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Photoelectron Spectroscopic Study of Ascorbate and Deprotonated Ascorbate Anions Using an Electrospray Ion Source and a Cryogenically Cooled Ion Trap

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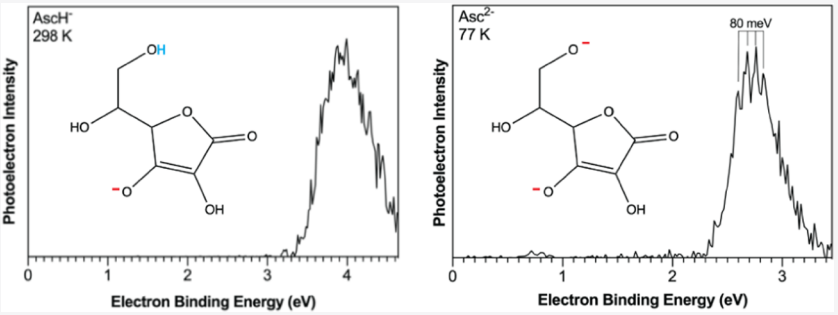

ABSTRACT: Reactive oxygen species (ROS) in biological systems are formed through a variety of mechanisms. These species are very reactive and have been associated with many diseases, including cancer and cardiovascular disease. One way of removing ROS from the body is through the use of radical scavengers, which are compounds capable of giving up an electron to neutralize the ROS yet form a stable radical species themselves. A common radical scavenger is ascorbic acid, also known as vitamin C. At physiological pH, ascorbic acid is predominately present as the ascorbate anion, $C_6H_7O_6^-$. The ascorbate anion, as well as the dianion ($C_6H_6O_6^{2-}$), is an effective antioxidant due to its ability to donate an electron from a lone pair generated by deprotonation. An electrospray ionization source was added to our pulsed anion photoelectron spectrometer to study ascorbate anions and deprotonated ascorbate dianions via photoelectron spectroscopy. The antioxidant behavior of the ascorbate anion and the deprotonated ascorbate dianion was confirmed based on the experimental vertical detachment energy (VDE), and, therefore, the ionization energy of the anions, 3.85 and 2.68 eV, respectively.

INTRODUCTION

Reactive oxygen species (ROS) are free radicals, or free-radical initiators, that are generated through a range of both normal metabolic processes, as well as through interactions of tissues with ultraviolet and ionizing radiation and other toxins.[1] These highly unstable, very reactive species damage important biological molecules, including DNA, lipids, and proteins. The subsequent radical chain reactions resulting from ROS interactions are capable of causing significant tissue damage due to oxidative stress. This process is often proposed as the cause of many diseases, such as cancer and cardiovascular and neurodegenerative diseases.[2−4] Additionally, it has been proposed that these radical reactions are major contributors to aging in organisms.[5]

Biological systems have several defensive processes for removing excessive ROS from the body. A common method is the employment of antioxidants. These radical scavengers are able to neutralize free radicals into stable molecules through electron or hydrogen atom donation.[6] The scavengers become stable, radical species and are degraded through different established pathways.[2,3] Ascorbic acid, also known as vitamin C, is an excellent, water-soluble radical scavenger due to its ability to react with a wide range of biologically relevant ROS, e.g., hydroxyl radicals and superoxide anion, in the blood and cytoplasm.[2,7,8]

Although not produced within the body, ascorbic acid can be easily consumed, as it is naturally present in many common fruits and vegetables.[9] It is an important nutrient that is attributed to many biological functions beyond its usefulness as an antioxidant, including the production of collagen, enzyme activity, and iron absorption.[10,11] At physiological pH, ascorbic acid ($AscH_2$) is mostly present in the form of the ascorbate anion ($AscH^-$), since the pK_a of the most acidic hydrogen is

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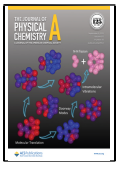

