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The $2H^+/2e^-$ free radical scavenging mechanisms of uric acid: thermodynamics of N—H bond cleavage

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ABSTRACT

Double ($2H^+/2e^-$) free radical scavenging mechanisms of the most abundant endogenous plasma antioxidant uric acid were theoretically studied using DFT method M05-2X/6-311++G(d,p) coupled with SMD solvation model. Calculations were performed for double, two sequential $1H^+/1e^-$ hydrogen atom transfer (HAT), double electron transfer followed by proton transfer (ET–PT) and double sequential proton loss electron transfer (SPLET) mechanisms in water as a solvent. It was found that inactivation of the first free radical by uric acid (the first $1H^+/1e^-$ mechanism) occurs at its 3-N site and inactivation of another one (the second $1H^+/1e^-$ mechanism) occurs at 7-N site of uric acid 3-N$^\bullet$ radical. The final product of all studied $2H^+/2e^-$ pathways is uric acid quinonoid diimine. Obtained results point to the SPLET mechanism as the favorable free radical scavenging mechanism by uric acid. Taking into account electronic properties of scavenged free radicals, double HAT mechanism is found to be competitive to double SPLET mechanism. Second mechanisms are less energy demanding than the first ones indicating $2H^+/2e^-$ processes as plausible. On the basis of exergonicity of the calculated reaction free energies, the reactivity of uric acid toward free radicals was predicted to decrease as follows: HO$^\bullet$ and $Cl_3COO^\bullet$ > alkoxyl, peroxyl ≫ superoxide radical.

1. Introduction

Regular fruit, vegetable, and tea consumption is associated with a decreased incidence of cardiovascular diseases, cancer, and neurodiseases, which all in their etiology include oxidative stress [1]. These natural products contain many macro- and micronutrients that may be responsible for their health promoting effects. In the last decades, one of the most popular explanations of such effects has been ascribed to antioxidant activity of flavonoids, molecules which are ubiquitous in plants and their products [2]. This notion has been questioned by studies on the bioavailability of flavonoids, which indicate that their plasma concentrations rarely exceed nM values [3,4]. Instead, it has been proposed that transient increase in plasma antioxidant capacity, which occurs after consumption of fruits, vegetables and beverages containing flavonoids could be a consequence of substantial amount of uric acid produced by metabolism of other food substances, e.g., fructose [5].

Uric acid is the final product of the endogenous and dietary purine metabolism in humans. It is only sparingly soluble in water and at the physiological pH of 7.4 the majority of uric acid (about 98%) circulates in the blood in the form of much more soluble ionized monosodium salt [6,7]. Uric acid is a weak diprotic acid (pK_{a1} = 5.4; pK_{a2} = 9.8) [8]. Its normal range in serum (as a monovalent anion) is <6.0 mg/dL (<360 μM) [6]. Uric acid is the main contributor to the antioxidant capacity of human plasma (40–55%), much higher than vitamin C (8–15%), vitamin E (<10%), and flavonoids (<2%) [3,9].

The physiological concentration of uric acid is close to its limit of solubility. When salt concentration exceeds solubility limit of 60 mg/L, the risk of monosodium urate crystal formation and precipitation increase. In the urinary tract, at pH below 5.7, uric acid crystal formation is even more favored [10]. Under normal conditions, uric acid production and excretion are carefully balanced processes [6,10]. An excess of uric acid is excreted in urine and

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feces. Out of balance, increased uric acid level in the bloodstream may cause hyperuricemia, a potentially harmful condition. It favors precipitation of uric acid crystals in joints and tissues, leading to illnesses such as gout and renal diseases. Hyperuricemia has been also associated with hypertension, diabetes mellitus, cardiovascular diseases, and metabolic syndrome [11–13].

Beneficial physiological action of uric acid has been related to its inherent antioxidant properties [14]. It may act as scavenger of harmful free radicals and chelator of metal ions involved in free radical production [15–17].

Free radicals are continuously produced in all cells as part of normal cellular function [18]. Those derived from oxygen represent the most important class of such species generated in living systems. Free radicals are essential for health because of their involvement in specific metabolic processes (such as energy production), intercellular signaling and destroying of pathogenic microbes. In healthy organisms, defence mechanisms ensure delicate balance between the production and the removal of free radicals, maintaining their optimal concentrations [19,20]. If this homeostasis is interrupted, oxidative stress occurs and an excess of free radicals can attack biological macromolecules, giving rise to protein, lipid and DNA damage. Because of the highest concentration in serum and ubiquitous presence in all cells and body fluids, uric acid has long been recognized as an important endogenous antioxidant capable to counteract oxidative stress [21]. Uric acid, i.e., its monovalent anion is able to inactivate various reactive oxygen and nitrogen species including peroxyl and hydroxyl radicals, peroxynitrite and nitric oxide [8,15,17,22–24]. It has been indicated that uric acid is much effective free radical scavenger in hydrophilic than in lipidic environment [22,25].

It should be noted that despite acting as an antioxidant under physiological conditions, depending on its chemical microenvironment, uric acid can also be pro-oxidant [26,27]. Like other redox-active molecules (e.g., polyphenols) uric acid also exert this paradoxical behavior. Uric acid may act as an antioxidant primarily in plasma or pro-oxidant mainly within the cell [28]. Under oxidative stress, uric acid generation is increased [11]. Pro-oxidative effects uric acid exert when it is present in blood at supranormal levels [13], what may contribute to development of chronic diseases [29].

