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The reactivity of dopamine precursors and metabolites towards $ABTS^{\bullet -}$: An experimental and theoretical study

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Abstract: The antiradical activity of L-3,4-dihydroxyphenylalanine (L-DOPA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid and tyrosine towards 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical ($ABTS^{\bullet -}$) was investigated experimentally and theoretically by UV–Vis spectroscopy and the DFT theory. The importance of the catechol moiety for this reaction was proven due to the formation of intramolecular hydrogen bond in the formed anions and radicals. The results indicated L-DOPA and DOPAC were more potent radical scavengers than homovanillic acid and tyrosine just because of intramolecular hydrogen bond formation. Based on experimental spectra, it was proved that electron transfer led to the reduction of $ABTS^{\bullet -}$. The values of thermodynamic parameters were used to predict the preferred mechanism. The reaction rates were calculated for the electron transfer processes and it was shown that these were both kinetically and thermodynamically driven processes. Based on the reaction rate values, thermodynamic parameters, and present species, as determined by electronic spectra, it was concluded that single proton loss electron transfer (SPLET) is the dominant reaction mechanism in the investigated system.

Keywords: antioxidant activity; UV–Vis spectroscopy; DFT; radicals.

INTRODUCTION

Oxidation processes in the human organism occur due to the presence of reactive species, molecules that are found externally and internally in the human body. Oxidative stress (OS) is caused by an imbalance between the production of reactive oxygen and the ability of biological systems to detoxify the reactive intermediates or easily repair the resulting damage. Oxidative stress is important from a biomedical point of view because it is related to a wide variety of human

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diseases, such as cardiovascular disease (muscular dystrophy), inflammatory disease (rheumatoid arthritis), allergies, immune system dysfunctions, diabetes and cancer. Oxidative stress is also recognized as a major contributor to ageing and is thought to be the primary cause of all neurodegenerative disorders such as Parkinson's, Alzheimer's, Huntington's diseases and ALS. It is assumed that the neural structures are under great influence of oxygen because the brain accounts for about 20 % of the basal oxygen consumption.[2,3]

Dopamine is a hormone in the humane body with various important functions.[4–6] It is characterized by the presence of the catechol moiety that is considered to be one of the most important structural parameters governing antioxidant activity.[7] The precursor for dopamine synthesis is L-3,4-dihydroxyphenylalanine (L-DOPA), a molecule synthesized from tyrosine that also acts as a neurotransmitter. Dopamine decomposes into several important products, among which homovanillic acid, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT) are used for the analysis of dopamine metabolism.

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) test is one of the standard tests for the description of the antioxidant activity of pure substances and their mixtures.[8] The use of this test is very simple, the formed radical is soluble both in water and organic solvents, is stable over a wide range of pH values, and it rapidly undergoes the reaction.[9] The ABTS assay measures the relative ability of antioxidants to scavenge the generated ABTS radical, as compared with Trolox (a water soluble vitamin E analogue). It is based on the reduction of the radical anion of ABTS by electron transfer or hydrogen atom transfer.[9,10] Campos and Lissi[11] suggested the following net reaction for the reduction of the radical by phenolic compounds:

$$ABTS^{\bullet-} + PhOH \leftrightarrows ABTS^{2-} + PhO^{\bullet} + H^{+} \quad (1)$$

The reaction itself is very complex, due to the coexistence of ABTS molecule in several protonation/deprotonation equilibria, which are pH-dependent. One of the major criticism of the use of the ABTS test is that the steric hindrance of the radical determines the activity of molecules, which means that smaller molecules have higher accessibility to the active part.[9,12] The high value of E^0 of ABTS increases the possibility of side reactions with natural products, such as alcohols, amino acids, and monophenols.[11]