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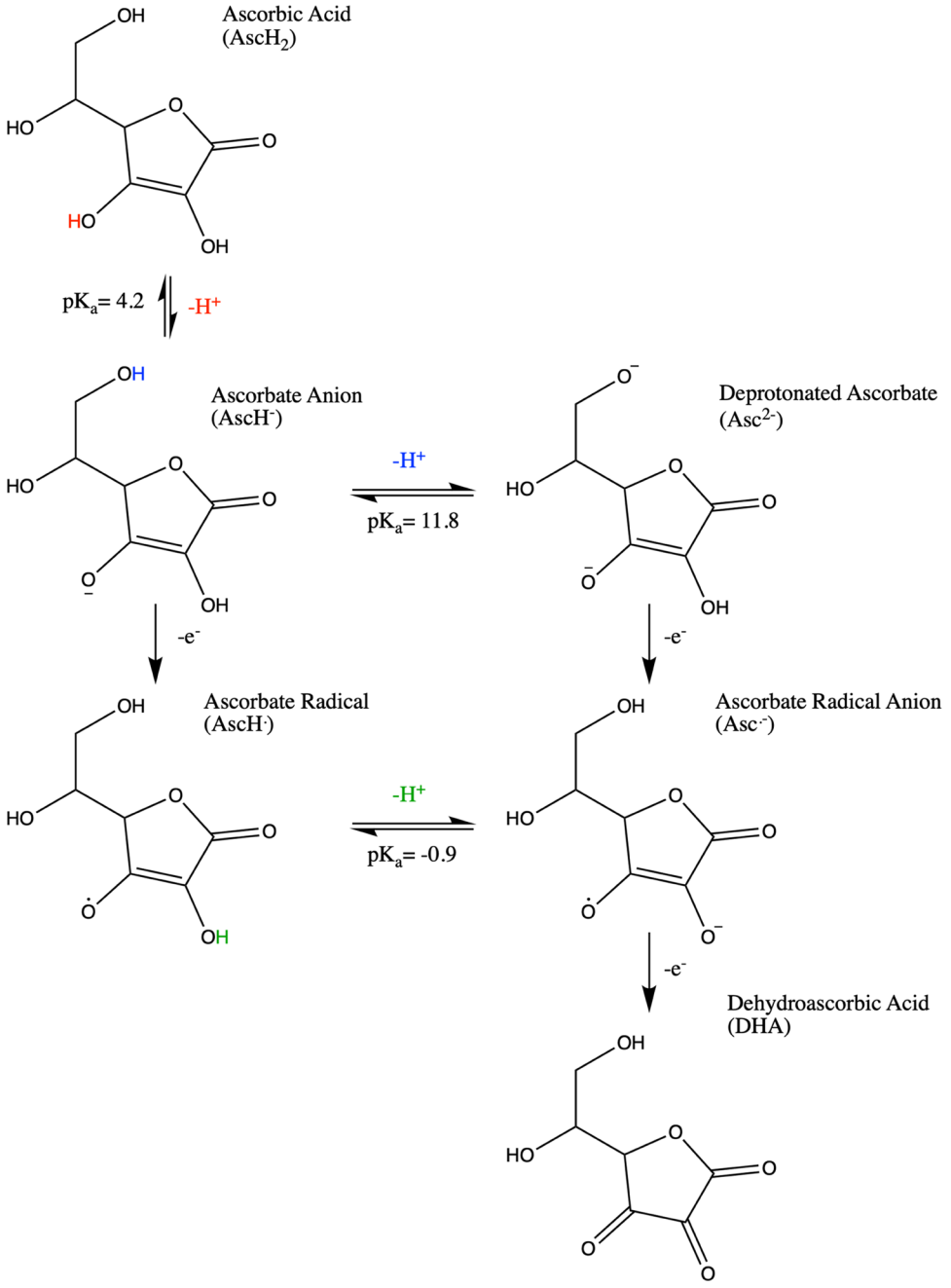