2. Computational details

Various reaction mechanisms involved in free radical scavenging have been proposed, mostly depending on the environment polarity and free radical characteristics. They can generally be grouped into the two types of processes: H-atom abstraction and radical adduct formation [30]. H-atom abstraction processes may occur via at least three different mechanisms [31]: hydrogen atom transfer (HAT), electron transfer followed by proton transfer (ET–PT) and sequential proton loss electron transfer (SPLET). HAT is defined as one step mechanism: electron and proton are transferred together as H-atom. ET–PT and SPLET are described as two step mechanisms: an electron is transferred prior to proton, and a proton is transferred prior to electron, respectively. Each of them may also be expected to proceed as two sequential $1H^+/1e^-$ processes, i.e., as double, $2H^+/2e^-$ transfer mechanisms. In this work, we have theoretically investigated energetics of homolytic and heterolytic N—H bond cleavage in uric acid molecule. Already published studies dealing with thermodynamics of N—H bond cleavage [31–34] and kinetic and thermodynamic aspects of free radical scavenging mechanisms of uric acid, have been based on single, $1H^+/1e^-$ processes [25]. Here, for the first time, two sequential $1H^+/1e^-$ processes have been studied as a pathway for double $2H^+/2e^-$ free radical scavenging mechanism of uric acid.

Uric acid ($UA(NH)_4$) is able to undergo double ($2H^+/2e^-$) free radical scavenging mechanisms, i.e., to scavenge two free radicals ($RO^\bullet$), with corresponding net reactions given by Eqs. (a–c) (Fig. 1) [35–37]:

$$UA(NH)_4 + RO^\bullet \rightarrow UA(NH)_3N^\bullet + ROH \quad (a)$$

$$UA(NH)_3N^\bullet + RO^\bullet \rightarrow UA(NH)_2N_2 + ROH \quad (b)$$

$$UA(NH)_4 + 2\,RO^\bullet \rightarrow UA(NH)_2N_2 + 2\,ROH \quad (c)$$

where $UA(NH)_3N^\bullet$ and $UA(NH)_2N_2$ are an uric acid radical and an uric acid quinonoid diimine, respectively.

We considered three double mechanisms: double HAT, double ET–PT, and double SPLET. Recently, such double mechanisms of homolytic and heterolytic O—H bond cleavage in flavonoids have been studied [38].

2.1. The double ($2H^+/2e^-$) HAT mechanism

It is assumed that in the first HAT mechanism one of the four N—H bonds of uric acid is broken and a hydrogen atom ($H^\bullet$) is transferred to a free radical, Eq. (1):

$$UA(NH)_4 \rightarrow UA(NH)_3N^\bullet + H^\bullet \quad (1)$$

Related first bond dissociation enthalpy (BDE1) is calculated according to Eq. (2):

$$BDE1 = H(UA(NH)_3N^\bullet) + H(H^\bullet) - H(UA(NH)_4) \quad (2)$$

where $H(UA(NH)_4)$, $H(UA(NH)_3N^\bullet)$ and $H(H^\bullet)$ are enthalpies of uric acid, uric acid radical, and H-atom, respectively. Uric acid free radical is able to transfer H-atom to another free radical which results in the formation of the final product, uric acid quinonoid diimine ($UA(NH)_2N_2$), Eq. (3):

$$UA(NH)_3N^\bullet \rightarrow UA(NH)_2N_2 + H^\bullet \quad (3)$$

Related second bond dissociation enthalpy (BDE2) is calculated using Eq. (4):

$$BDE2 = H(UA(NH)_2N_2) + H(H^\bullet) - H(UA(NH)_3N^\bullet) \quad (4)$$

where $H(UA(NH)_2N_2)$ is an enthalpy of uric acid quinonoid diimine. A lower BDE value, usually attributed to a greater ability to donate a hydrogen atom released by the homolytic cleavage of the N—H bond, results in an easier free radical scavenging reaction. In general, HAT is favored for radicals with high H-atom affinity and is preferred in non-polar solvents because it does not involve charge separation [39].

2.2. The double ($2H^+/2e^-$) ET–PT mechanism

In the ET–PT mechanism, where heterolytic cleavage of the N—H bond occurs, quenching of a free radical begins with the transfer of an electron from uric acid to a free radical, Eq. (5):

$$UA(NH)_4 \rightarrow UA(NH)_4^{\bullet+} + e^- \quad (5)$$

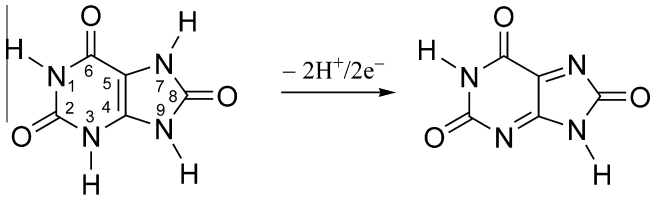


Fig. 1. Molecular structures of the most stable tautomer of uric acid and its product of double ($2H^+/2e^-$) free radical scavenging mechanisms – quinonoid diimine.

