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A theoretical study of ascorbic acid oxidation and $HOO^{\bullet}/O_2^{\bullet-}$ radical scavenging†

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Ascorbic acid is a well-known antioxidant and radical scavenger. It can be oxidized by losing two protons and two electrons, but normally loses only one electron at a time. The reactivity of the ascorbate radical is unusual, in that it can either disproportionate or react with other radicals, but it reacts poorly with non-radical species. To explore the oxidation mechanism of ascorbic acid, the pK_a's and reduction potentials have been calculated using the B3LYP/6-31+G(d,p) and CBS-QB3 levels of theory with the SMD implicit solvent model and explicit waters. Calculations show that the most stable form of dehydroascorbic acid in water is the bicyclic hydrated structure, in agreement with NMR studies. The possible oxidation reactions at different pH conditions can be understood by constructing a potential-pH (Pourbaix) diagram from the calculated pK_a's and standard reduction potentials. At physiological pH disproportionation of the intermediate radical is thermodynamically favored. The calculations show that disproportionation proceeds *via* dimerization of ascorbate radical and internal electron transfer, as suggested by Bielski. In the dimer, one of the ascorbate units cyclizes. Then protonation and dissociation yields the fully reduced and bicyclic fully oxidized structures. Calculations show that this mechanism also explains the reaction of the ascorbic acid radical with other radical species such as superoxide. Ascorbate radical combines with the radical, and intramolecular electron transfer followed by cyclization and hydrolysis yields dehydroascorbic acid and converts the radical to its reduced form.

Introduction

Free radicals in the body can damage DNA, cells, and tissues *via* oxidation, as they react quickly with non-radical species to form new radicals and propagate free-radical chain reactions.[1] In contrast, antioxidants can protect against oxidative damage by breaking free radical chains. Since its identification by Albert Szent-Györgyi and Walter Norman Haworth, ascorbic acid (vitamin C) has become popularly acclaimed as a preeminent antioxidant and free-radical scavenger.[2–4] Due to its remarkable behavior, the chemistry of ascorbic acid has been studied extensively, but the oxidation mechanism is complex and still poorly understood. Ascorbic acid can lose two electrons, as observed by voltammetry.[5] However, it commonly functions as a one-electron reducing agent because the ascorbate radical ($\mathbf{A}^{\bullet-}$ in Scheme 1), the one-electron oxidation product of ascorbic acid, is relatively unreactive with non-radical species and reacts preferentially with itself and other radicals.[6–8] These properties of ascorbate radical are crucial to the biological function of ascorbic acid, and a more comprehensive understanding of these properties would be helpful.

Dehydroascorbic acid (**DHA**) is the fully oxidized form of ascorbic acid and can be formed by two-electron oxidation of ascorbic acid, by one-electron oxidation of ascorbate radical or by a disproportionation reaction between two ascorbate radicals. Pulse radiolysis studies by Bielski *et al.* showed that, in the presence of protons, two ascorbate radicals react together to form an unspecified dimer that disproportionates to a fully reduced ascorbic acid and a fully oxidized dehydroascorbic acid.[6] The structure of **DHA** is commonly shown as **DHA1** (Scheme 1) even though it is known that this structure is energetically unstable and can be detected only in "dry" aprotic solvent.[9] In aqueous media, the primary **DHA** structure is actually found to be the bicyclic hydrated form **DHA4**.[10–14] Understanding the mechanism of formation of **DHA4** has significant importance for exploring the likely reaction pathways for oxidation of ascorbic acid. Extensive studies of the thermodynamics and kinetics of ascorbic acid oxidation have been conducted experimentally.[6,7,9,11,15] The structures related to these reactions have also been studied theoretically[11,16–36] by

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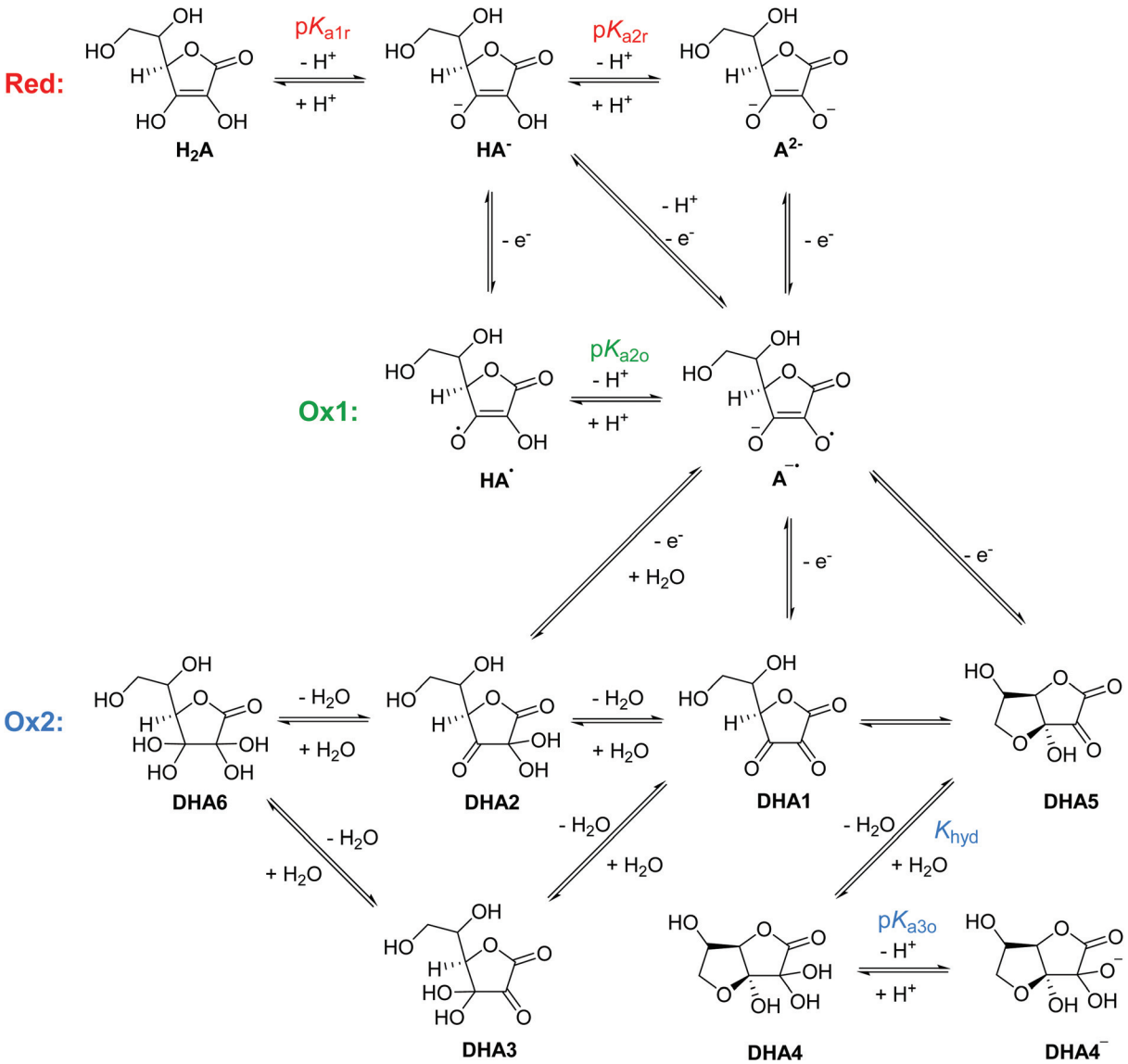
†Electronic supplementary information (ESI) available: Structures and energies of low energy conformers of ascorbic acid in its various protonated and oxidized states; energetics for the hydration of formaldehyde and acetaldehyde and energy diagrams for disproportionation of ascorbate radical and the reaction of ascorbate radical with superoxide; energy diagrams associated with the various schemes; Cartesian coordinates for reactants, intermediates, transition states, and products (PDF file). See DOI: 10.1039/c7ob00791d



Scheme 1 Electrochemical equilibria and structures of ascorbic acid and its derivatives in water.

several groups; however, the role of the **DHA4** structure is seldom discussed in these studies. In the disproportionation reaction, it is generally assumed that the ascorbate radical obtained from the first oxidation undergoes one-electron transfer to form **DHA1** as an intermediate, which then quickly undergoes ring closure and hydration to form the stable bicyclic hydrated form **DHA4**.[11,15] However, the rate for this process would be expected to be very slow because the energy required to form **DHA1** is so high that it occurs rarely. Another suggested pathway is that ascorbate radicals obtained from the first oxidation undergo an initial dimerization and then disproportionation to form the fully oxidized **DHA** and regenerate the fully reduced **HA**$^-$.[6,7]

Reactions of hydroperoxyl radical (**HOO**$^{\bullet}$)/superoxide (**O**$_2^{\bullet-}$) can be considered representative of the radical scavenging reactions of ascorbic acid. Bielski and coworkers[7,37] have used stop-flow and pulse radiolysis to measure the reaction rates of **HOO**$^{\bullet}$/**O**$_2^{\bullet-}$ with ascorbic acid and ascorbate radical. Because the rate for the latter reaction is considerably higher, as soon as ascorbate radical is formed, it reacts with any available **HOO**$^{\bullet}$/**O**$_2^{\bullet-}$. Galano *et al.*[38] have used density functional calculations to study the radical scavenging reaction rates of several antioxidants including ascorbic acid. However, the reaction of **HOO**$^{\bullet}$/**O**$_2^{\bullet-}$ with ascorbate radical has not yet been studied by computational methods.

The calculation of pK_a's and reduction potentials can provide useful thermodynamic information for these reactions and comparison with experimental data can validate the calculational methodology. Reduction potentials of ascorbic acid in aqueous solution have been measured by pulse radiolysis and electrochemical techniques, and the values range from +0.06 V to +0.40 V depending on the experimental conditions.[5,14,15]

Several studies have calculated pK_a's and reduction potentials of ascorbic acid.[26,30] Although these theoretical studies predict reliable pK_a values, they have not considered the intermediate one electron oxidized state and have not examined the hydrated and bicyclic structures of the fully oxidized form. The disproportionation reaction has also not been examined computationally.