Figure 1. Structures of different anions, radicals, and neutral molecules associated with vitamin C. The structures represent those that are most stable in the gas phase.[15]

4.24 (Figure 1).[12] The ascorbate anion is one of the most active antioxidant forms of vitamin C. It is classified as a donor antioxidant due to of its ability to donate an electron from the lone pair generated by deprotonation, i.e., Scheme 1, where R is the ROS.[3,12]

$AscH^{\bullet}$ is transient; the second hydrogen has a pK_a of −0.9 and will rapidly deprotonate to $Asc^{\bullet-}$.[12] Although not present in high concentrations in biological systems since $AscH^-$ has a pK_a of 11.8, the doubly deprotonated form of ascorbic acid, Asc^{2-}, is also an effective antioxidant. The dianion transfers an electron to a free-radical species to form the stable radical anion, $Asc^{\bullet-}$, e.g., Scheme 2.

Scheme 1

$$AscH^- + R^{\bullet} \rightarrow AscH^{\bullet} + R^-$$

Scheme 2

$$Asc^{2-} + R^{\bullet} \rightarrow Asc^{\bullet-} + R^-$$

$Asc^{\bullet-}$ is a stable radical species that can convert back into the ascorbate anion as well as into dehydroascorbic acid (DHA) in biological systems, as shown in Figure 1.[12] Additionally, $Asc^{\bullet-}$ can be a source of hydrated electrons, which are considered a universal reductant.[13]

The most stable structures of ascorbic acid, the ascorbate anion, and deprotonated ascorbate have been determined

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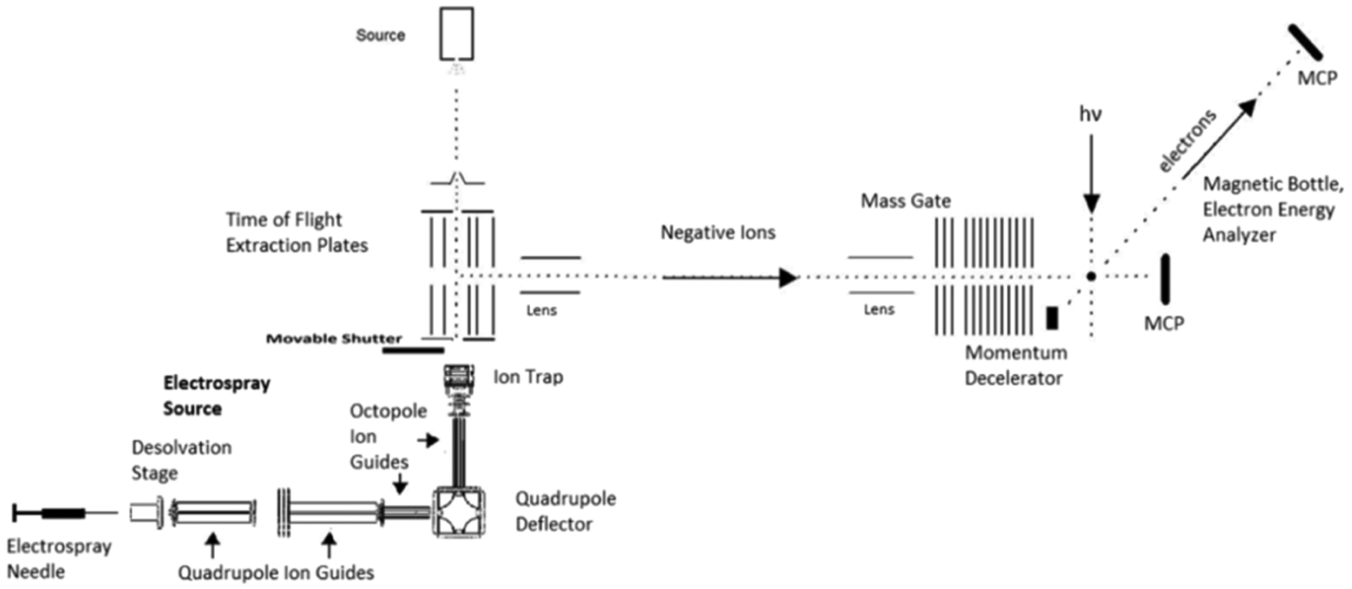