where $UA(NH)_4^{\bullet+}$ is an uric acid radical cation. Related first ionization potential (IP1) is calculated using Eq. (6):

$$IP1 = H(UA(NH)_4^{\bullet+}) + H(e^-) - H(UA(NH)_4) \quad (6)$$

where $H(UA(NH)_4^{\bullet+})$ and $H(e^-)$ are enthalpies of uric acid radical cation and electron, respectively. Uric acid radical cation is assumed to readily release a proton in the next step, Eq. (7):

$$UA(NH)_4^{\bullet+} \rightarrow UA(NH)_3N^{\bullet} + H^+ \quad (7)$$

Related first proton dissociation enthalpy (PDE1) is given by Eq. (8):

$$PDE1 = H(UA(NH)_3N^{\bullet}) + H(H^+) - H(UA(NH)_4^{\bullet+}) \quad (8)$$

where $H(H^+)$ is enthalpy of proton. The uric acid free radical may undergo second ET–PT mechanism *via* formation of an uric acid cationic intermediate ($UA(NH)_3N^+$) according to Eqs. (9) and (10), respectively:

$$UA(NH)_3N^{\bullet} \rightarrow UA(NH)_3N^+ + e^- \quad (9)$$

$$UA(NH)_3N^+ \rightarrow UA(NH)_2N_2 + H^+ \quad (10)$$

Associated second ionization potential (IP2) and second proton dissociation enthalpy (PDE2) can be calculated by Eqs. (11) and (12), respectively:

$$IP2 = H(UA(NH)_3N^+) + H(e^-) - H(UA(NH)_3N^{\bullet}) \quad (11)$$

$$PDE2 = H(UA(NH)_2N_2) + H(H^+) - H(UA(NH)_3N^+) \quad (12)$$

where $H(UA(NH)_3N^+)$ is enthalpy of uric acid intermediate. The net result of the double ET–PT mechanism is the same as in the double HAT mechanism.

2.3. The double ($2H^+/2e^-$) SPLET mechanism

The double SPLET mechanism starts with deprotonation of uric acid, i.e., by heterolytic cleavage of one of its N—H bonds, Eq. (13):

$$UA(NH)_4 \rightarrow UA(NH)_3N^- + H^+ \quad (13)$$

Related first proton affinity (PA1) is calculated using Eq. (14):

$$PA1 = H(UA(NH)_3N^-) + H(H^+) - H(UA(NH)_4) \quad (14)$$

where $H(UA(NH)_3N^-)$ is enthalpy of uric acid anion. Uric acid anion then gives an electron to a free radical, Eq. (15):

$$UA(NH)_3N^- \rightarrow UA(NH)_3N^{\bullet} + e^- \quad (15)$$

Related first electron transfer enthalpy (ETE1) is calculated using Eq. (16):

$$ETE1 = H(UA(NH)_3N^{\bullet}) + H(e^-) - H(UA(NH)_3N^-) \quad (16)$$

Uric acid radical generated by the first $1H^+/1e^-$ process then undergoes second $1H^+/1e^-$ process *via* uric acid radical anion ($UA(NH)_2N^{\bullet}N^-$) according to Eqs. (17) and (18), respectively:

$$UA(NH)_3N^{\bullet} \rightarrow UA(NH)_2N^{\bullet}N^- + H^+ \quad (17)$$

$$UA(NH)_2N^{\bullet}N^- \rightarrow UA(NH)_2N_2 + e^- \quad (18)$$

Associated second proton affinity (PA2) and second electron transfer enthalpy (ETE2) can be calculated by Eqs. (19) and (20), respectively:

$$PA2 = H(UA(NH)_2N^{\bullet}N^-) + H(H^+) - H(UA(NH)_3N^{\bullet}) \quad (19)$$

$$ETE2 = H(UA(NH)_2N_2) + H(e^-) - H(UA(NH)_2N^{\bullet}N^-) \quad (20)$$

where $H(UA(NH)_2N^{\bullet}N^-)$ is enthalpy of uric acid radical anion. The net result of all three mechanisms is the same, that is, the production of quinonoid diimine (Eqs. (a)–(c)). In general, ET–PT and SPLET mechanisms are preferred in the case of reaction with radicals with high electron affinity [39]. They are favoured in polar media, since they involve charge separation processes.

Given a reactive N—H group, all three double mechanisms studied have the same reactants and the same products (Eq. (c)), and hence they have the same net thermodynamic balance, Eq. (21) [40]:

$$\Delta H_{HAT} = \Delta H_{ET-PT} = \Delta H_{SPLET} \quad (21)$$

Accordingly, all three mechanisms of free radical scavenging may proceed in parallel and could be competitive. Calculation of the energy requirements for each mechanism, BDEs for double HAT, IPs and PDEs for double ET–PT as well as PAs and ETEs for double SPLET may indicate thermodynamically dominant one and point out the active site for radical inactivation [31,41]. This approach, with included influence of the solvent, takes into consideration only characteristics of free radical scavenger, here uric acid and its species involved in studied mechanisms.

2.4. Influence of nature of free radicals scavenged on double mechanisms

Scavenging of free radicals is a complex process influenced by many factors [42]. Among them, characteristics of free radicals scavenged should not be neglected because these influence the scavenging mechanism [25,43–46]. To explore free radical scavenging potential of uric acid and its monoanion, a set of free radicals has been chosen. It embraces oxygen-centered radicals with very different reactivities: $^{\bullet}OH$ (hydroxyl radical), $^{\bullet}OOH$ (hydroperoxyl radical), $^{\bullet}OCH_3$ (methoxyl radical), $^{\bullet}OC(CH_3)_3$ (*t*-butoxyl radical), $O_2^{\bullet-}$ (superoxide radical anion), $CH_2{=}CH{-}O{-}O^{\bullet}$ (vinyl peroxyl radical), $CH_2{=}CH{-}CH_2{-}O{-}O^{\bullet}$ (allyl peroxyl radical), and $Cl_3C{-}O{-}O^{\bullet}$ (trichloromethyl peroxyl radical). The $^{\bullet}OH$ radical is the main source of biological damage in living organisms because it is so electrophilic that it may strip an electron or H-atom from almost any compound with which it comes in contact [47]. Radicals $^{\bullet}OCH_3$ and $^{\bullet}OC(CH_3)_3$ are examples of alkoxyl radicals. The smallest alkoxyl radical $^{\bullet}OCH_3$ is abundant damaging free radical in the human body. In comparison, peroxyl radicals $^{\bullet}OOH$, $CH_2{=}CH{-}O{-}O^{\bullet}$ and $CH_2{=}CH{-}CH_2{-}O{-}O^{\bullet}$ are less reactive. They may mimic lipid peroxyl radicals $LOO^{\bullet}$ which are abundantly formed in biological systems. $Cl_3C{-}O{-}O^{\bullet}$ is very electronegative, highly reactive peroxyl radical. $O_2^{\bullet-}$ is an important radical with rather low reactivity that can occur during *in vivo* metabolism.