The present study comprises experimental and theoretical approaches proposing the possible reaction pathways between dopamine precursors and metabolites and $ABTS^{\bullet-}$. The experimental results for the antiradical activity of selected molecules are explained from the theoretical point of view and the rate constant values were calculated for reaction with $ABTS^{\bullet-}$. L-DOPA, DOPAC, homovanillic acid and tyrosine (Fig. 1) are selected so that important structural parameters of antioxidant activity were covered: carboxyl group and substituents on the aro-

matic ring.[13] The antiradical activity of these molecules towards different radical species has been previously examined both theoretically[13–15] and experimentally.[16–18] Based on theoretical parameters, these molecules could be considered as very good antiradical scavengers.[19]

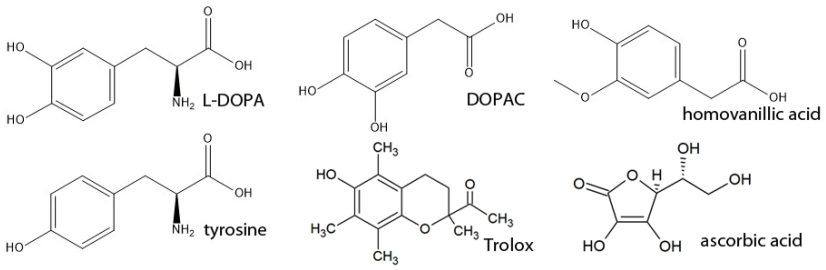

Fig. 1. The structures of the investigated molecules.

To the best of our knowledge, this is the first time that theoretical considerations of this important test have been performed and combined with experimental results.

EXPERIMENTAL

The used chemicals: tyrosine, homovanillic acid, L-DOPA, DOPAC, ascorbic acid, Trolox, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and potassium persulphate were purchased from Sigma–Aldrich. All chemicals were of reagent grade. The spectrophotometric measurements were performed on a Thermo Scientific Evolution 220 UV–Vis spectrophotometer in the range between 250 and 800 nm.

The ABTS was generated by reacting with a strong oxidizing agent (*e.g.*, potassium permanganate or potassium persulphate) with ABTS. The procedure for the preparation of $ABTS^{\bullet-}$ was taken from the literature.[20] The standard solutions were 7 mM aqueous solution of ABTS and 2.45 mM aqueous solution of potassium persulphate. The mixture was left in the dark for 12–14 h. The working concentration of generated radical was determined from the absorptivity coefficients (ε_{415} = 3.6×10^4 M^{-1} cm^{-1} and ε_{734}=1.5×10^4 M^{-1} cm^{-1}).[16] The stock solution of the investigated molecules was 5 mM. The activity of the investigated substances was obtained as the slope of the plot showing the dependence of the percentage reduction on the substance concentration.[9] The Trolox equivalent antioxidant capacity (*TEAC*) was determined as the ratio of the slopes between the substance of interest and Trolox.

Computational

The optimization of structures was performed using the DFT functional M06-2X[21] in conjunction with the 6-311++G(d,p) basis set.[22] For this purpose, the Gaussian program package[23] was employed. In order to mimic the experimental conditions, the SMD solvent model was applied.[24] The absence of imaginary frequencies after the optimization of neutral molecules, their radical cations, radicals and anions proved that the structures with energy minima were found. natural bond orbital (NBO)[25] analysis was used to examine the charge and spin distribution in the formed radicals and anions, as implemented in the NBO 6.0 program package.[26]

The kinetics of electron transfer (ET) between species was investigated by the Marcus' theory.[27] The activation barrier for this process depends on two parameters – the change of Gibbs energy of the reaction (ΔG^0_{ET}) and the nuclear reorganization energy (λ):

$$\Delta G^{\ddagger}_{ET} = \frac{\lambda}{4}\left(1+\frac{\Delta G^0_{ET}}{\lambda}\right)^2 \quad (2)$$