In the present study, we explore the mechanisms for ascorbic acid oxidation and the reactivity of intermediates by using electronic structure methods to calculate structures, energetics, pK_a's and reduction potentials. Recently developed computational protocols have allowed us to calculate the pK_a's and reduction potentials for guanine oxidation in good agreement with the experimental measurements.[39–41] For this study, we follow the same methodology to predict pK_a's and reduction potentials ($E°$ and E^7) of ascorbic acid and the intermediates along the oxidation pathways. From these calculated values, we can construct a Pourbaix diagram for each one-electron oxidation process to identify the thermodynamically favored species and reactions under selected pH conditions. Then possible reaction pathways are explored to understand the most likely oxidation mechanism of ascorbic acid under physiological conditions. In particular, we explore the possibility that dimerization of the ascorbate radical, followed by cyclization and then dissociation yields the stable bicyclic form of dehydroascorbic acid directly, avoiding formation of the high-energy DHA1 intermediate. Understanding of oxidation mechanism allows us to further study how ascorbate radical is able to interact with reactive oxygen species such as $\mathbf{HOO^{\bullet}}/\mathbf{O_2^{\bullet -}}$, which may shed light on its unique antioxidant properties.

Computational methods

Calculations were performed with the development version of the Gaussian series of programs.[42] Equilibrium geometries and transition states were optimized using the SMD implicit solvation model[43] and the B3LYP functional[44–48] with the 6-31+G(d,p) basis set.[49–54] Vibrational frequency calculations were used to confirm that the optimized structures are minima or first order saddle points on the potential energy surface. Because ascorbic acid and its derivatives have multiple conformations, the ensemble averaged values of pK_a's and reduction potentials were evaluated using Boltzmann weighting. The ensemble-averaged pK_a's and reduction potentials were very close to the values calculated from the most stable structures. The most stable structures for each protonated and oxidized state were used as starting structures for CBS-QB3 calculations to obtain more accurate values for the pK_a's and reduction potentials.

Implicit solvation models like SMD do not treat specific hydrogen bonding interactions between the solute and water. This can lead to significant problems for anionic solutes, which can be alleviated by solute cavity scaling[39,40] or by including explicit water molecules.[41] Our previous study found that the solvent cavity for negatively charged solutes needed to be scaled to obtain good agreement between calculated and experimental pK_a values for nucleobases.[39,40] For dianions such as $\mathbf{A^{2-}}$, a cavity scaling parameter of 0.90 was used for B3LYP and CBS-QB3. The solvent cavity was not scaled for dimers of ascorbate radical since the negative charges in the dimers are far enough from each other. Solvent cavity scaling was not needed for monoanions of ascorbic acid and its derivatives since they have significant intramolecular hydrogen bonding interactions. The solvent cavity was also not scaled for neutral species. The B3LYP/6-31+G(d,p) level of theory was used to determine the placement of explicit water molecules to augment the SMD solvation model. More accurate energies for pK_a's and reduction potential and for hydration and cyclization reactions were calculated with the CBS-QB3 method and SMD implicit solvation with explicit waters.

The pK_a for a deprotonation reaction in aqueous solution,

$$\mathrm{HA_{(aq)}} \xrightarrow{\Delta G_{\mathrm{deprot\,(aq)}}} \mathrm{A^-_{(aq)}} + \mathrm{H^+_{(aq)}} \quad (1)$$

is calculated from the Gibbs free energy of deprotonation, $\Delta G_{\mathrm{deprot\ (aq)}}$,

$$\mathrm{p}K_a = \frac{\Delta G_{\mathrm{deprot\,(aq)}}}{2.303RT} \quad (2)$$

where R is the gas constant and T is the temperature. The Gibbs free energy for aqueous deprotonation can be calculated by

$$\Delta G_{\mathrm{deprot\ (aq)}} = G_{(\mathrm{aq})}(\mathrm{A^-}) + G_{(\mathrm{aq})}(\mathrm{H^+}) - G_{(\mathrm{aq})}(\mathrm{HA}) \quad (3)$$

The aqueous phase Gibbs energy for a proton ($G_{(\mathrm{aq})}(\mathrm{H^+})$) is

$$\begin{aligned} G_{(\mathrm{aq})}(\mathrm{H^+}) &= G^{\circ}_{(\mathrm{g})}(\mathrm{H^+}) + \Delta G^{1\,\mathrm{atm}\rightarrow 1\,\mathrm{M}} + \Delta G^{*}_{(\mathrm{aq})}(\mathrm{H^+}) \\ &= -270.3\ \mathrm{kcal\,mol^{-1}} \end{aligned} \quad (4)$$

where $G^{\circ}_{(\mathrm{g})}(\mathrm{H^+}) = -6.287\,\mathrm{kcal\,mol^{-1}}$ is the gas phase free energy of a proton at 298 K (obtained from $H^{\circ}_{(\mathrm{g})}(\mathrm{H^+}) = 1.48\,\mathrm{kcal\,mol^{-1}}$ and $S^{\circ}_{(\mathrm{g})}(\mathrm{H^+}) = 26.05\,\mathrm{cal\,(mol\,K)^{-1}}$), $\Delta G^{1\ \mathrm{atm}\rightarrow 1\ \mathrm{M}} = 1.89$ kcal mol^{-1} is the change in the standard state from 1 atm to 1 M, and $\Delta G^{*}_{(\mathrm{aq})}(\mathrm{H^+}) = -265.9\ \mathrm{kcal\,mol^{-1}}$ is the literature value for the aqueous solvation free energy for a proton.[55] Superscripts ° and * denote the standard state of 1 atm and 1 mol L^{-1}, respectively.

Ascorbic acid can lose two electrons (Scheme 1) in two sequential oxidation steps forming first the intermediate Ox1 (eqn (5)) and then the fully oxidized product Ox2 (eqn (6)).

$$\mathrm{Ox1_{(aq)}} + \mathrm{e^-_{(aq)}} = \mathrm{Red_{(aq)}} \quad E_{\mathrm{ox1}} \quad (5)$$

$$\mathrm{Ox2_{(aq)}} + \mathrm{e^-_{(aq)}} = \mathrm{Ox1_{(aq)}} \quad E_{\mathrm{ox2}} \quad (6)$$

The oxidation process can also occur as a concerted 2e$^-$ oxidation forming the fully oxidized Ox2 state directly.

$$\mathrm{Ox2_{(aq)}} + 2\mathrm{e^-_{(aq)}} = \mathrm{Red_{(aq)}} \quad E_{\mathrm{ox}} \quad (7)$$

For a reduction reaction,

$$\mathrm{A_{(aq)}} + n\mathrm{e^-_{(aq)}} \xrightarrow{\Delta G^{*}_{\mathrm{red(aq)}}} \mathrm{A^{n-}_{(aq)}} \quad (8)$$

the standard reduction potential is

$$E^{\circ}_{\mathrm{red\,(aq)}} = \frac{-\Delta G^{*}_{\mathrm{red\,(aq)}}}{nF} - \mathrm{SHE} \tag{9}$$

where $\Delta G^{*}_{\mathrm{red\,(aq)}}$ is the Gibbs free energy of the reduction under standard conditions, n is the number of electrons transferred in the reduction process, F is Faraday's constant (23.06 kcal (mol V)$^{-1}$), and SHE is the absolute potential of the standard hydrogen electrode (4.281 V).[56–59] The Gibbs free energy for reduction is

$$\Delta G^{*}_{\mathrm{red\,(aq)}} = G_{\mathrm{(aq)}}(\mathrm{A}^{n-}) - G_{\mathrm{(aq)}}(\mathrm{A}) - nG^{\circ}_{\mathrm{(g)}}(\mathrm{e}^{-}) \tag{10}$$

where $G^{\circ}_{\mathrm{(g)}}(\mathrm{e}^{-})$ is the gas phase free energy of an electron. At 298 K, the gas phase Gibbs energy of an electron is $G^{\circ}_{\mathrm{(g)}}(\mathrm{e}^{-}) = -0.867\,\mathrm{kcal\,mol^{-1}}$ and is obtained from the literature values of $H^{\circ}_{\mathrm{(g)}}(\mathrm{e}^{-}) = 0.752\,\mathrm{kcal\,mol^{-1}}$ and $S^{\circ}_{\mathrm{(g)}}(\mathrm{e}^{-}) = 5.434\,\mathrm{cal\,(mol\,K)^{-1}}$.[60,61] The Nernst equation

$$E = E^{\circ} - \frac{RT}{F}\ln\left(\frac{[\mathrm{Red}]}{[\mathrm{Ox}]}\right) \tag{11}$$

can be applied to convert reduction potentials under the standard condition (E°) to other experimental conditions.

For the first one electron oxidation of ascorbic acid, the reduced form has two K_{a}'s and the oxidized form has one K_{a}. The pH dependent reduction potential is

$$\begin{aligned} E^{\mathrm{pH}} = {} & E^{\circ}(\mathbf{HA}^{\bullet}, \mathbf{HA}^{-}) + \frac{RT}{F}\ln\left(\frac{1}{K_{\mathrm{a1r}}}\right) \\ & + \frac{RT}{F}\ln\left(\frac{K_{\mathrm{a1r}}K_{\mathrm{a2r}} + K_{\mathrm{a1r}}10^{-\mathrm{pH}} + 10^{-2\mathrm{pH}}}{K_{\mathrm{a2o}} + 10^{-\mathrm{pH}}}\right) \end{aligned} \tag{12}$$

assuming low ionic strength of the solute. $\mathbf{HA}^{-}$ is the reduced monoanion form and $\mathbf{HA}^{\bullet}$ is the oxidized radical that has one less electron than $\mathbf{HA}^{-}$. The acid dissociation constants K_{a1r}, K_{a2r}, and K_{a2o} are shown in Scheme 1. Depending on K_{a2o} and the pH, the radical may deprotonate yielding $\mathbf{A}^{\bullet-}$. Njus and coworkers[62] have shown that at physiological pH this first oxidation step of ascorbic acid occurs *via* proton-coupled electron transfer ($\mathbf{HA}^{-}$ to $\mathbf{A}^{\bullet-}$ in Scheme 1).