To take into account characteristics of selected free radicals on reaction with uric acid, calculations of Gibbs free energy of reactants and products involved in studied three double mechanisms have been performed. In general, Gibbs free energy for a reaction represents the criterion of the thermodynamically preferred process. The reaction between uric acid and particular free radical is thermodynamically favorable if it is exergonic [48,49]:

$$\Delta_r G = [G(\text{products}) - G(\text{reactants})] < 0$$

In the double HAT mechanism, the first, i.e., the most abstractable imino H-atom is transferred from uric acid to the free radical $RO^{\bullet}$, and then second imino H-atom is transferred from the uric acid radical to another free radical $RO^{\bullet}$ (Eqs. (1) and (3)). $\Delta_r G_{BDE1}$ and $\Delta_r G_{BDE2}$ represent the free energies of the first and the second HAT mechanism (Fig. 2), respectively, and are calculated by Eqs. (22) and (23), respectively:

$$\Delta_r G_{BDE1} = [G(UA(NH)_3N^{\bullet}) + G(ROH)] - [G(UA(NH)_4) + G(RO^{\bullet})] \quad (22)$$

$$\Delta_r G_{BDE2} = [G(UA(NH)_2N_2) + G(ROH)] - [G(UA(NH)_3N^{\bullet}) + G(RO^{\bullet})] \quad (23)$$

Lower $\Delta_r G_{BDE}$ value is assumed to correspond to a greater reactivity of uric acid with a free radical considered.

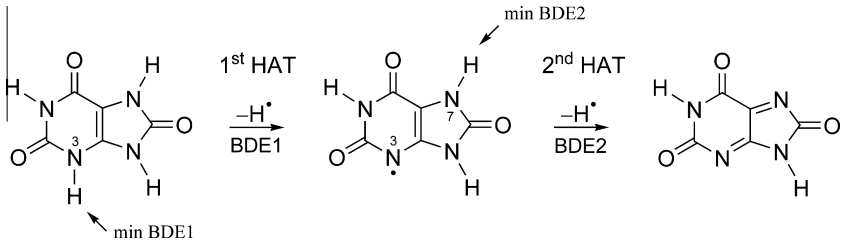


Fig. 2. Double ($2H^+/2e^-$) HAT mechanism.

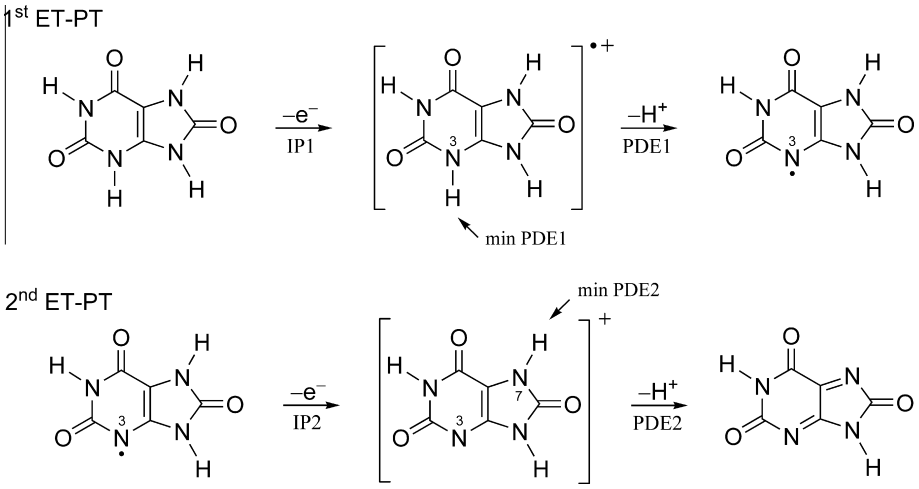


Fig. 3. Double ($2H^+/2e^-$) ET–PT mechanism.

Similarly, the first ET–PT mechanism (Eqs. (5) and (7), Fig. 3) is characterized by $\Delta_r G_{IP1}$ and $\Delta_r G_{PDE1}$, which are calculated by Eqs. (24) and (25), respectively:

$$\Delta_r G_{IP1} = [G(UA(NH)_4^{\bullet+}) + G(RO^-)] - [G(UA(NH)_4) + G(RO^{\bullet})] \quad (24)$$

$$\Delta_r G_{PDE1} = [G(UA(NH)_3N^{\bullet}) + G(ROH)] - [G(UA(NH)_4^{\bullet+}) + G(RO^-)] \quad (25)$$

The second ET–PT mechanism (Eqs. (9) and (10), Fig. 3) is characterized by $\Delta_r G_{IP2}$ and $\Delta_r G_{PDE2}$, which are calculated by Eqs. (26) and (27), respectively:

$$\Delta_r G_{IP2} = [G(UA(NH)_3N^{+}) + G(RO^-)] - [G(UA(NH)_3N^{\bullet}) + G(RO^{\bullet})] \quad (26)$$

$$\Delta_r G_{PDE2} = [G(UA(NH)_2N_2) + G(ROH)] - [G(UA(NH)_3N^{+}) + G(RO^-)] \quad (27)$$

