAT), pre nego SPLET ili SET-PT mehanizam, favorizovan u pogledu antioksidativne aktivnosti alizarina red S u gasnoj fazi. U vodenom rastvoru, sva tri mehanizma su konkurentna.

Ključne reči: alizarin red S, DFT, antioksidativnost, BDE, IP

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