The nuclear reorganization energy was calculated as the difference between the non-adiabatic energy difference between products and reactants (ΔE_{ET}) and the activation barrier:

$$\lambda \approx \Delta E_{ET} - \Delta G^0_{ET} \quad (3)$$

The reaction rate was calculated as follows:

$$k_{ET} = \frac{k_B T}{h}\exp -\frac{\Delta G^{\ddagger}_{ET}}{RT} \quad (4)$$

where k_B is the Boltzmann's constant, T is temperature, h is the Planck's constant and R is the universal gas constant. The ET reaction rates are usually comparable to diffusion limited rate constants. The apparent rate constant values were calculated by the Collins–Kimball theory[28] (Eq. (5)). The Smoluchowski rate constant values were calculated under the assumption of an irreversible bimolecular diffusion-controlled reaction (Eq. (6)):

$$k_{app} = \frac{k_D k}{k_D + k} \quad (5)$$

$$k_D = 4\pi R D_{AB} N_A \quad (6)$$

In the previous equation, D_{AB} is the diffusion coefficient of reaction, N_A is Avogadro's constant. The calculation of the diffusion coefficients can be found in the rature.[7]

RESULTS AND DISCUSSION

Throughout this paper, $ABTS^{\bullet-}$ is denoted as a radical anion according to the work of Scott and coworkers,[29,30] although some authors suggest that the radical species is positively charged.[9] The radical cation notation is more common so that the reduction to neutral form, due to the electron transfer, is explained. The spectrophotometrically determined pK_a of $HABTS^-$ is 2.08±0.02,[30] therefore, more than 99.9 % of ABTS is in the deprotonated form at the experimental pH value of 7.2. The measurements in this study were performed at 734 nm as the investigated molecules do not absorb in this region.[7] The experimentally determined radical scavenging activities are given in Table I.

TABLE I. Antiradical activities of the selected molecules

Substance	*TEAC*
L-DOPA	1.92
DOPAC	1.86
Homovanillic acid	0.08
Tyrosine	0.04
Ascorbic acid	1.36
Trolox	1

It should be noted that ascorbic acid is added to the group as the standard antioxidant, and the obtained value of 1.36 is very close to the one reported by Walker and Everette.[29]

Based on the results presented in Table I, the investigated molecules could be divided into two groups. The first group consists of L-DOPA and DOPAC, with TEAC values being higher than 1.4. This leads to the conclusion that selected molecules are more potent radical scavengers than Trolox and ascorbic acid. The scavenging activity of dopamine towards $ABTS^{\bullet-}$ is given in a paper by Miura and coworkers.[31] The TEAC values from this study are comparable to those for chlorogenic, caffeic and ferulic acids,[29] the molecules structurally characterized by a catechol moiety, which is a common structural element of good antioxidants. In a previous study, the reactivity of L-DOPA and DOPAC was also proven towards another standard radical, $DPPH^{\bullet}$.[7] The obtained activity was higher than for the standard antioxidants, Trolox, ascorbic acid and (+)-α-tocopherol.[7]

The second group includes homovanillic acid and tyrosine, molecules with either one hydroxy group or a hydroxy and methoxy groups attached directly to the aromatic ring. Their activity is two orders of magnitude lower than for the first group of molecules. The structure of the radical formed from these two molecules does not contain a hydrogen bond that additionally stabilizes the formed radical and, consequently, causes the lower activity. When the structures of L-DOPA/tyrosine and DOPAC/homovanillic acid pairs are compared, it could be concluded that they differ only in the number of OH groups. The backward analysis proves that the majority of the activity comes from the catechol moiety, while a single OH group does not play an important role, although some activity was observed. The theoretical part of the paper describes the possible mechanism because carboxyl groups in molecules from this study could be deprotonated at the experimental pH value and therefore, electron transfer from the anions is to be expected.

In order to understand better the mechanism behind the reduction of $ABTS^{\bullet-}$, the structures of H_2ABTS, $ABTS^{\bullet-}$ and $ABTS^{2-}$ were optimized at the M06-2X/6-311++G(d,p) level of theory. The optimized structure of $ABTS^{\bullet-}$ with an enumeration of the atoms of interest is given in Fig. 2. The values of the NBO charge and spin density in the mentioned structures are presented in Table II.