In the case of double SPLET, $\Delta_r G_{PA1}$ and $\Delta_r G_{ETE1}$ are related to the first SPLET mechanism (Eqs. (13) and (15), Fig. 4), and are calculated by Eqs. (28) and (29), respectively:

$$\Delta_r G_{PA1} = [G(UA(NH)_3N^-) + G(ROH)] - [G(UA(NH)_4) + G(RO^-)] \quad (28)$$

$$\Delta_r G_{ETE1} = [G(UA(NH)_3N^{\bullet}) + G(RO^-)] - [G(UA(NH)_3N^-) + G(RO^{\bullet})] \quad (29)$$

$\Delta_r G_{PA2}$ and $\Delta_r G_{ETE2}$ are related to the second SPLET mechanism (Eqs. (17) and (18), Fig. 4), and are calculated by Eqs. (30) and (31), respectively:

$$\Delta_r G_{PA2} = [G(UA(NH)_2N^{\bullet}N^-) + G(ROH)] - [G(UA(NH)_3N^{\bullet}) + G(RO^-)] \quad (30)$$

$$\Delta_r G_{ETE2} = [G(UA(NH)_2N_2) + G(RO^-)] - [G(UA(NH)_2N^{\bullet}N^-) + G(RO)] \quad (31)$$

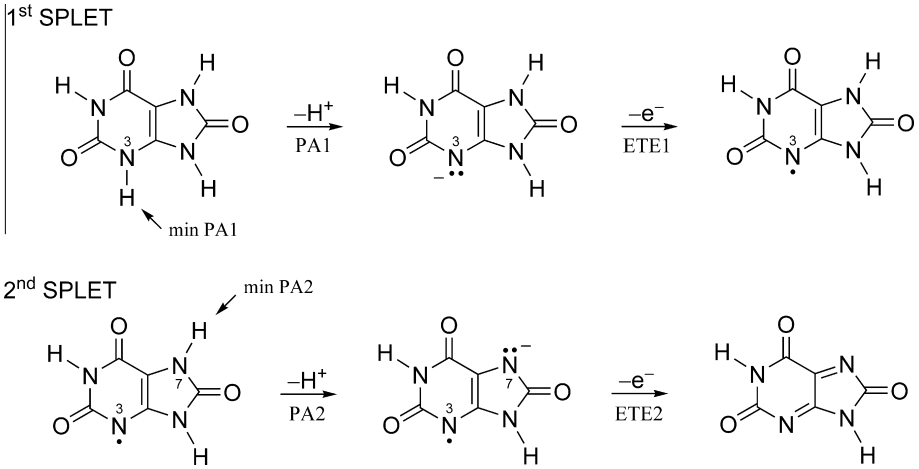


Fig. 4. Double ($2H^+/2e^-$) SPLET mechanism.

2.5. Methodology

All calculations were performed using the Gaussian 09 program package [50]. Density functional theory (DFT) represents a powerful tool for studying the free radical scavenging mechanisms of natural compounds [51,52]. Geometry optimizations and frequency calculations for uric acid, its radical cation, radicals, anions, radical anions, quinonoid diimine, as well as for eight selected free radicals and their species involved in radical scavenging pathways were carried out using the M05-2X functional [53] in conjunction with the 6-311++G(d,p) basis set. Among the suite of density functionals, M05-2X, M06-2X and M-11L Minnesota functionals are particularly suitable for systems where thermochemistry and kinetics are important [53–55]. The M05-2X functional has been chosen because it is designed for main-group chemistry and recommended for kinetic and thermodynamic calculations by its developers [53]. It is among the best performing functionals for modeling reaction energies involving free radicals [56] and its reliability has been independently confirmed by other authors [57]. The influence of water as a solvent was calculated with an implicit continuum solvation model, SMD [58] which takes into account the full solute electron density in estimation of free energy of solvation. SMD is a universal solvation model applicable to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known (in particular, dielectric constant, refractive index, bulk surface tension, and acidity and basicity parameters). In conjunction with the M05-2X density functional, SMD model has been successfully used for study of thermodynamics and kinetics of free radical scavenging mechanisms [30,59–61]. Spin unrestricted calculations were used for open-shell systems. Relative enthalpies and free energies were calculated at 298.15 K. Published values of the gas-phase enthalpy of proton and electron [62] as well as of the hydration enthalpy of hydrogen atom, proton and electron were used [31,63].

3. Results and discussion

3.1. Estimation of thermodynamically preferred $2H^+/2e^-$ mechanism based on electronic properties of free radical scavenger, i.e., uric acid

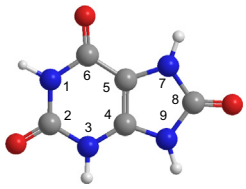
Uric acid exhibits prototropic tautomerism because it possesses four H-atoms that can move as protons between seven heteroatoms (three exo O-atoms and four endo N-atoms) and also between heteroatoms and five endo C-atoms [64]. Among numerous tautomers [64], a structure with four N—H (imino) and three C=O (carbonyl) groups, shown in Fig. 1, is the lowest-energy tautomer [25,65–67]. All calculations in this report were performed using this most stable tautomer of uric acid.

3.1.1. The first $1H^+/1e^-$ processes

The results of the SMD/M05-2X/6-311++G(d,p) calculations in water as the solvent, related to the energetics of double ($2H^+/2e^-$) processes, i.e., for the first $1H^+/1e^-$ and second $1H^+/1e^-$ HAT, ET–PT and SPLET mechanisms, are summarized in Tables 1 and 2.