Following the first electron oxidation, ascorbic acid can lose a second electron to form dehydroascorbic acid (**DHA**). Dehydroascorbic acid has several hydrated forms in water or alcohol, involving cyclization and the hydration of the carbonyl groups (see Scheme 1). The pH dependent reduction potential of **DHA** is

$$\begin{aligned} E_{\mathrm{ox2}} = {} & E^{\circ}(\mathbf{A}^{\bullet-}, \mathbf{DHA}) - \frac{RT}{F}\ln\left(\frac{[\mathbf{A}^{\bullet-}]}{[\mathbf{DHA}]}\right) \\ = {} & E^{\circ}(\mathbf{A}^{\bullet-}, \mathbf{DHA}) + \frac{RT}{F}\ln\left(\frac{K_{\mathrm{a2o}} + 10^{-\mathrm{pH}}}{K_{\mathrm{a2o}}}\right) \\ & + \frac{RT}{F}\ln\left(\frac{10^{-\mathrm{pH}}}{K_{\mathrm{a3o}} + 10^{-\mathrm{pH}}}\right) \end{aligned} \tag{13}$$

where **DHA** can be any of the structures listed in Scheme 1, and K_{a2o} and K_{a3o} are the acidic dissociation constants of $\mathbf{HA}^{\bullet}$ and **DHA**, respectively.

Ascorbic acid and its oxidation products have many conformations because of numerous rotatable OH groups and the alkyl side chain. Since these conformations can be close in energy, their populations may need to be considered in calculating pK_{a}'s and reduction potentials. The Boltzmann population for a given conformer is

$$f_1 = \frac{\exp\left(\frac{-G_{1(\mathrm{aq})}}{RT}\right)}{\sum_n \exp\left(\frac{-G_{n(\mathrm{aq})}}{RT}\right)}, \quad \text{where } \sum_n f_n = 1 \tag{14}$$

The ensemble averaged pK_{a} value is

$$\mathrm{p}K_{\mathrm{a}} = \mathrm{p}K_{\mathrm{a}}{}^{ij} - \log(f_i) - \log(f'_j) \tag{15}$$

where $\mathrm{p}K_{\mathrm{a}}{}^{ij}$ is the specific pK_{a} value for the deprotonation of conformer i resulting in the formation of conformer j, f_i is the population of the i-th conformer of the protonated species, and f'_j is the population of the j-th conformer of the deprotonated species. Similarly, the ensemble averaged reduction potential for a reduction reaction is calculated by

$$E = E^{xy} + \frac{RT}{F}\ln(f_x) - \frac{RT}{F}\ln(f'_y) \tag{16}$$

where E^{xy} is the specific reduction potential, f_x is the population of the oxidized species which is reduced to form the species which has the population f'_y.

As described in the Results and discussion section, calculating the energies of hydration and cyclization reactions of **DHA** can be problematic. The free energy for hydration of acetone ($CH_3COCH_3 + H_2O \rightarrow CH_3C(OH)_2CH_3$) calculated by CBS-QB3 with SMD solvation and an additional explicit water molecule is in error by 2.1 kcal mol^{-1} when compared to experiment.[63] CBS-APNO[64] and G4[65] calculations are in better agreement with experiment but are not currently practical for systems the size of ascorbic acid. Therefore, the CBS-QB3 calculated free energies for the hydration and cyclization reactions of **DHA** have been corrected for the difference between the CBS-QB3 and experimental free energies for the hydration of acetone.

$$\begin{aligned} \Delta G_{\mathrm{cor}} = {} & \Delta G(\text{CBS-QB3}) + (\Delta G(\text{exp. acetone hydration}) \\ & - \Delta G(\text{CBS-QB3 acetone hydration}) \\ = {} & \Delta G(\text{CBS-QB3}) + 2.1\,\mathrm{kcal\,mol^{-1}} \end{aligned} \tag{17}$$

The disproportionation reaction of ascorbate radical,

$$\mathbf{A}^{\bullet-}{}_{\mathrm{(aq)}} + \mathbf{A}^{\bullet-}{}_{\mathrm{(aq)}} + \mathrm{H}^{+}{}_{\mathrm{(aq)}} \xrightarrow{\Delta G_{\mathrm{disp\,(aq)}}} \mathbf{HA}^{-}{}_{\mathrm{(aq)}} + \mathbf{DHA}_{\mathrm{(aq)}} \tag{18}$$

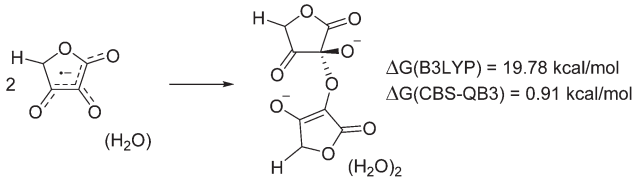
where $\mathbf{DHA}_{\mathrm{(aq)}}$ is one of the forms of dehydroascorbic acid. The reaction energy (ΔG_{disp}) is governed by the extent of the potential inversion between the first and second oxidation.

$$\Delta G_{\mathrm{disp}} = -RT\ln K_{\mathrm{disp}} = F(E_{\mathrm{ox1}} - E_{\mathrm{ox2}}) \tag{19}$$

In the first step of the disproportionation reaction, two ascorbate radicals form a dimer.

$$\mathbf{A}^{\bullet -}{}_{(aq)} + \mathbf{A}^{\bullet -}{}_{(aq)} \rightarrow \mathbf{A}_2{}^{2-}{}_{(aq)} \quad (20)$$

Unfortunately, the dimer is too large to calculate readily at the CBS-QB3 level of theory. However, if the ethyldiol side-chain of $\mathbf{A}^{\bullet -}$ is removed, the dimerization energy of this smaller system can be calculated by CBS-QB3.



(21)

The CBS-QB3 energy for dimerization for two ascorbate radicals can be estimated in the following way:

$$\begin{aligned}\Delta G(\text{rx } 20,\ \text{est CBS-QB3}) &= \Delta G(\text{rx } 20,\ \text{B3LYP}) \\ &+ \Delta G(\text{rx } 21,\ \text{CBS-QB3}) - \Delta G(\text{rx } 21,\ \text{B3LYP}) \\ &= 17.57 + 0.91 - 19.78 = -1.28\ \text{kcal mol}^{-1}\end{aligned} \quad (22)$$

This is equivalent to a QM/QM calculation of the dimerization reaction using an ONIOM approach.[66] Similarly, the cyclization reactions for unprotonated ascorbate dimer (**1** → **2**) and protonated ascorbate dimer (**3** → **4**) can be calculated with a QM/QM approach using the CBS-QB3 energies of the corresponding cyclization reactions of the monomers (see Scheme 3)

$$\begin{aligned}\Delta G(\mathbf{1} \rightarrow \mathbf{2},\ \text{est CBS-QB3}) &= \Delta G(\mathbf{1} \rightarrow \mathbf{2},\ \text{B3LYP}) \\ &+ \Delta G(\mathbf{DHA2}^- \rightarrow \mathbf{DHA4}^-,\ \text{CBS-QB3}) \\ &- \Delta G(\mathbf{DHA2}^- \rightarrow \mathbf{DHA4}^-,\ \text{B3LYP}) \\ &= -0.84 + (-4.18) - (-1.94) = -3.09\ \text{kcal mol}^{-1}\end{aligned} \quad (23)$$

$$\begin{aligned}\Delta G(\mathbf{3} \rightarrow \mathbf{4},\ \text{est CBS-QB3}) &= \Delta G(\mathbf{3} \rightarrow \mathbf{4},\ \text{B3LYP}) \\ &+ \Delta G(\mathbf{DHA2} \rightarrow \mathbf{DHA4},\ \text{CBS-QB3}) - \Delta G(\mathbf{DHA2} \rightarrow \mathbf{DHA4},\ \text{B3LYP}) \\ &= -2.73 + (-5.48) - (-2.97) = -5.24\ \text{kcal mol}^{-1}\end{aligned} \quad (24)$$

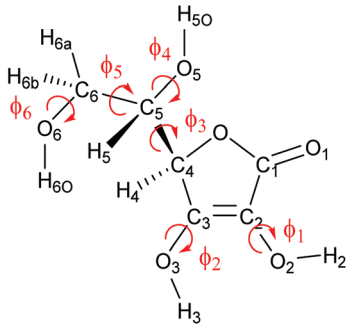
Results and discussion

The overall oxidation of ascorbic acid typically involves the transfer of two electrons and two protons to form dehydroascorbic acid (**DHA**): $\mathbf{H_2A} \rightarrow \mathbf{DHA} + 2e^- + 2H^+$. The process is seen as a single $2e^-$ irreversible wave by cyclic voltammetry, but the actual oxidation reaction is a complex mechanism that involves several chemical reactions as shown in Scheme 1. To develop a comprehensive understanding of the oxidation mechanism of ascorbic acid, we need to examine the individual one-electron oxidation steps. To understand the pH effect on these oxidation steps, we need the pK_a's for the various structures shown in Scheme 1. We start by examining the energies of the conformations of ascorbic acid, ascorbate radical and dehydroascorbic acid in their different protonated and hydrated forms. The most stable conformers of ascorbic acid and its derivatives are then used to calculate pK_a's and reduction potentials of ascorbic acid and its derivatives. With the calculated pK_a's and reduction potentials, we can explore the possible reaction pathways for the oxidation mechanism of ascorbic acid and its reaction with other radical species.

