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Essential features for antioxidant capacity of ascorbic acid (vitamin C)

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Abstract

Vitamin C or ascorbic acid is an indispensable micronutrient for human health found principally on citrus species such as lemon and orange fruits, and vegetables. It was involved in the production of proteins such as collagen. Its biochemical mechanism is related to its antioxidant capacity, however its function at the cellular level is still unclear. Several theoretical studies about antioxidant and redox mechanisms for ascorbic acid were suggested, however no derivative was proposed. Thereby, an electronic study of antioxidant capacity for ascorbic acid derivatives was performed using theoretical chemistry at the DFT/ B3LYP/6-311++(2d,2p) level of theory. Simplified derivatives show that enol hydroxyls are more important than any other functional group. The vicinal enolic hydroxyl on β -position are more important for antioxidant capacity of ascorbic than hydroxyl on α -position. According to our molecular modifications, the keto-alkene compound showed the best values when compared to ascorbic acid in some molecular characteristics. New promising structural derivatives related to ascorbic acid can be developed in the future.

Keywords Ascorbic acid • Antioxidant mechanism • Pharmacophore • DFT • Redox capacity.

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Introduction

Ascorbic acid (AA) or vitamin C is an essential micronutrient for human health, acquired mainly through citrus fruits, such as lemon and orange, and vegetables, with antioxidant activity and involved in the production of proteins such as collagen, although its function at the cellular level is still unclear [1, 2]. Its deficiency can cause hemorrhagic condition (scurvy) of the skin and gums with an attack on collagen in the connecting tissues [3, 4].

Chemically, the structure (Figure 1) is composed of a lactone ring (in red) and two enolic hydroxyls (in green), in addition to a primary and a secondary alcohol group (in blue). The two intermolecular hydrogen bonds contribute substantially to the stability and chemical qualities of the molecule [5]. The enolic form of α -ketolactone, known as (R)-3,4-dihydroxy-5-((S)-1,2-dihydroxyethyl)-furan-2(5H)-one, is easily oxidized in the form of diketo (dehydroascorbic acid) and converted to oxalic acid, diketogulonic acid or threonic acid [5]. AA is an electron donor and therefore a reducing agent, resulting from all its known physiological and biochemical actions. It has an ability to donate a hydrogen atom and form a relatively stable ascorbyl free radical, thanks to its low electron potential and resonance stability. Yet, it donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule. In addition, it plays a role in purifying reactive oxygen species (ROSs) and nitrogen, superoxide, ozone, among others, maintaining the intracellular redox balance and minimizing the oxidative damage caused by these free radicals [1, 3, 5–9].

Fig. 1 Redox cycle of ascorbic acid (AA) in anion (a), semiquinone (sq) and ascorbate quinone (q).

AA has been widely used in the treatment and prevention of cancer, although its clinical results are still inconclusive. At low concentrations, it has an antioxidant role, preventing oxidation, which induces apoptosis [1, 10]. Physiological concentrations of ascorbate demonstrated inhibition of LDL oxidation and a synergistic action with vitamin E preventing lipid oxidation of cell membranes [1, 11, 12].

Based on their activity, antioxidants can be categorized as enzymatic and non-enzymatic. Enzymatic antioxidants work by breaking down and removing free radicals. To understand the mechanism of action of antioxidants, it is necessary to understand the generation of free radicals and their damaging reactions [13].

AA changes to the ascorbate radical by donating an electron to the lipid radical to terminate the lipid peroxidation chain reaction. The pairs of ascorbate radicals react quickly to produce an ascorbate molecule and a dehydroascorbate molecule (without antioxidant activity), the latter being converted back to ascorbate by the addition of two electrons, probably due to the action of oxidoreductase [13, 14].