The BDE could serve as a general parameter for estimation of free radical scavenging potency [68]. The minimal value of BDE of N—H bonds (minBDE) indicates which N—H group of uric acid possesses the most abstractable hydrogen, that is, which N—H group is active site for inactivation of damaging free radicals. As can be seen from Table 1, the minimal energy requirement for homolytic bond cleavage, i.e., minBDE1 (340.4 kJ/mol) is related to the 3-NH group of uric acid. Therefore, if the HAT1 mechanism occurs, the thermodynamically preferred site for radical inactivation would be the 3-NH group (Fig. 2). The minimal energy

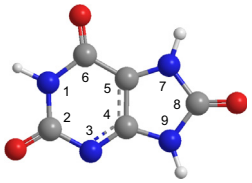
Table 1
Parameters for free radical scavenging potency of uric acid (kJ/mol) in water as a solvent calculated using SMD/M05-2X/6-311++G(d,p) approach. Preferred site for radical inactivation and related energetics are indicated in italics.



Uric acid molecule

Site	HAT1	SET-PT1		SPLET1	
	BDE1	IP1	PDE1	PA1	ETE1
		343.1			
1	392.8		49.6	100.8	291.9
3	*340.4*		*−2.9*	*66.0*	*274.2*
7	345.6		2.3	100.9	244.6
9	342.3		−0.9	73.6	268.6

Table 2
Parameters for free radical scavenging potency of uric acid radical 3-N$^\bullet$ (kJ/mol) in water as a solvent calculated using SMD/M05-2X/6-311++G(d,p) approach. Preferred site for radical inactivation and related energetics are indicated in italics.



Uric acid radical 3-N$^\bullet$

Site	HAT2	SET-PT2		SPLET2	
	BDE2	IP2	PDE2	PA2	ETE2
		374.6			
1	414.2		39.4	96.8	317.3
7	*308.6*		*−66.2*	*42.6*	*265.8*
9	367.7		−7.2	62.9	304.6

requirements for all three first $1H^+/1e^-$ mechanisms, i.e., HAT1 (minBDE1), ET–PT1 [min(IP + PDE)] and SPLET1 [min(PA + ETE)] are associated with the same 3-NH group of uric acid and the final product of all three processes is the same: thermodynamically the most stable uric acid 3-N$^\bullet$ radical. Calculated spin densities of uric acid 3-N$^\bullet$ radical are presented in Fig. 5. It is obvious that an unpaired electron is delocalized over the whole structure conferring to stability of the radical. It should be noted that the 5-C atom is characterized with the highest spin density value.

In polar solvent (such as water) the homolytic bond cleavage is expected to be less probable than the heterolytic N—H bond cleavage [39] indicating that the contribution of HAT mechanism to the radical scavenging is much less than the contributions of ET–PT and/or SPLET mechanisms. To conclude which radical scavenging mechanism represents the most probable reaction pathway from the thermodynamic point of view, IP1 as well as PA1 and ETE1 values related to 3-N site of uric acid should also be considered. Because both PA1 (66.0 kJ/mol) and ETE1 (274.2 kJ/mol) are significantly lower than IP1 (343.1 kJ/mol) and BDE1 (340.4 kJ/mol) values (Table 1), SPLET mechanism is expected to be more probable than HAT and ET–PT mechanisms.

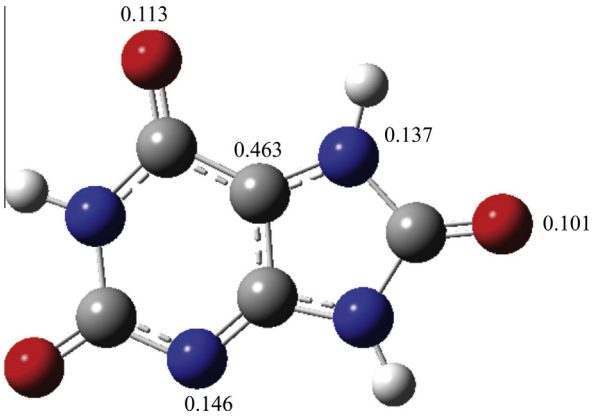


Fig. 5. Spin densities of uric acid 3-N• radical.

The first step of $1H^+/1e^-$ SPLET mechanism is deprotonation which occurs at 3-N site of uric acid because it possesses the lowest PA1 value (Table 1). This result is in agreement with previously published findings that imino-hydrogen atom in position 3-N is the most acidic [25,67,69–74]. Product of this deprotonation is designated as 3-N^- anion (Fig. 4). However, it should be emphasized that negative charge of this monoanion is delocalized over the oxygen and nitrogen atoms, where three oxygen atoms bear larger partial negative charges than nitrogen atoms of purine core (Fig. 6). As mentioned in *Introduction section* uric acid at physiological pH of 7.4 is present in serum and body fluids almost completely as a monoanion. This 3-N^- monoanion is the main specie involved in the free radical scavenging activity of uric acid. Electron transfer from 3-N^- anion results in formation of the most stable 3-N• radical (Fig. 4).