Conformations of ascorbic acid (H_2A) and its derivatives

H_2A contains an ethyldiol side chain, two hydroxyl groups and a carbonyl group around the γ-lactone ring. The hydroxyl groups can be proton donors or acceptors in hydrogen bonds and the carbonyl group can act as proton acceptor. Rotation of the ethyldiol and hydroxyl groups provides a variety of intramolecular hydrogen bond arrangements which affect the pK_a's and electrochemical potentials of ascorbic acid. To explore possible conformations for ascorbic acid and its derivatives, we followed the approach of Juhasz *et al.*[29] and Ebrahimi *et al.*[67] by optimizing the **H_2A** structures with the six different dihedral angles defined in Table 1. Next, the optimized **H_2A** conformers were deprotonated, first from the C3 hydroxyl group and then from the C2 hydroxyl group, and reoptimized to obtain the lowest energy structures for $\mathbf{HA}^-$ and $\mathbf{A}^{2-}$. Structures for the oxidized forms ($\mathbf{HA}^{\bullet}$, $\mathbf{A}^{\bullet -}$, and **DHA1** in Scheme 1) were obtained by starting with the optimized structures of $\mathbf{HA}^-$ and $\mathbf{A}^{2-}$ and reoptimizing the corresponding oxidation states. All **H_2A** conformers have multiple hydrogen bonds, and the most stable conformers at different deprotonated and oxidation states exhibit somewhat different arrangements of intramolecular hydrogen bonds. The optimized structures of these conformers are shown in Fig. 1. For **H_2A**, the most stable conformer has three hydrogen bonds: H2···O3, H3···O6, and H5···O. For the deprotonated forms of ascorbic

Table 1 The most stable conformations of ascorbic acid and its derivatives optimized at SMD/B3LYP/6-31+G(d,p)



		ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6
Red	$\mathbf{H_2A}$	−3.3	160.7	50.5	−63.9	65.3	76.1
	$\mathbf{HA}^-$	−2.9		55.1	−57.0	74.6	−56.4
	$\mathbf{A}^{2-}$			54.8	−54.1	73.0	−54.2
Ox1	$\mathbf{HA}^{\bullet}$	−0.80		55.6	−64.4	−57.5	−64.5
	$\mathbf{A}^{\bullet -}$			55.3	−58.2	−57.4	−63.2
Ox2	**DHA1**			53.3	−70.7	−57.8	−65.7

ϕ_1: H2–O2–C2–C3; ϕ_2: H3–O3–C3–C2; ϕ_3: O5–C5–C4–O; ϕ_4: H5O–O5–C5–C4; ϕ_5: O6–C6–C5–C4; ϕ_6: H6O–O6–C6–C5 (structures and energies of all conformations considered can be found in Tables S1–S6 of the ESI).

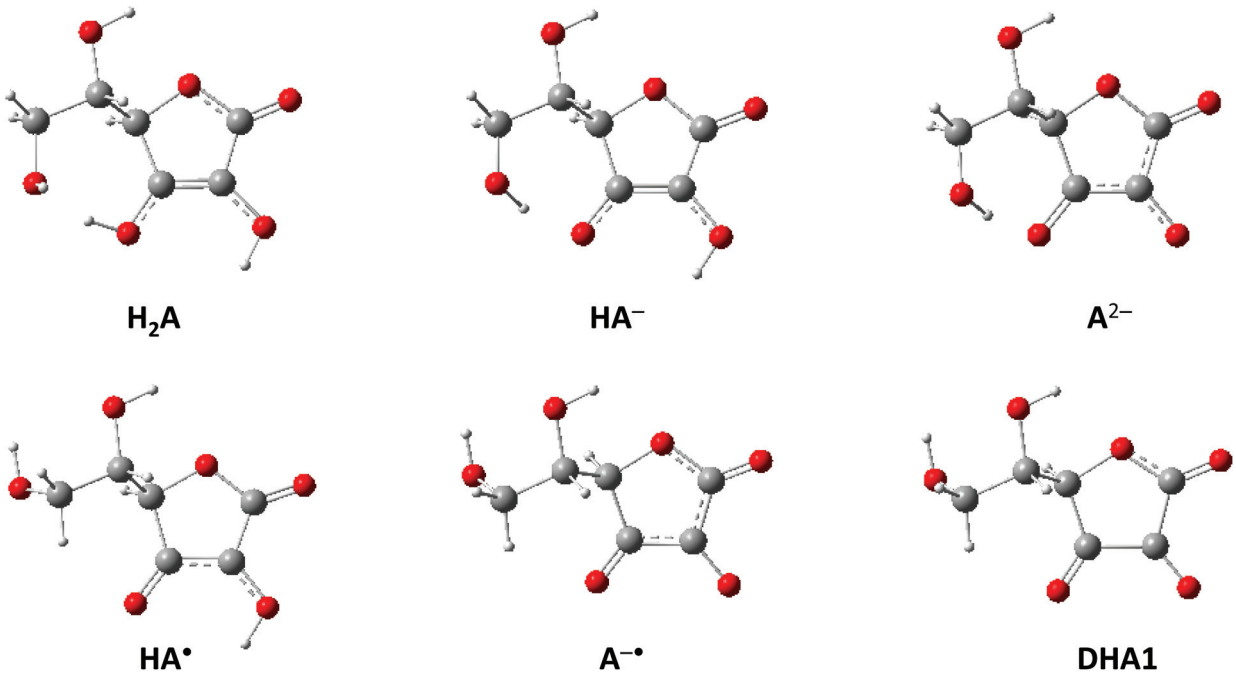


Fig. 1 The B3LYP/6-31+G(d,p) optimized structures of the most stable conformers for ascorbic acid and its derivatives using the SMD implicit solvent model. (**HA**$^{\bullet}$ and **A**$^{\bullet-}$ with the same conformations as **HA**$^{-}$ and **A**$^{2-}$ are less than 2 kcal mol^{-1} higher in energy than the most stable **HA**$^{\bullet}$ and **A**$^{\bullet-}$ conformers).

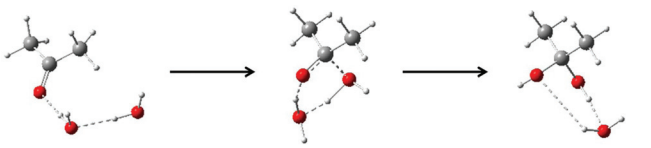
acid (**HA**$^{-}$ and **A**$^{2-}$ in Scheme 1), the most stable **HA**$^{-}$ conformer also shows hydrogen bonds at H2···O3, H6···O3, and H5···O, and the most stable **A**$^{2-}$ conformer has almost the same conformation as **HA**$^{-}$ except for the loss of the H2···O3 hydrogen bond. For the 1e^{-} oxidized forms of ascorbic acid, the most stable conformer of **HA**$^{\bullet}$ has hydrogen bonds at H2···O3, H6···O5, and H5···O, and its deprotonated form, **A**$^{\bullet-}$, also shows the hydrogen bonds at H6···O5 and H5···O. For the 2e^{-} oxidized ascorbic acid, the most stable **DHA1** conformer has almost the same conformation as **A**$^{\bullet-}$. These most stable conformers along with one or two explicit water molecules were used for the calculation of the pK_a's and reduction potentials, and for the hydration and cyclization reactions.

Relative energies of hydrated and bicyclic forms of dehydroascorbic acid

The conventional form of dehydroascorbic acid is **DHA1**, a structure with three carbonyls. However, this structure is detectable by NMR spectroscopy only in dry DMSO[9] but not in water or alcohols.[10–14] In water or alcohol, the second 1e^{-} oxidation of ascorbic acid is accompanied by hydration and ring closure. The possible structures of **DHA** in water are presented in Scheme 1, but the calculation of their relative free energies is surprisingly troublesome. Therefore we first examined the hydration of some simple carbonyls to calibrate the computational methodology.

Only a few experimental free energies for the hydration of carbonyls are available for comparison with calculations. Table 2 shows the results for the hydration of acetone (corresponding data for formaldehyde and acetaldehyde can be found in Tables S7 and S8 in the ESI†). For B3LYP/6-31+G(d,p) calculations with SMD implicit solvation, the calculated ΔG is in error by nearly 12 kcal mol^{-1}. Increasing the basis set does not solve the problem. Methods like CBS-QB3, CBS-APNO and G4 typically yield gas phase energy differences that are accurate to within 1–2 kcal mol^{-1}. With implicit solvation these methods still have large errors for the free energy of hydration of acetone. If explicit water molecules are included along with

Table 2 Reaction enthalpy and free energy for the hydration of acetone

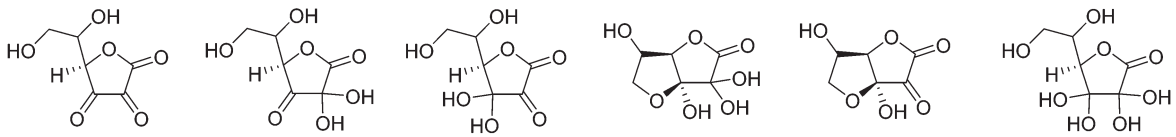


	Implicit		Inclusion of explicit water	
	ΔH (kcal mol^{-1})	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔG (kcal mol^{-1})
B3LYP/6-31+G(d,p)	2.73	15.67	5.13	9.42
B3LYP/CBSB7	−0.36	13.06		
B3LYP/CBSB7+	4.02	16.82		
CBS-QB3	−3.30	10.10	−1.58	1.56
CBS-APNO	−2.75	9.94	−1.10	2.70
G4	−3.07	9.10	−0.86	3.78
Experimental				3.68 (ref. 63)

implicit solvation, the calculated free energies are in much better agreement with experiment. To determine the appropriate placement of the explicit waters, we started by optimizing the six-centered transition structure for the hydration of acetone using two water molecules. Then steepest decent reaction path following with the DVV method[68] followed by full optimization of the minima at the B3LYP/6-31+G(d,p) level of theory yielded the positions and orientations of the explicit waters in the reactant and product for the hydration reaction (these structures were used as starting points for the higher level calculations). The G4 and CBS-APNO levels of theory are within 1 kcal mol^{-1} of the experimental free energy of hydration. However, these methods are too costly for most of the calculations in our study of ascorbic acid oxidation. The CBS-QB3 method is affordable but overstabilizes the hydrated form of acetone by 2.1 kcal mol^{-1}. As shown in eqn (17), we apply this correction factor to the CBS-QB3 results for each hydration and ring closure step in relative free energy calculations of the hydrated and bicyclic forms of dehydroascorbic acid. These corrected free energies are also used in the calculation of pK_a's, reduction potentials and disproportionation reactions.