The molecule of H_2ABTS is a highly symmetric molecule. Once it is deprotonated from sulphate groups, the charge is delocalized over the whole molecule, which leads to the small change on the atoms of interest. As could be seen, the changes are negligible on the sulphur and nitrogen atoms of the thiazole rings. The dianion is stabilized through delocalization over the aromatic and thiazole rings. The radical anion is formed when one of the electrons is removed from dianion, $ABTS^{2-}$, by an oxidizing agent, in this case potassium persulfate. This

induces more noticeable changes in charge on the nitrogen and sulphur atoms, with a decrease in negative charge on the nitrogen atoms, especially on N2 and N3, and an increase in positive charge on the sulphur atoms. The consequence of this is spin density delocalization through nitrogen and sulphur atoms. It should be noted that most of the spin density is found localized on the N2 and N3 atoms. Such good delocalization of unpaired electrons leads to stability of the formed radical for a long time. These results are consistent with literature data.[10,32]

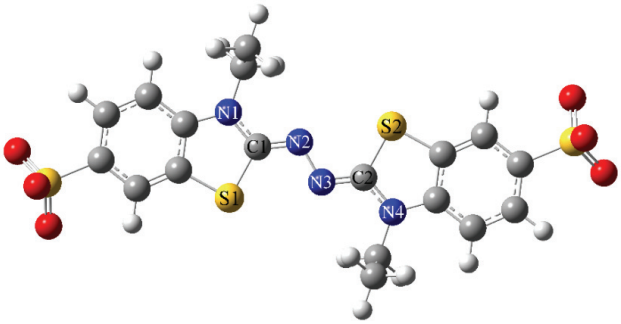

Fig. 2. The structure of $ABTS^{\bullet-}$ (with enumeration of the atoms of interest).

TABLE II. NBO charge and spin density on the atoms of interest in the optimized structure of H_2ABTS, $ABTS^{\bullet-}$ and $ABTS^{2-}$

Atom	H2ABTS	$ABTS^{\bullet-}$		$ABTS^{2-}$
	Charge	Charge	Spin density	Charge
N1	–0.471	–0.385	0.143	–0.479
C1	0.334	0.316	–0.025	0.332
S1	0.365	0.473	0.067	0.348
N2	–0.443	–0.332	0.255	–0.454
N3	–0.443	–0.332	0.255	–0.454
C2	0.336	0.316	–0.025	0.335
S2	0.364	0.474	0.067	0.351
N4	–0.471	–0.385	0.143	–0.479

As mentioned earlier, the reduction of $ABTS^{\bullet-}$ occurs when an electron is transferred from a radical scavengers to the radical. There are two commonly investigated mechanisms that include electron transfer: single electron transfer--proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET). SET-PT and SPLET are two-step mechanisms, the first one includes the transfer of an electron followed by the transfer of a proton (Eqs. (7a) and (7b)). On the other hand, SPLET consists of proton exchange between an antioxidant and $ABTS^{\bullet-}$ in the first and electron exchange in the second step (Eqs. (8a) and (8b)). The formed radical from DOPAC and L-DOPA can be further oxidized forming the corresponding quinones:[33]

$$AOH + ABTS^{\bullet-} \leftrightarrows AOH^{\bullet+} + ABTS^{2-} \quad (7a)$$

$$AOH^{\bullet+} + ABTS^{2-} \leftrightarrows AO^{\bullet} + HABTS^{-} \quad (7b)$$

$$AOH + ABTS^{\bullet-} \leftrightarrows AO^{-} + HABTS^{\bullet} \quad (8a)$$

$$AO^{-} + HABTS^{\bullet} \leftrightarrows AO^{\bullet} + HABTS^{-} \quad (8b)$$

As presented by Eqs. (7) and (8), $ABTS^{2-}$ and $HABTS^{-}$ are the species formed after the electron transfer to the radical species. UV–Vis spectrophotometry can be used to distinguish[30] between the following forms of ABTS: $HABTS^{-}$, $ABTS^{\bullet-}$ and $ABTS^{2-}$. Only one absorption maximum is characteristic for $ABTS^{2-}$ and $HABTS^{-}$ positioned at 340 and 310 nm, respectively, while the spectrum of $ABTS^{\bullet-}$ contains four absorption maxima positioned at 417, 645, 728 and 810 nm. The electronic spectra before and after the addition of a sufficient amount of DOPAC to reduce the present $ABTS^{\bullet-}$ are shown in Fig. 3.