Despite the instability of ascorbic acid in solution has been known for almost a century, the kinetics and mechanism of degradation, along with the influence of experimental conditions are still debated and controversial. Product studies indicate that, under aerobic conditions in an aqueous environment, the stages of degradation of the formal oxidation of two electrons by molecular oxygen produces dehydroascorbic acid, which is hydrolyzed to diketogluconic acid and, subsequently transformed into a variety of products [14–16].

Methodology

All the calculations were performed with the Gaussian 09 molecular package [17]. The PM3 geometries [18] were optimized with the DFT/B3LYP hybrid density functional [19–21] by using the 6-311++G(2d,2p) basis set [22]. Based on lowest energy structures of neutrals (Mol or AA), cation free radicals (Mol^{•+} or AA^{•+}), semiquinones (Mol[•] or AA[•]), and anion free radicals (Mol^{•-}), four antioxidant mechanisms were explored (Figure 2). Reactive species such as cation free radical (RS^{•+}) or semiquinone (RS[•]) are disassembled by means of electron or hydrogen scavenging. Usually, the single electron transfer (SET) and the hydrogen atom transfer (HAT) are the main antioxidant mechanisms, however there are many other [23]. The ionization potential (IP) was calculated as the energy difference between a neutral molecule (Mol) and its respective cation free radical (1). The SET was calculated, as showed in equation 2, as a difference between ascorbic acid neutral and cation free radical (AA and AA^{•+}) minus all other derivatives (Mol and Mol^{•+}) [24–30]. The sequential proton lost electron transfer (SPLET) values were calculated as the difference between semiquinone (Mol[•]) and its respective anionic form (Mol^{•-}) (3). The bond dissociation energy of enol moiety (BDE_{OH}) was calculated as the energy difference between a neutral molecule (Mol) and its respective semiquinone plus hydrogen (4). The HAT was calculated, as showed in equation 5, as a difference between ascorbic acid (AA) minus all other derivatives (Mol). The single electron acceptance (SEA) values were calculated as the difference between anion free radical (Mol^{•-}) and its respective quinone form (MolQ) (6).

$$SET = EMol^{\bullet+} - EMol \quad (1)$$

$$SET = (EAA^{\bullet+} + EMol) - (EAA + EMol^{\bullet+}) \quad (2)$$

$$SPLET = EMol^{\bullet} - EMol^{\bullet-} \quad (3)$$

$$BDE_{OH} = (EMol^{\bullet} + EH^{\bullet}) - (EMol) \quad (4)$$

$$HAT = (EAA^{\bullet+} + EMol) - (EAA + EMol^{\bullet+}) \quad (5)$$

$$SEA = EMol^{\bullet-} - EMol \quad (6)$$

Fig. 2 Proposed antioxidant mechanism on redox process of ascorbic acid and simplified derivatives.

Results and discussion

In this study, the molecular modifications of ascorbic acid (1) are compared with their simplified derivatives by using molecular simplification on furanone system (2-5) and compared to furan ring derivatives (6-9). These compounds are showed in Fig. 3.

Fig. 3 Chemical structures of ascorbic acid (1) and its simplified derivatives (2-9).

According to theoretical results described in Table 1, the ionization potential (IP) and single electron transfer (SET) values have indicated a lesser variation between ascorbic acid (1) and derivative 5 of 193.51 and 202.15 kcal/mol, when compared to compounds (2 and 3) without a hydroxyl (211.49 and 206.14 kcal/mol) or non-hydroxylated (210.17 kcal/mol) as observed in molecule 4, linked to the lactone ring. The same trend was observed for the transfer of a hydrogen, with enolic bond dissociation energy (BDE_{OH}) values of 80.97 and 86.24 kcal/mol for ascorbic acid (1) and 82.75 and 87.20 kcal/mol for derivative 5, considering the β and α position of the enolic group. In addition, according to the BDE_{OH} values, 2 and 3 compounds showed the importance of the diol system due to the increase of BDE_{OH} values to 91.70 and 99.99 kcal/mol. This agrees to IP values and explains the higher value of 210.17 kcal/mol for the PI of derivative 4 of ascorbic acid, which does not have the enolic hydroxyls.