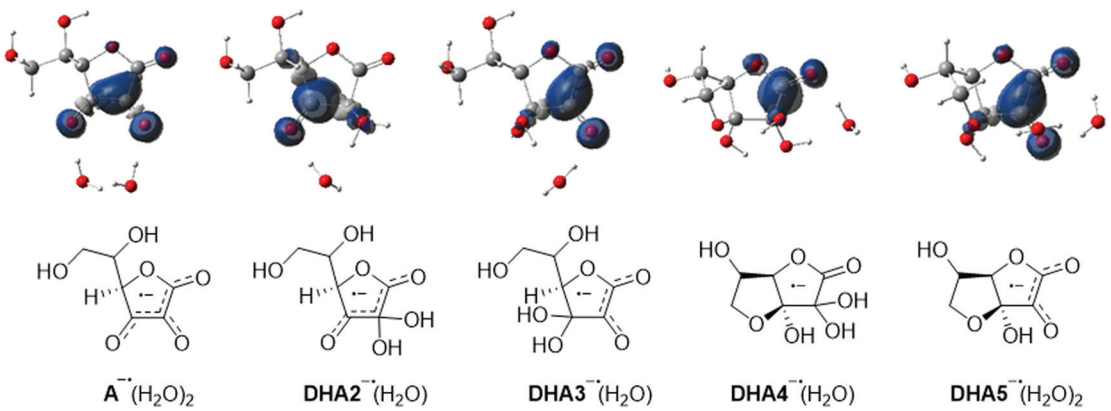
The relative free energies of the **DHA** structures computed with CBS-QB3, SMD implicit solvation and explicit water molecules are summarized in Scheme 2. Hydration is preferred at O2 and cyclization is preferred at O3. The most stable form of dehydroascorbic acid is the hydrated bicyclic structure, **DHA4**. The CBS-QB3 calculated barriers are 16.99 kcal mol^{-1} for **DHA1** → **DHA2**, 11.1 kcal mol^{-1} for **DHA1** → **DHA5** and 17.2 kcal mol^{-1} for **DHA5** → **DHA4** (see Fig. S1 in the ESI† for details).

By contrast to dehydroascorbic acid, hydration and cyclization are not favored for ascorbate radical. The spin density plots in Scheme 3 show this is because hydration and cyclization limit the π delocalization of the unpaired electron. The most stable form of ascorbate radical is $\mathbf{A}^{\bullet-}$. This is consistent with ESR results indicating that the unpaired electron of ascorbate radical is delocalized over the three carbonyl groups.[69]



	DHA1 $(H_2O)_2$	DHA2 (H_2O)	DHA3 (H_2O)	DHA4 (H_2O)	DHA5 $(H_2O)_2$	DHA6
ΔH	0.00 [a]	-14.51 [a]	-4.98 [b]	-24.21 [a]	-15.20 [a,c]	-19.81 [a]
$\Delta G_{(cor)}$ [d]		-6.33	-0.66	-11.81	-8.71	-6.28

Scheme 2 The relative enthalpies and free energies of the **DHA** structures calculated at CBS-QB3 with inclusion of explicit water molecules. For the placement of the waters, see Fig. S1 in the ESI.† [a] With respect to **DHA1a** in Fig. S1.† [b] With respect to **DHA1b** in Fig. S1.† [c] Using **DHA5b** in Fig. S1.† [d] Δ*G* is corrected using eqn (17).



	$A^{\bullet-}(H_2O)_2$	DHA2$^{\bullet-}$ (H_2O)	DHA3$^{\bullet-}$ (H_2O)	DHA4$^{\bullet-}$ (H_2O)	DHA5$^{\bullet-}$ $(H_2O)_2$
ΔH	0.00	28.05	11.07	34.66	5.30
ΔG	0.00	29.96	14.73	41.54	9.30

Scheme 3 The spin density plots (isovalue = 0.04 au), relative enthalpies and free energies of the ascorbate radical structures calculated at CBS-QB3 with SMD implicit solvation and inclusion of explicit water molecules. The positions of explicit waters were initially determined from the optimized DHA structures shown in Scheme 2.

pK_a's of ascorbic acid and its one and two electron oxidized forms

The calculated pK_a's for ascorbic acid and its derivatives are summarized in Table 3. Since the calculations at the B3LYP level of theory show that the pK_a's computed with the lowest energy structures are in good agreement with the ensemble averaged pK_a's, the CBS-QB3 calculations used only the lowest energy structures. For the fully reduced ascorbic acid, the two pK_a's of **H_2A** (pK_{a1r} and pK_{a2r} in Scheme 1) computed at CBS-QB3 are 4.16 and 13.79. The calculated pK_{a1r} is in good agreement with the experimental value of 4.04, while the second one is overestimated by about 2 units compared to the experimental pK_{a2r} of 11.34.[70] With inclusion of explicit water and cavity scaling for the dianion, the computed pK_{a1r} and pK_{a2r} are 4.45 and 10.51 at the CBS-QB3 level of theory, in good agreement with the experimental values. At physiological conditions, **HA^-** is the predominant species for the fully reduced form. One electron oxidation of ascorbic acid yields ascorbate radical. For **$HA^\bullet$**, pK_{a2o} calculated at CBS-QB3 is −2.26 which is at least 1.5 pK_a units lower than the experimental value of −0.45.[69] A better pK_a value of −1.21 can be obtained when an explicit water is included at the CBS-QB3 level of theory. For dehydroascorbic acid, the fully oxidized form of ascorbic acid, several structures need to be considered. With explicit waters, the pK_a's calculated for **DHA2**, **DHA3** and **DHA4** are 7.90, 8.57 and 8.86, respectively, compared the experimental pK_a of ∼8 for **DHA**.[71] (however, **DHA** decomposes rapidly when pH is higher than 8 (ref. 69)).

Reduction potentials and Pourbaix diagrams for ascorbic acid

Table 4 lists the calculated and experimental standard reduction potentials of various forms of ascorbic acid. The standard reduction potential for **HA^-**/**$HA^\bullet$** couple computed at CBS-QB3 with the SMD implicit model is 0.72 V which is in good agreement with the experimental values of 0.72 V (ref. 70) and 0.766 V.[62] The reduction potential of 0.68 V calculated with inclusion of explicit water is also in good agreement with the experiment. The variation of the reduction potential with pH can be illustrated in a Pourbaix diagram. Fig. 2 shows the Pourbaix diagram for the first 1e$^-$ oxidation of ascorbic acid (Red → Ox1 + e$^-$, E_{ox1}). This is constructed from the reduction potential for **HA^-**/**$HA^\bullet$** couple (0.68 V calc., 0.72 V exp.) and the pK_a's of **H_2A**, **HA^-**, and **$HA^\bullet$** using the Nernst equation in eqn (11). When pH < pK_{a2o} for **$HA^\bullet$** (−1.21 calc., −0.45 exp.), the line has a slope of −0.059 V/pH, and ascorbic acid undergoes 1H$^+$, 1e$^-$ oxidation from **H_2A** to **$HA^\bullet$**. When the pH is between pK_{a2o} for **$HA^\bullet$** and pK_{a1r} for **H_2A** (4.45 calc., 4.04 exp.), the slope increases to −0.118 V/pH, indicating the oxidation involves a 2H$^+$, 1e$^-$ transfer. When pH is between pK_{a1r} and pK_{a2r} for **H_2A** (10.51 calc., 11.43 exp.), the slope decreases to −0.059 V/pH again, corresponding to a 1H$^+$, 1e$^-$ oxidation from **HA^-** to **$A^{\bullet-}$**. When pH > pK_{a2r} of **H_2A**, **$A^{\bullet-}$** is formed

Table 3 pK_a's for ascorbic acid and its derivatives in aqueous solution

Deprotonation reactions	B3LYP/6-31+G(d,p)[a]	CBS-QB3[c]	Experiment
H_2A → **HA^-** + H$^+$; pK_{a1r}	3.69 (3.78)[b]	4.16; 4.45[d]	4.04 (ref. 70)
HA^- → **A^{2-}** + H$^+$; pK_{a2r}	14.74 (14.69)[b]	13.79; 14.16;[d] 10.51[e]	11.34 (ref. 70)
$HA^\bullet$ → **$A^{\bullet-}$** + H$^+$; pK_{a2o}	−1.32 (−1.43)[b]	−2.34; −1.21[d]	−0.45 (ref. 69)
DHA2 → **DHA2**$^-$ + H$^+$; pK_{a3o}		7.90[f]	
DHA3 → **DHA3**$^-$ + H$^+$; pK_{a3o}		8.57[f]	
DHA4 → **DHA4**$^-$ + H$^+$; pK_{a3o}		8.86[f]	
DHA			∼8 (ref. 71)

[a] Using the most stable conformers optimized at B3LYP/6-31+G(d,p) with SMD. [b] Using an ensemble average of the conformers optimized at B3LYP/6-31+G(d,p) with SMD. [c] Using CBS-QB3 optimized structures starting with the most stable B3LYP/6-31+G(d,p) optimized structures. [d] Calculated with two explicit water molecules. [e] Calculated with one explicit water and 0.90 for cavity scaling for the **A^{2-}** dianion. [f] Calculated with one explicit water molecule.