Figure 2. Schematic view of the electrospray ionization source that was incorporated into the preexisting pulsed ion photoelectron spectrometer.

using X-ray crystallography with the aid of calculations.[14,15] The vertical ionization energies and electron affinities (EA) have been theoretically calculated for ascorbic acid and the ascorbate anion, as well as deprotonated ascorbate and their radicals in both the gas phase and when solvated by water.[16−18] Another radical scavenger, vitamin E, has previously been studied via photoelectron studies.[19] In this work, we report the anion photoelectron spectra of the ascorbate anion ($AscH^-$) and deprotonated ascorbate (Asc^{2-}) generated by an electrospray ionization (ESI) source. The photoelectron study of $AscH^-$ and Asc^{2-} sheds light on energetic aspects of vitamin C as an antioxidant.

EXPERIMENTAL SECTION

The ascorbate anion ($AscH^-$) and the deprotonated ascorbate dianion (Asc^{2-}) were generated via electrospray ionization, a source recently added to one of our pulsed anion photoelectron spectrometers. This system is highly amenable to ionization by ESI due to water solubility and facile tuning of the pH conditions in the solution. The electrospray ionization source is modeled on the previous designs of Wang, Wang, and Johnson.[20−22] Millimolar solutions of L-(+)-ascorbic acid (Sigma-Aldrich, BioXtra, ≥99.0%) diluted in 3:1 MeOH/H_2O in a range of pH values (5 < pH < 12) were prepared. The ascorbic acid solution was sprayed through a ∼10 μm pulled silica capillary floated at negative 3−5 kV into a humidity-controlled, ambient atmosphere chamber. The ions entered a stainless steel desolvation capillary, which was heated to help remove solvent from the droplets formed via the ionization process. After exiting the capillary, the anions were guided through differentially pumped chambers by ion guides and ion optics into an ion trap. The ion trap has the capability to be cooled to 77 K by a liquid nitrogen flow cryostat (Advanced Research Systems, Inc.), and the temperature is monitored via a silicon diode. A buffer gas of 20% H_2 with a balance of He was used in the ion trap to facilitate the cooling of the ions, as well as collisional focusing.[20] The ions were accumulated and cooled in the trap for 100 ms before being pulsed out of the trap into a time of flight mass spectrometer. The ions from the electrospray ionization source enter into that time of flight mass spectrometer, which has been described elsewhere,[23,24] on the opposite side of the Wiley−McLaren extraction plates as compared to our tradition pulsed anion sources, as shown in Figure 2.

Anion photoelectron spectroscopy was conducted by crossing a mass-selected packet of negative ions with a fixed frequency photon beam and energy analyzing the resultant photodetached electrons. These photoelectrons are governed by the energy-conserving relationship: $h\nu$ = EBE + EKE, where $h\nu$ is the photon energy, EBE is the electron binding energy, and EKE is the measured electron kinetic energy. The anion photoelectron spectrometer consists of a magnetic bottle electron energy analyzer and a Nd:YAG laser. The magnetic bottle has a resolution of ∼50 meV at an EKE of 1 eV. The spectra were collected with the third and fourth harmonic of the Nd:YAG laser (355 nm, 3.49 eV and 266 nm, 4.66 eV, respectively). The photoelectron spectrometer was calibrated against the known transitions of I^-.[25] Photoelectron spectra were obtained with the ion trap at room temperature as well as when cooled to 77 K.