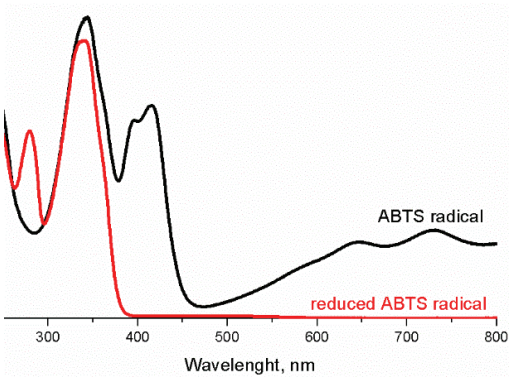

Fig. 3. The electronic spectrum of $ABTS^{\bullet-}$ before and after the addition of DOPAC.

As it can be seen in the spectra, before the reduction, absorption maxima characteristic both for $ABTS^{2-}$ and $ABTS^{\bullet-}$ are present, which is due to the partial oxidation of ABTS in the presence of potassium persulfate.[34] After the reduction, only the maximum of $ABTS^{2-}$ exists, which indicates radical reduction by electron transfer. Both mechanisms have $HABTS^{-}$ as one of the products, but because of the low pK_a of this anion, it could be expected that deprotonation occurs to a large extent. Therefore, the experimental results cannot be helpful in determining the actual mechanism.

The preferability of a mechanism could be determined based on the thermodynamic parameters for antioxidant activity, as previously explained.[7] When the structures of L-DOPA, DOPAC, homovanillic acid and tyrosine are compared, it is obvious that there are several positions from which a hydrogen atom could be transferred, hydroxyl groups and a carboxyl group. Careful analysis proved that the OH group in the *para*-position is the most probable position for hydrogen atom loss and only the results for this position are given in this contribution.[7,13] Energy requirements related to the mentioned radical scavenging mechanisms are

governed by different molecular properties: the ionization potential (*IP*) of ArOH and proton dissociation enthalpy (*PDE*) of $ArOH^{\bullet+}$ in the SET-PT mechanism, and proton affinity (*PA*) of ArOH and electron transfer enthalpy (*ETE*) of ArO^- in the SPLET mechanism. The values for thermodynamic parameters were calculated by employing Eqs. (9)–(12):

$$IP = H(AOH^{\bullet+}) + H(e^-) - H(AOH) \quad (9)$$

$$PDE = H(AO^{\bullet}) + H(H^+) - H(AOH^{\bullet+}) \quad (10)$$

$$PA = H(AO^-) + H(H^+) - H(AOH) \quad (11)$$

$$ETE = H(AO^{\bullet}) + H(e^-) - H(AO^-) \quad (12)$$

The same procedure is repeated for the carboxylate anionic form of the investigated molecules because their significant deprotonation is expected due to the low pK_a values. The results are presented in Table III.

TABLE III. The thermodynamic parameters for the antiradical activity of L-DOPA, DOPAC, homovanillic acid and tyrosine (in kJ mol^{-1})

Molecule	Neutral				Anion			
	SET-PT		SPLET		SET-PT		SPLET	
	IP	*PDE*	*PA*	*ETE*	*IP*	*PDE*	*PA*	*ETE*
L-DOPA	471	24	131	364	476	29	149	356
DOPAC	487	22	140	369	471	31	146	356
HVA	482	35	154	363	466	44	160	349
Tyrosine	506	28	156	378	495	34	158	371