Table 4 Standard reduction potentials for ascorbic acid and its derivatives in aqueous solution

Redox reactions	B3LYP/6-31+G(d,p)	CBS-QB3[d]	Experiment
$HA^\bullet$ + e$^-$ → **HA^-**; E° (**HA^-**/**$HA^\bullet$**)	0.59 V[a] (0.58 V)[b]	0.72; 0.68 V[e]	0.72 V;[70] 0.766 V (ref. 62)
$A^{\bullet-}$ + e$^-$ → **A^{2-}**; E° (**A^{2-}**/**$A^{\bullet-}$**)	−0.36 V[c] (−0.37 V)[b]	−0.01 V[f]	0.015 V (at pH 13.5); 0.085 V (at pH ∼11)[72]
DHA1 $(H_2O)_2$ + e$^-$ → **$A^{\bullet-}$** $(H_2O)_2$; E° (**$A^{\bullet-}$**/**DHA1**)		0.33 V[e]	0.22 V; 0.24 V (ref. 73)
DHA2 (H_2O) + e$^-$ → **$A^{\bullet-}$** $(H_2O)_2$; E° (**$A^{\bullet-}$**/**DHA2**)		0.05 V[g]	
DHA4 (H_2O) + e$^-$ → **$A^{\bullet-}$** $(H_2O)_2$; E° (**$A^{\bullet-}$**/**DHA4**)		−0.18 V[g]	
DHA5 $(H_2O)_2$ + e$^-$ → **$A^{\bullet-}$** $(H_2O)_2$; E° (**$A^{\bullet-}$**/**DHA5**)		−0.05 V[g]	
DHA			−0.14 V;[15] −0.174 V;[70] −0.21 V (ref. 74)

[a] Using the most stable conformers optimized at B3LYP/6-31+G(d,p) with SMD. [b] Using an ensemble average of the conformers optimized at B3LYP/6-31+G(d,p) with SMD. [c] Using the most stable conformers optimized at B3LYP/6-31+G(d,p) and SMD with 0.90 for cavity scaling for the **A^{2-}** dianion. [d] Using CBS-QB3 optimized structures starting with the most stable B3LYP/6-31+G(d,p) optimized structures. [e] Calculated with two explicit water molecules. [f] Calculated with two explicit water and 0.90 for cavity scaling for the **A^{2-}** dianion. [g] Calculated with corrected ΔG's for hydration and cyclization of DHA (see eqn (17)).

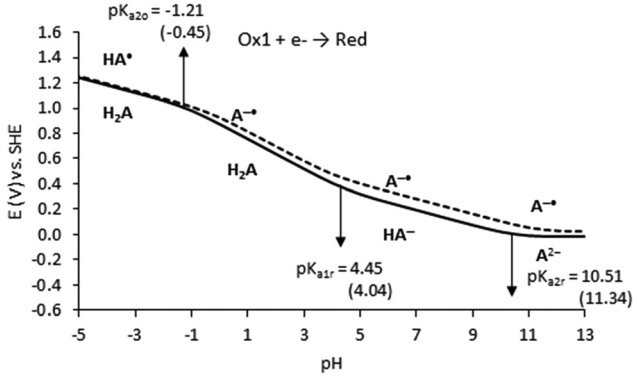


Fig. 2 Pourbaix diagram for the first 1e$^-$ oxidation of ascorbic acid in water. Solid line: The pH-dependent reduction potential calculated at the CBS-QB3 level of theory with SMD implicit solvent and inclusion of explicit waters (pK_{a1r} = 4.45; pK_{a2r} = 10.51; pK_{a2o} = −1.21; $E°$ (HA$^-$/HA$^•$) = 0.68 V). Dashed line: The pH-dependent reduction potentials obtained from experiment values (pK_{a1r} = 4.04; pK_{a2r} = 11.34; pK_{a2o} = −0.45; $E°$ (HA$^-$/HA$^•$) = 0.72 V). The values in parenthesis are the experimental pK_a's.

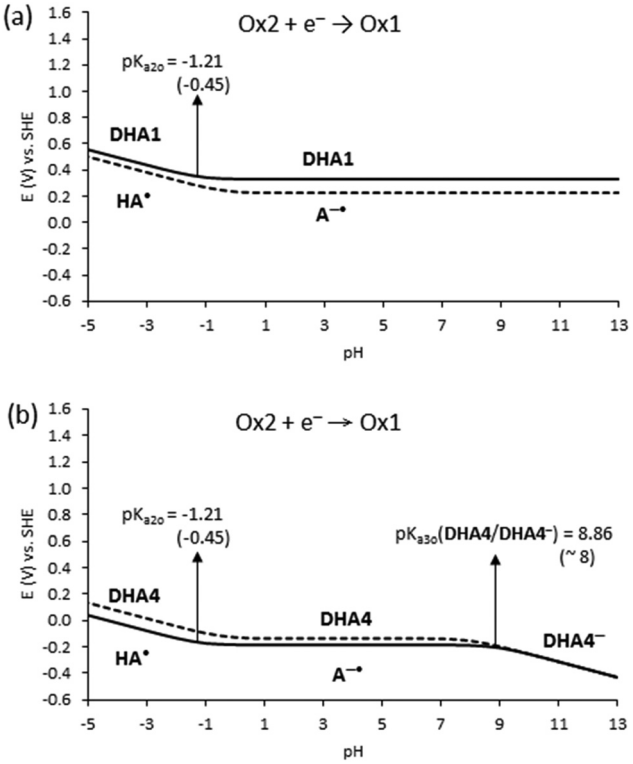


Fig. 3 Pourbaix diagram for 1e$^-$ oxidation of HA$^•$ or A$^{•-}$ to produce (a) **DHA1** and (b) **DHA4** in water. Solid line: The values for pK_a's and redox potentials are calculated at the CBS-QB3 level of theory with SMD implicit solvent and explicit water molecules (for **DHA1**, pK_{a2o} = −1.21 and $E°$ (A$^{•-}$/**DHA1**) = 0.33 V; for **DHA4**, pK_{a2o} = −1.21, pK_{a3o} = 8.86, and $E°$ (A$^{•-}$/**DHA4**) = −0.18 V). Dashed line: The pH-dependent reduction potentials obtained from experiments (for **DHA1**, pK_{a2o} = −0.45 and $E°$ (A$^{•-}$/**DHA1**) = 0.23 V; for **DHA4**, pK_{a2o} = −0.45; pK_{a3o} = 8; $E°$ (A$^{•-}$/**DHA4**) = −0.14 V).

via 1e$^-$ oxidation of **A**$^{2-}$ without a proton transfer, so the slope of the potential-pH curve is zero. The Pourbaix diagrams using the calculated and experimental potentials are in good agreement. At pH 7, the dominant reaction is the oxidation of **HA**$^-$ to form **A**$^{•-}$, and the calculated value for E^7 is 0.20 V, in acceptable agreement with experimental values of 0.33 V (ref. 74) from EPR results and 0.28 V (ref. 70) (derived from reduction potential of 0.72 V for **HA**$^-$/**HA**$^•$; pK_{a1r} = 4.04; pK_{a2r} = 11.34; pK_{a2r} = −0.45).

In water, the second 1e$^-$ oxidation of ascorbic acid from **A**$^{•-}$ to **DHA** can be accompanied by hydration and ring closure. The two Pourbaix diagrams in Fig. 3 correspond to the oxidation of ascorbate radical to the **DHA1** and **DHA4** forms of dehydroascorbic acid. When **A**$^{•-}$ undergoes 1e$^-$ oxidation without hydration or ring closure, the reduction potential for **A**$^{•-}$/**DHA1** couple computed at CBS-QB3 is +0.29 V (Table 4) and is in good agreement with the experimental values of +0.22 and +0.24 V obtained by polarography and high speed cyclic voltammetry, respectively.[73] To test the CBS-QB3 level of theory for the oxidation of a radical anion, we calculated the redox potential for 4-methylcatechol semiquinone (Q$^{•-}$) to *o*-quinone (Q) (the *ortho* enediol group of 4-methylcatechol similar to ascorbic acid). The reduction potential of −0.056 V calculated at CBS-QB3 with SMD implicit solvation (no explicit waters) is in very good agreement with the experimental value of −0.046 V.[5] Inclusion of explicit waters changes the reduction potential for **A**$^{•-}$/**DHA1** by only a small amount (0.33 V *vs.* 0.29 without explicit water). The Pourbaix diagram for the oxidation of **A**$^{•-}$ to **DHA1** is in good agreement with the one derived from the experimental values (Fig. 3a). Formation of **DHA1** was confirmed by the reversible trace from cyclic voltammetry when very fast scan rates (several hundred volts per second) were applied.[75] However, **DHA1** structure is unstable. Once it is formed, it can undergo hydration or cyclization. **DHA4** is calculated to be the most stable structure, 12 kcal mol^{-1} lower in energy than the conventional **DHA1** structure at the CBS-QB3 level of theory (Scheme 2). This is consistent with NMR data, which show that the predominant form of the oxidized ascorbic acid in water is a bicyclic hydrated structure, **DHA4**.[10–14] The predicted reduction potential at pH 7 for oxidation of **A**$^{•-}$ to **DHA4** is −0.18 V, which is good agreement with the experimental values of −0.14 V,[15] −0.174 V (ref. 70), and −0.21 V (ref. 74) (Fig. 3b).