According to these results, the 3,4-dihydro-furan-2-one system (5) is the most important and essential group for the antioxidant effect observed in ascorbic acid [30–33]. Consequently, similar structures that can be more potent than ascorbic acid in free radicals scavenging were proposed through molecular modifications [24, 27] that included: i) the molecular simplification by removal of primary and secondary alcohol from the saturated chain, ii) the removal of carbonyl for the formation of the ether in simplified ring (6), iii) the addition of a second carbonyl in the δ position for the formation of the anhydride derivative (7), and the modification of the lactone group to obtain an aliphatic (8) and olefinic ketone (9).

According to these proposals, the absence of the carbonyl group decreased the values of IP and BDE_{OH} to 184.30 and 76.94 kcal/mol (**6**) and the presence of an extra carbonyl increased these values to 225.99 and 97.64 kcal/mol (**7**). These results are accompanied by the lower SET and HAT values of -9.20 and -4.03 kcal/mol (**6**) and higher values of 32.48 and 16.67 kcal/mol (**7**), respectively. The presence of the keto-alkene group (**9**) showed the best values of IP and BDE_{OH} (186.29 and 77.48 kcal/mol) when compared to the keto-alkane compound (**8**), whose values increased to 199.01 and 89.77 kcal/mol. These results are also accompanied by the lower SET and HAT values of -7.21 and -3.49 kcal/mol for keto-alkene (**9**) and higher SET and HAT values of 5.50 and 8.80 kcal/mol for keto-alkane (**8**).

Table 1 Theoretical parameters for ascorbic acid and related derivatives.

Compound	IP	SET	BDE _{OH} ^a	BDE _{OH} ^b	HAT	SPLET	BDE _{OH} ^c	SEA
1	193.51	0	86.24	80.97	0	72.12	70.14	-68.41
2	211.49	17.97	—	91.70	10.73	87.66	—	—
3	206.14	12.63	—	99.99	19.02	70.41	—	—
4	210.17	16.66	—	—	—	—	—	—
5	202.15	8.63	97.20	82.75	1.78	60.43	69.90	-60.43
6	184.30	-9.20	76.94	76.94	-4.03	35.75	59.59	-35.75
7	225.99	32.48	97.64	97.64	16.67	79.36	71.97	-79.36
8	199.01	5.50	94.48	89.77	8.80	55.72	72.73	-55.72
9	186.29	-7.21	77.61	77.48	-3.49	46.60	63.02	-46.60

BDE_{OH} values: abstraction of hydrogen for a) β position, b) α position, and c) quinone formation.

The same disposition was observed for the SPLET mechanism and for the quinone (MolQ) formation, revealing **6** and **9** derivatives have the best performance by the lowest values for these properties of 35.75 and 59.59 kcal/mol (**6**) and 46.60 and 63.02 kcal/mol (**9**), respectively. Finally, according to the single electron acceptance (SEA) values for the quinone derivatives, the presence of the carbonyl group is important for the best values [34]. The SEA values followed the order: dicarbonyl **7** (-79.36 kcal/mol) > carbonyl **5** (-60.43 kcal/mol) > keto-alkane **8** (-55.72 kcal/mol) > keto-alkene **9** (-46.60 kcal/mol) and ketone **6** (-35.75 kcal/mol), when compared to ascorbic acid **1** (-68.41 kcal/mol). Thus, the keto-alkene **9** can be a good strategy on design of potent antioxidant related to ascorbic acid.

Conclusion

According to our obtained theoretical results, the keto-alkene compound showed the best values when compared to ascorbic acid. However, it is surprising the superiority of the redox

property of ascorbic acid in comparison to the derivatives proposed in this work. Except for SEA, all suggested molecules showed better values for the studied parameters. In this sense, it is possible that the best natural compounds that act as antioxidants have an average value for all properties, and ascorbic acid can be act by several antioxidant mechanisms.

***Declarations**

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