When the thermodynamic parameters for antiradical activity are compared, a lower value of the parameter describes the more probable mechanism or reaction position. The *IP* parameter denotes electron loss and the formation of the respective radical cation and usually molecules with lower *IP* are readily engaged in chemical reactions with radical species.[7] The lowest value of *IP* (471 kJ mol^{-1}) was calculated for L-DOPA when neutral molecules are concerned. DOPAC and HVA have similar values of *IP*, which proves that this parameter does not depend on the substituents on the aromatic ring. The highest value of *IP* was obtained for tyrosine. When anions are concerned, the values of *IP* are lower in all cases except for L-DOPA, but their values are still much higher than 450 kJ mol^{-1}. Nenadis and coworkers also concluded that the ionization potential cannot be correlated with TEAC values for phenolic compounds,[36] probably due to the very complex reaction mechanism. The second step of the SET-PT mechanism requires less than 30 kJ mol^{-1} of energy for neutral molecules and less than 45 kJ mol^{-1} of energy for anions.

Proton affinity is the parameter used for the first step of the SPLET mechanism. Its value is much lower than that of *IP*, indicating a higher probability of the SPLET mechanism over the SET-PT. Again, the lowest value of *PA* was

calculated for L-DOPA, proving its highest activity towards radicals. The second order perturbation theory allowed the investigation of the stabilization interactions in the formed anions. Both L-DOPA and DOPAC interact with the lone pair of oxygen and the OH group with a value of 2.20 kJ mol^{-1}, which additionally stabilizes the structure. The values of *PA* for homovanillic acid and tyrosine are higher, by 15–25 kJ mol^{-1}, than the same parameter for DOPAC and L-DOPA. When anions are investigated, the *PA* values are higher by several kJ mol^{-1}, which was expected because the electron is lost from anions and dianions are formed. The values of *PA* for anions still show good correlation with the experimental data. The *PA* parameter is very dependent on the substituents on the aromatic ring. The exchange of one OH group with OCH_3 increases *PA* value for 15 kJ mol^{-1}, while the loss of one OH group increases *PA* value for 25 kJ mol^{-1}. This is a consequence of the hydrogen bond formation between the deprotonated OH group and the neighbouring groups.

The values of *PA* are lower than the *IP* values and therefore, this is not a limiting step in the process of antiradical activity. The second step includes electron transfer from an anion to a radical species and the formation of the respective radicals. The thermodynamic parameter governing this step is more positive than the first one, on average 230 kJ mol^{-1} (Table III). The kinetic parameters for this process were calculated using the Marcus theory, Eqs. (4) and (5). The changes in the Gibbs energy of reaction and apparent rate constants are given in Table IV for both neutral molecules and anions.

TABLE IV. The change in Gibbs energy of reaction and reaction rates for the electron transfer step in the SPLET mechanism

Molecule	Neutral		Anion	
	ΔG_{ET} / kJ mol^{-1}	k_{app} / M^{-1} s^{-1}	ΔG_{ET} / kJ mol^{-1}	k_{app} / M^{-1} s^{-1}
L-DOPA	–53.04	3.21×10^9	–59.58	3.21×10^9
DOPAC	–46.75	3.20×10^9	–60.90	3.21×10^9
HVA	–52.03	3.20×10^9	–67.80	3.22×10^9
Tyrosine	–39.04	3.19×10^9	–45.70	3.21×10^9

The electron transfer reactions, as the second step in the SPLET mechanism, are exothermic as shown by the change in Gibbs free energy of the reaction. The values of this thermodynamic parameter are lower by several kJ mol^{-1} for anionic than for neutral forms. The values of ΔG_{ET} are around –60 kJ mol^{-1} for L-DOPA, DOPAC and homovanillic acid, while for tyrosine, it is –46 kJ mol^{-1}. The values for reaction rates are of the order 10^9 M^{-1} s^{-1} for the investigated molecules both in neutral and anionic forms. This result proves that the electron transfer reactions are diffusion controlled and therefore not limiting step either. When the actual values for reaction rates are compared, it is clear that the differences are negligible. This shows that reaction rate values cannot be taken as the

only parameter of activity because a clear correlation with the experimental results is not possible. Only together with *PA* values and stabilization interactions in formed anions and radicals do the reaction rate values give a complete picture of reactivity. The net reaction is consistent with the findings of Campos and Lissi.[11]