Disproportionation reaction of ascorbic acid

At pH 7, the reduction potential for **HA**$^-$/**A**$^{•-}$ (E^7 = 0.32 V exp, 0.33 V calc) is higher than the reduction potential for **A**$^{•-}$/**DHA** (E^7 = −0.20 V exp, −0.18 V calc for **DHA4**, the most stable form of **DHA**). Fig. 4 shows the changes in the concentration for each oxidation state when the applied potential is increased from −0.5 V to 0.6 V. Because the reduction potentials are inverted, ascorbic acid is converted to dehydroascorbic acid in an apparent two electron process as the potential is increased, while the concentration of the one electron oxidized ascorbate radical remains small. Fig. 4 also shows that in the absence of

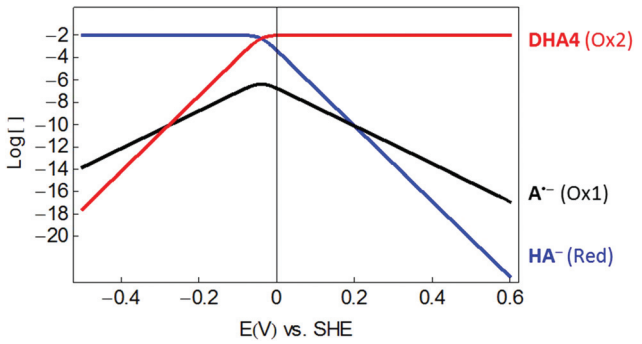
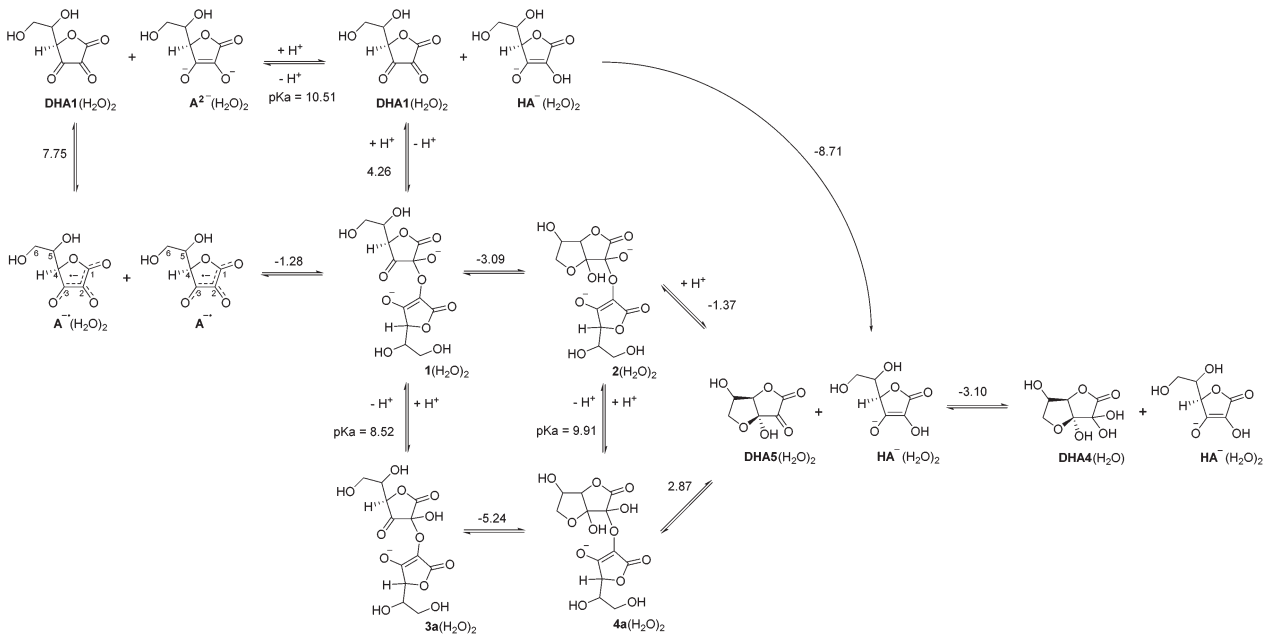


Fig. 4 Logarithm of the concentration of ascorbic acid in the Red, Ox1, and Ox2 states at pH 7 when the applied potential is in the range of −0.5 V to +0.6 V.

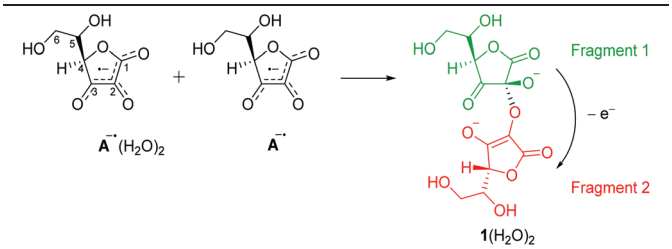
an applied potential, it is thermodynamically favorable for two ascorbate radicals to disproportionate into ascorbic acid and dehydroascorbic acid: $2\mathbf{A}^{\bullet-} + H^+ \rightarrow \mathbf{HA}^- + \mathbf{DHA4}$. The equilibrium constant for the disproportionation reaction, $K = 5.1 \times 10^{-9}$, has been determined at pH 6.4 by combining ascorbic acid and dehydroascorbic acid, and measuring the concentration of ascorbate radical by EPR.[76] The free energy for the disproportionation reaction calculated with CBS-QB3, ΔG = −9.7 kcal mol^{-1} is in good agreement with ΔG = −11.3 kcal mol^{-1} obtained from the experimental equilibrium constant. Fast disproportionation of ascorbate radicals is also supported by the NMR studies which provide evidence for the interconversion between ascorbic acid and **DHA4**.[13,14]

To determine the energetically favored mechanism for the disproportionation of ascorbic radical, possible mechanisms shown in Scheme 4 were compared (for the corresponding energy diagram, see Fig. S2 in the ESI†). Direct electron transfer would yield $\mathbf{A}^{2-}$ + **DHA1**, which is endothermic (ΔG = 7.75 kcal mol^{-1} by CBS-QB3). Protonation and/or cyclization before electron transfer are also unfavorable (see Table 3 and Scheme 3). Bielski[6] proposed that two ascorbate radicals disproportionate by forming a dimer. We propose the dimer structure shown in Scheme 4 because it allows transfer of the unpaired electron from one unit to the other and permits cyclization of the donor unit. Using a QM/QM approach (see eqn (22) in the Computational methods section), the estimated CBS-QB3 energy for dimerization is ΔG = −1.28 kcal mol^{-1}. This is in agreement with Bielski's estimate of the equilibrium constant for dimerization,[6] $K \approx 10^3$ which corresponds to ΔG = −4.1 kcal mol^{-1}. The calculated pK_a of the dimer is 8.5, which is in accord with the pH dependence of disproportionation rate observed by Bielski.[6] Dissociation of the uncyclized dimer to form **DHA1** + $\mathbf{HA}^-$ is unfavorable (ΔG = 4.26 kcal mol^{-1}). However, one of the components of the dimer can cyclize. The estimated CBS-QB3 energies for cyclization are −3.09 kcal mol^{-1} for the unprotonated dimer and −5.24 kcal mol^{-1} for the protonated dimer (see eqn (23) and (24)). Protonation of the bridging oxygen of the unprotonated dimer (or transfer of the C2–O proton to the bridging oxygen in the protonated dimer) leads to dissociation of the dimer to **DHA5** + $\mathbf{HA}^-$. This process is exothermic for the unprotonated dimer (estimated CBS-QB3 ΔG = −1.37 kcal mol^{-1} obtained by completing the thermodynamic cycle) but endothermic for the protonated dimer (estimated CBS-QB3 ΔG = 2.87 kcal mol^{-1}). Hydration of the C2–O bond in **DHA5** produces the final products, **DHA4** + $\mathbf{HA}^-$.



Scheme 4 The mechanism for the disproportionation of ascorbate radical. Free energies calculated by CBS-QB3 at pH 7 (see Computational methods section for details).

Table 5 Bond distances (angstrom) of ascorbic acid in fully reduced (HA^-), partially oxidized ($A^{\bullet-}$), fully oxidized (DHA2) and dimerized forms (Fragments 1 and 2)[a]



	$A^{\bullet-}$	Dimer 1 (fragment 1/fragment 2)	DHA2/HA^-
C1–C2	1.477	1.546/1.424	1.538/1.426
C2–C3	1.443	1.542/1.392	1.544/1.383
C1–O1	1.227	1.216/1.241	1.213/1.238
C3–O3	1.251	1.218/1.273	1.213/1.283

[a] The structures are optimized with SMD solvation model and explicit waters at the B3LYP/6-31+G(d,p) level of theory.

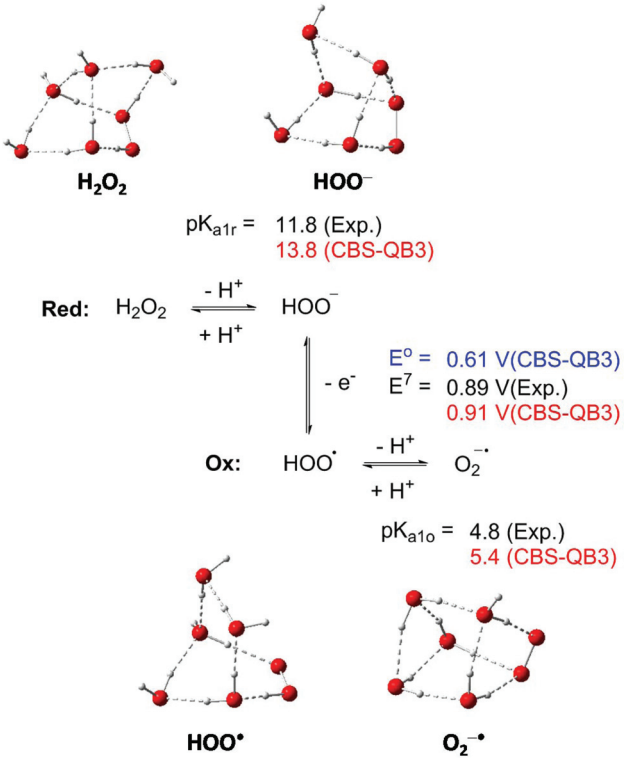
The change in the geometry on formation of the dimer provides evidence for the transfer of an electron from one fragment to the other (Table 5). The C1–C2 and C2–C3 bonds in fragment 1 elongate to resemble **DHA2** while the corresponding bonds in fragment 2 shorten to resemble HA^-. The trend in the C=O bonds is similar but the changes are smaller.