3.1.2. The second $1H^+/1e^-$ processes

The uric acid 3-N• radical formed after the first $1H^+/1e^-$ mechanisms is assumed to undergo the second $1H^+/1e^-$ mechanisms by scavenging another free radical. Results presented in Table 2 indicate that active site of 3-N• radical is the 7-N site characterized by minimal energy requirements. Hence, the second HAT mechanism (Fig. 2) occurs with H-atom transfer from 7-N site which possesses minBDE2 value (308.6 kJ/mol). In the second ET–PT mechanism, electron transfer from uric acid 3-N• radical is followed by the proton transfer from 7-N site of cationic intermediate which possesses minPDE2 value (Fig. 3). The second SPLET mechanism starts with deprotonation at 7-N site of 3-N• radical which possesses minPA2 value (Fig. 4). As can be seen from Fig. 7 an unpaired electron of the formed radical anion is delocalized over the five-membered ring including the 4-C, 5-C, 7-N and 8-O atoms. This result is consistent with results of experimental ESR measurements and provides additional evidence that this radical anion could be best described as a delocalized π radical [74]. Negative charge (expressed as negative numerical values in parentheses in Fig. 7) is also highly delocalized over the O and N atoms, where O-atoms bear larger partial negative charges. Electron transfer from radical anion results in formation of quinonoid diimine (Fig. 4).

The final product of all three mechanisms (HAT2, ET-PT2 and SPLET2) is the same, i.e., quinonoid diimine. Comparison of minBDE2, IP2, PA2 and ETE2 values show that the second $1H^+/1e^-$ SPLET mechanism is the most favorable for 3-N• radical (Table 2). Presented results indicate that preferred reaction pathway for inactivating of free radicals by uric acid in both the first $1H^+/1e^-$ and second $1H^+/1e^-$ radical scavenging processes is the SPLET mechanism. The minimal energy requirements for the second single $1H^+/1e^-$ free radical scavenging mechanisms (308.6 kJ/mol at 7-N site) are less than for the first single $1H^+/1e^-$ processes (340.4 kJ/mol at 3-N site) which could be the driving force for the double $2H^+/2e^-$ mechanisms.

3.2. Estimation of thermodynamically preferred $2H^+/2e^-$ mechanism based on electronic properties of uric and scavenged free radicals

As the scavenging mechanisms are highly influenced by the properties of the scavenged free radical species, we also calculated the free energy of reactions ($\Delta_r G$) of uric acid with each of eight selected free radicals (•OH, •OOH, $•OCH_3$, $•OC(CH_3)_3$, $O_2^{•-}$, $CH_2{=}CH{-}O{-}O•$, $CH_2{=}CH{-}CH_2{-}O{-}O•$, and $Cl_3C{-}O{-}O•$) for double ($2H^+/2e^-$) HAT, ET–PT and SPLET mechanisms (Eqs. (22)–(31)). Results are presented in Table 3. As expected, because reactants and products in reactions of uric acid with particular free radical

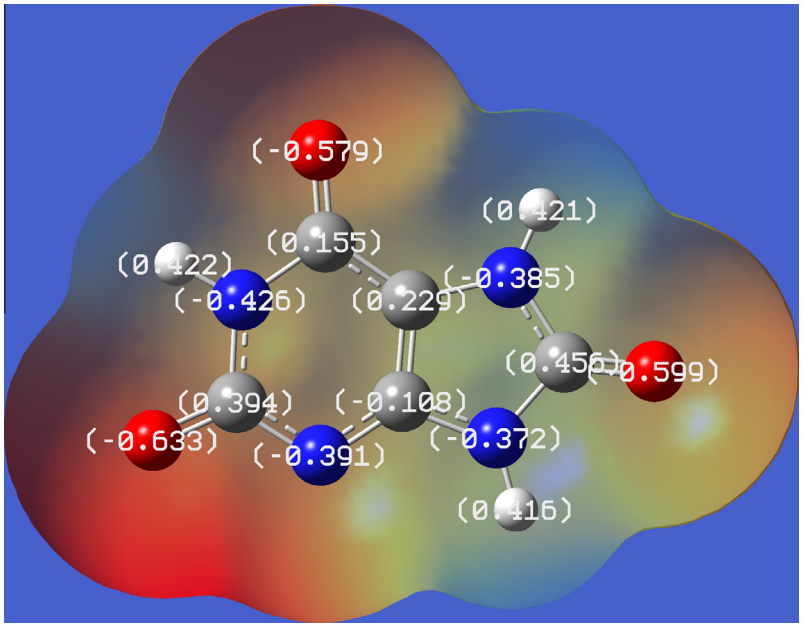


Fig. 6. Electron density map of 3-N^- anion of uric acid. The red regions indicate the negative area. Numbers in parentheses denotes atomic charges. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
SMD/M05-2X/6-311++G(d,p) reaction free energies (kJ/mol) for double HAT, ET–PT and SPLET mechanisms of free radical scavenging by uric acid in aqueous medium.

Free radical	Δ_rG_{HAT1}	Δ_rG_{HAT2}	Δ_rG_{ET-PT1}		Δ_rG_{ET-PT2}		Δ_rG_{SPLET1}		Δ_rG_{SPLET2}	
	Δ_rG_{BDE1}	Δ_rG_{BDE2}	Δ_rG_{IP1}	Δ_rG_{PDE1}	Δ_rG_{IP2}	Δ_rG_{PDE2}	Δ_rG_{PA1}	Δ_rG_{ETE1}	Δ_rG_{PA2}	Δ_rG_{ETE2}
HO$^\bullet$	−149.0	−180.4	35.8	−184.8	67.1	−247.4	−115.4	−33.6	−138.8	−41.6
CCl_3—O—O$^\bullet$	−42.3	−73.6	46.1	−88.4	77.4	−151.0	−19.0	−23.3	−42.3	−31.3
$(CH_3)_3C$—O$^\bullet$	−93.2	−124.6	102.5	−195.7	133.8	−258.3	−126.3	33.1	−149.7	25.1
CH_3—O$^\bullet$	−84.8	−116.2	109.3	−194.1	140.6	−256.7	−124.7	39.9	−148.0	31.9
CH_2=CH—O—O$^\bullet$	−11.2	−42.6	111.2	−122.4	142.5	−185.0	−53.0	41.8	−76.4	33.8
HOO$^\bullet$	−12.3	−43.6	132.2	−144.6	163.5	−207.1	−75.1	62.8	−98.5	54.8
H_2C=CH—CH_2—O—O$^\bullet$	−5.5	−36.8	137.0	−142.5	168.3	−205.1	−73.1	67.6	−96.4	59.6
O—O$^{\bullet-}$	55.2	23.9	289.5	−234.3	320.7	−296.9	−164.9	220.1	−188.2	212.1