The obtained results imply that the reactions between the dopamine precursors and metabolites and the ABTS radical preferentially go through the SPLET mechanism. The thermodynamic parameters governing different mechanisms are lower for DOPAC and L-DOPA, due to hydrogen bond formation in the corresponding anions and radicals, which proves the importance of the catechol moiety. The presence of a carboxylic group and the possibility of its deprotonation does not cause significant changes in the preferred mechanism. Once the electron transfer occurs, the formed radicals could possibly react with another ABTS radical or undergo self-combination.[11] As this is a first attempt to give a theoretical explanation of the experimental data on ABTS reduction measurements, it is believed that additional considerations will be needed in the future.

CONCLUSIONS

The radical scavenging activity of DOPAC, L-DOPA, tyrosine and homovanillic acid towards $ABTS^{\bullet-}$ was determined by means of UV–Vis spectroscopy and the DFT theory. The molecules containing the catechol moiety, and having TEAC values larger than 1.4 were shown to be better scavengers than ascorbic acid and Trolox. Once the second hydroxy group is lost or exchanged by the methoxy group, the activity is two orders of magnitude lower. The absence of absorption maxima of $HABTS^{-}$ species in electronic spectra confirmed the reduction of $ABTS^{\bullet-}$ upon electron transfer from the radical scavenger. The thermodynamic parameters, namely ionization potential (*IP*) and proton affinity (*PA*) were employed to discuss the preferability of the mechanism. The *PA* values reflect well the presence of various substituents and the possibility of the formation of intramolecular hydrogen bonds in the formed anions. These values were lower than the *IP*, which makes sequential proton loss electron transfer (SPLET) the thermodynamically preferred mechanism. Due to low pK_a values of the investigated molecules, their deprotonation to the large extent was expected, but the thermodynamic parameters did not show a change in the order of parameters. The second step of SPLET, electron transfer, was analyzed by the Marcus theory. The reaction rates, both for neutral and anionic species, are of the order 10^9 M^{-1} s^{-1}, which makes them diffusion controlled processes. Therefore, the suggested mechanism of $ABTS^{\bullet-}$ reduction includes the exchange of protons, electron transfer and later the deprotonation of $HABTS^{-}$, which is consistent with the experimental observations. Since this is the first time that the reactions with this radical were theoretically analyzed, further research is certainly needed.

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ИЗВОД

РЕАКТИВНОСТ ПРЕКУРСОРА И МЕТАБОЛИТА ДОПАМИНА ПРЕМА ABTS$^{\bullet-}$: ЕКСПЕРИМЕНТАЛНА И ТЕОРИЈСКА СТУДИЈА

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Антирадикалска активност L-3,4-дихидроксифенилаланина (L-DOPA), 3,4-дихидроксифенилсирћетне киселине (DOPAC), хомованилинске киселине и тирозина према ABTS$^{\bullet-}$ је испитана експериментално и теоријски применом UV–Vis спектроскопије и метода теорије функсионала густине. Важност катехолне групе доказана је формирањем интрамолекулске водоничне везе у формираним анјонима и радикалима. Резултати су показали да су молекули L-DOPA и DOPAC бољи гасиоци радикала у односу на хомованилну киселину и тирозин што се тумачи као последица формирања интрамолекулске водоничне везе. На основу експериментално добијених спектара закључено је да пренос електрона доводи до редукције ABTS$^{\bullet-}$. Вредности термодинамичких параметара су искоришћене за одређивање вероватнијег механизма редукције. Израчунате су вредности константе брзине за процес преноса електрона и показано да термодинамички и кинетички параметри реакције утичу на дати процес. На основу вредности констате брзине, термодинамичких параметара и присутних врста, одређених на основу електронских спектара, закључено је да је губитак једног протона праћен преносом електрона (sequential proton loss electron transfer (SPLET)) доминантан реакциони механизам у испитиваним системима.

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