In summary, direct electron transfer between two ascorbate radicals is not favorable because it would produce higher energy products, **DHA1** and A^{2-}. Hydration and/or cyclization of ascorbate radical before electron transfer is also endothermic because these reactions destroy the delocalization of the unpaired electron in the radical. However, the dimerization of two ascorbate radicals is thermodynamically favorable, and internal electron transfer on dimerization leads to a structure with a **DHA2**-like fragment and a HA^--like fragment. The **DHA2** fragment can cyclize, and dissociation to **DHA5** and HA^- is exothermic. Hydration of **DHA5** then yields the final **DHA4** and HA^- products.

Reaction with superoxide

Reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$) and peroxyl radical ($HO_2^{\bullet}$), can cause oxidative damage to DNA bases and other biological macromolecules. Ascorbic acid can play a protective role preventing this damage by reducing reactive oxygen species. Since the experimental pK_a for $HO_2^{\bullet}$ is 4.8, both $HO_2^{\bullet}$ and $O_2^{\bullet-}$ could contribute to the reactivity.[37] With the inclusion of 5 explicit water molecules, the pK_a's and reduction potential for $HO_2^{\bullet}$ computed with CBS-QB3 are in good agreement with the experimental values (Scheme 5).

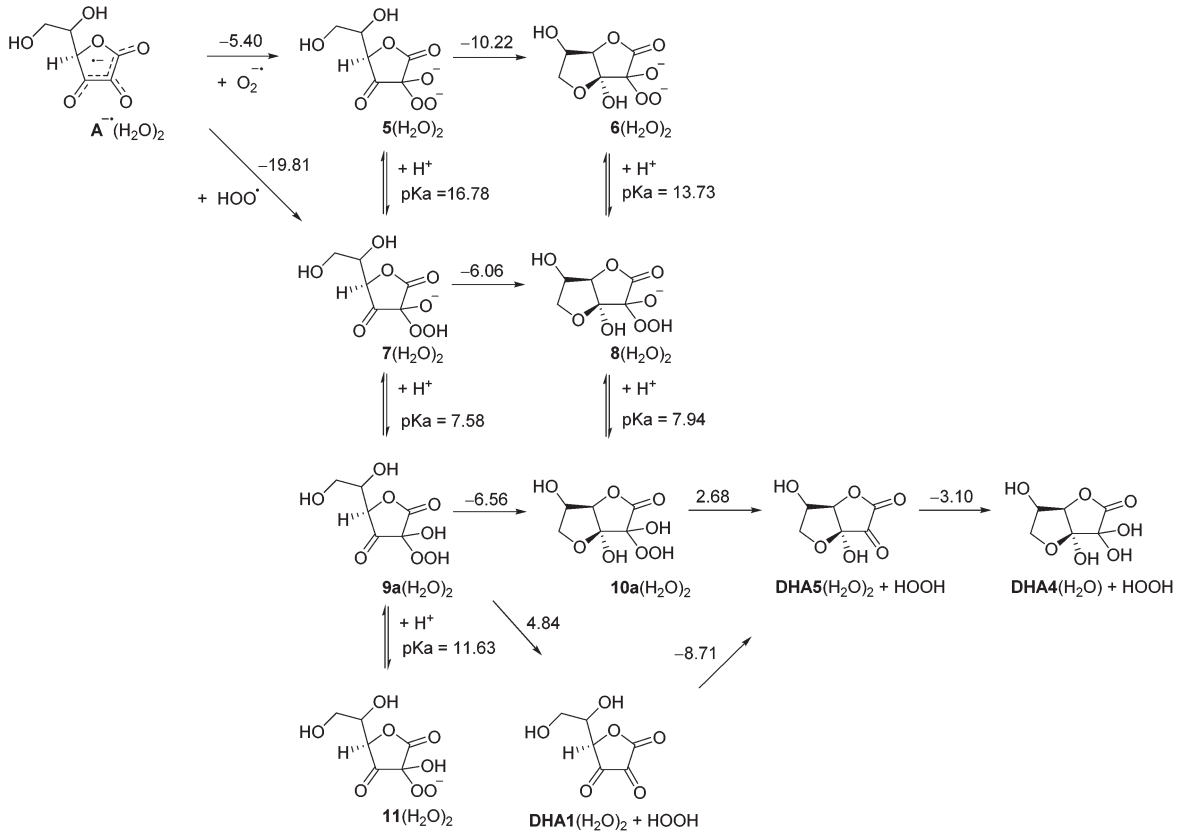
The reaction rates of $HOO^{\bullet}/O_2^{\bullet-}$ with ascorbic acid have been measured by Bielski and coworkers[7,37] and calculated by Galano *et al.*[38] Bielski also found that ascorbate radical reacts with $HOO^{\bullet}/O_2^{\bullet-}$ even faster than ascorbic acid,[7,37] but this reaction has not yet been studied by computational methods. Hydrogen atom transfer from ascorbate radical can be ruled out (no hydrogens on the relevant oxygens), and electron



Scheme 5 The pK_a values and reduction potential for oxygen species at pH 7 are calculated at CBS-QB3 with SMD implicit solvent and inclusion of 5 explicit water molecules.

transfer from ascorbate radical to $HOO^{\bullet}$ producing HOO^- and **DHA1** is thermoneutral. However, an exothermic pathway analogous to the disproportionation reaction can explain the high reactivity of the ascorbate radical with superoxide as shown in Scheme 6 (for the corresponding energy diagram, see Fig. S3 in the ESI†). The reaction pathway starts with the addition of $O_2^{\bullet-}$ or $HOO^{\bullet}$ to C2 of ascorbate radical to form adduct **5** or **7** (ΔG = –5.40 kcal mol^{-1} for $A^{\bullet-} + O_2^{\bullet-} \rightarrow$ **5** and –19.81 kcal mol^{-1} for $A^{\bullet-} + HOO^{\bullet} \rightarrow$ **7** at CBS-QB3), followed by protonation to form **9a**. The calculated pK_a for deprotonation from the OH group of adduct **9a** is 7.58, similar to the pK_a's 7.90–8.86 of **DHA**s in Table 2. The pK_a for deprotonation the OOH group of adduct **9a** is 11.63, similar to the pK_a value of H_2O_2 (13.8 (CBS-QB3) and 11.8 (exp.); Scheme 5). Therefore, both the OH and OOH groups will be protonated at pH 7. The overall reaction of $A^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow$ **9a** is calculated to be exothermic by 38.60 kcal mol^{-1}. The geometry of **9a** closely resembles **DHA2** (see Table S9 in the ESI†) demonstrating that the addition process results in the oxidation of ascorbate and reduction of superoxide.

Adduct **9a** can cyclize to form adduct **10a** with ΔG = –6.56 kcal mol^{-1} and a barrier of 7.7 kcal mol^{-1} when assisted by two explicit water. The OH group of adduct **10a** has a pK_a of 7.94, similar to 7.90–8.86 for the pK_a's of **DHA**s in Table 3; deprotonation of the OOH group has a pK_a of 11.34, similar to the pK_a value of H_2O_2 (13.8 (CBS-QB3) and 11.8 (exp.); Scheme 5). Hydrolysis of adduct **10a**, assisted by two explicit



Scheme 6 The calculated energies and pK_a's for the reaction of ascorbate radical with $O_2^{\bullet-}$ or $HO_2^{\bullet}$.

waters has a barrier of 19.13 kcal mol^{-1} and produces **DHA5** and $\mathbf{H_2O_2}$. Hydration of **DHA5** to form the most stable form of dehydroascorbic acid, **DHA4**, has a barrier of 17.2 kcal mol^{-1}. The alternative pathway, involving the hydrolysis of **9a** to form **DHA1** is endothermic by 4.84 kcal mol^{-1} and has a higher barrier of 18.1 kcal mol^{-1}. Cyclization of **DHA1** to form **DHA5** has a barrier of 11.05 kcal mol^{-1}.

In summary, the reaction of ascorbate radical with superoxide proceeds in a manner similar to the disproportionation reaction of ascorbate radical. The formation of an adduct between superoxide and ascorbate radical is followed by the addition of two protons to form a neutral intermediate. This intermediate cyclizes before dissociating to **DHA5** and $\mathbf{H_2O_2}$. Hydration of **DHA5** then yields the final **DHA4** and $\mathbf{H_2O_2}$ products.

Conclusions

In the present study, we have used B3LYP and CBS-QB3 calculations with the SMD implicit solvent model and explicit waters to explore the oxidation of ascorbic acid and its reaction with superoxide. The lowest energy conformations have been determined for ascorbic acid in its various protonation and oxidation states. The bicyclic hydrated isomer of dehydroascorbic acid was found to be the most stable structure of the fully oxidized form of ascorbic acid in aqueous solution, in agreement with NMR studies. The calculated pK_a's, reduction potentials and Pourbaix diagrams are in good agreement with the experimental values. The disproportionation of ascorbate radical into fully reduced ascorbic acid and fully oxidized dehydroascorbic acid is thermodynamically favored at physiological pH. In agreement with Bielski's proposed mechanism, the calculations show that disproportionation proceeds *via* dimerization of ascorbate radical and internal electron transfer. One of the ascorbate units in the dimer cyclizes before dissociating to yield a fully reduced ascorbic acid and fully oxidized dehydroascorbic acid. This mechanism is also found for the reaction of the ascorbic acid radical with superoxide. Ascorbate radical and superoxide combine to form an adduct which cyclizes before dissociating to dehydroascorbic acid and hydrogen peroxide.

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