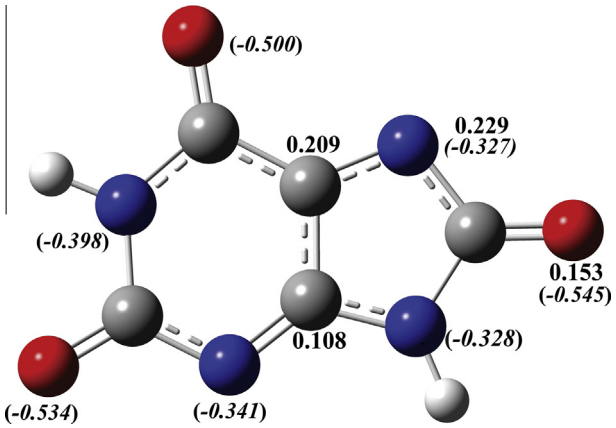


Fig. 7. Spin density and atomic charge values (in parentheses) of uric acid anion radical.

are the same, results presented in Table 3 confirm that $\Delta_rG_{HAT} = \Delta_rG_{ET-PT} = \Delta_rG_{SPLET}$. It means that all studied mechanisms have equal energy requirements and that the preferred one should be deduced from values of underlying processes, that is Δ_rG_{BDE}, Δ_rG_{IP}, Δ_rG_{PA} and Δ_rG_{ETE}.

The second and third columns in Table 3 are related to free energy change of reactions of inactivation of free radicals by uric acid *via* the first HAT (Δ_rG_{BDE1}) and second HAT (Δ_rG_{BDE2}) mechanisms, respectively. These values are also total energy requirements for the first and the second ET–PT as well as SPLET mechanisms, as it has already been discussed. More negative values indicate thermodynamically more preferred reactions. Therefore, it is clear that uric acid is the best scavenger of OH radicals (the most exergonic processes), but it is not able to scavenge $O_2^{\bullet-}$ radicals (endergonic processes).

Uric acid is able to effectively scavenge $^\bullet$OH and Cl_3C—O—O$^\bullet$ free radicals by SPLET and HAT mechanisms because these processes are exergonic in both first and second $1H^+/1e^-$ processes (Δ_rG_{PA}, Δ_rG_{ETE} and Δ_rG_{BDE}). The first step of ET–PT pathways (Δ_rG_{IP}) is endergonic and consequently ET–PT mechanism is least probable.

Alkoxyl radicals ($^\bullet OCH_3$ and $^\bullet OC(CH_3)_3$) could be scavenged by uric acid by SPLET and HAT mechanisms because Δ_rG_{PA} and Δ_rG_{BDE} are exergonic processes. In the SPLET pathway (first and second) exergonicity of Δ_rG_{PA} overwhelms endergonicity of Δ_rG_{ETE}, and according to Hess's law these pathways in summary are exergonic (thermodynamically unfeasible reaction can be driven by a thermodynamically feasible reaction that is coupled to it).

Due to lower exergonicity of mechanisms involved in inactivation of peroxyl radicals ($^\bullet$OOH, CH_2=CH—O—O$^\bullet$ and CH_2=CH—CH_2—O—O$^\bullet$) uric acid has less potential to scavenge these radicals than alkoxyl ones. Operative mechanisms could be double SPLET and double HAT.

As already mentioned, uric acid is not able to scavenge $O_2^{\bullet-}$ radicals by any mechanism because of endergonicity of these processes. This is in line with experimental ESR results that uric acid is not effective in scavenging of superoxide [17].

Obviously, here presented thermodynamic parameters may be important factors governing the radical scavenging reactions of uric acid, while a more complete understanding would require kinetic analysis. It should be noted that small positive values of Δ_rG (<10 kcal/mol) do not necessarily mean that the corresponding free radical scavenging reactions should be neglected. Such processes may represent notable reaction pathways if they take place at significant rates [75].

4. Conclusion

Double ($2H^+/2e^-$) mechanisms (HAT, SET-PT and SPLET) of free radical scavenging by uric acid in water have been studied by using SMD/M05-2X/6-311++G(d,p) calculations. Inactivation of the first free radical (by the first $1H^+/1e^-$ processes) occurs at 3-N site of uric acid and inactivation of another one (by the second $1H^+/1e^-$ processes) occurs at 7-N site of uric acid 3-N$^\bullet$ radical. The product of all studied $2H^+/2e^-$ pathways is uric acid quinonoid diimine. Among three studied mechanisms, double SPLET mechanism is thermodynamically preferred process in water as a solvent, due to the lowest values of PA and ETE in comparison with BDE and IP values (Tables 1 and 2). However, taking into account characteristics of scavenged free radicals, double HAT mechanism is also thermodynamically feasible. Exergonicity of double SPLET and HAT mechanism reactions in aqueous solution indicates that uric acid is more efficient in scavenging of HO$^\bullet$ and $Cl_3COO^\bullet$ then other investigated free radicals (Table 3).

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