RESULTS

The ascorbate anion ($AscH^-$), deprotonated ascorbate (Asc^{2-}), and fragments of ascorbate were seen in the mass spectrum, as presented in Figure 3. The relative intensities of the ascorbate anion versus deprotonated ascorbate were strongly dependent on the pH of the solution. In line with the pK_a of ascorbate and deprotonated ascorbate, deprotonated ascorbate was favored at pH higher than 11.8.[12] The other fragments in the spectrum were likely generated in the ionization process or during the ion transport through the ion guide system via collisions with background gas molecules.

The photoelectron spectrum (PES) of the ascorbate anion ($AscH^-$) was obtained using the fourth harmonic of a Nd:YAG laser (266 nm, 4.66 eV) with the ion trap at ambient temperature (Figure 4). The broad feature has an onset at EBE ∼3.5 eV, corresponding to the electron affinity (EA) of the ascorbate radical, $AscH^•$, with a vertical detachment energy (VDE) value of 3.85 eV. While there appear to be vibrational bands, they are not well resolved in the spectrum.

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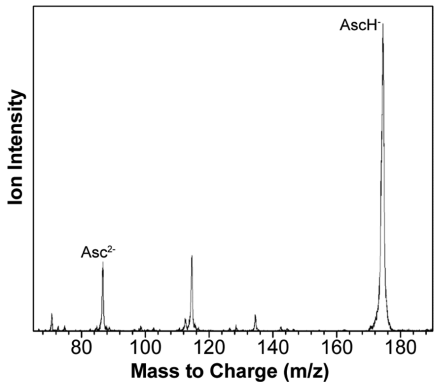

Figure 3. Mass spectrum of an ascorbic acid solution with a pH of 8 generated by ESI.

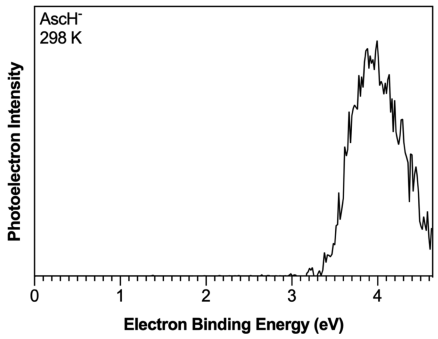

Figure 4. Photoelectron spectrum of the ascorbate anion ($AscH^-$) taken with the ion trap at ambient temperature using the fourth harmonic of a Nd:YAG laser (266 nm, 4.66 eV).

The deprotonated ascorbate (Asc^{2-}) was photodetached at an ion trap temperature of 77 K using the third harmonic of a Nd:YAG laser (355 nm, 3.49 eV), as well as at ambient temperature in the ion trap using the fourth harmonic of a Nd:YAG laser (266 nm, 4.66 eV), Figures 5 and 6,

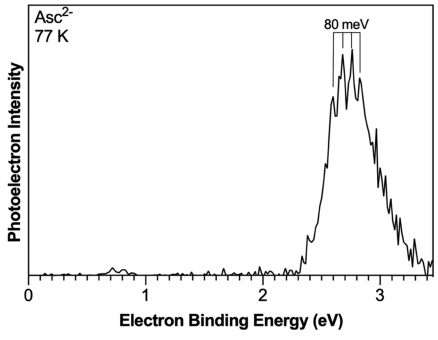

Figure 5. Photoelectron spectrum of deprotonated ascorbate (Asc^{2-}) taken at an ion trap temperature of 77 K using the third harmonic of a Nd:YAG laser (355 nm, 3.49 eV).

respectively. The observed VDE value, when photodetachment was conducted with 355 nm photons, is 2.68 eV. The vibrational structure, 80 meV, is easily discernable under these cold anion conditions. The spectrum collected using higher energy photons and with the ion trap at room temperature displays two broad bands. The VDE value of the first peak appears to have shifted to slightly higher electron binding energy under these circumstances. Instead of the 2.68 eV VDE value observed in the third harmonic spectrum, the VDE value

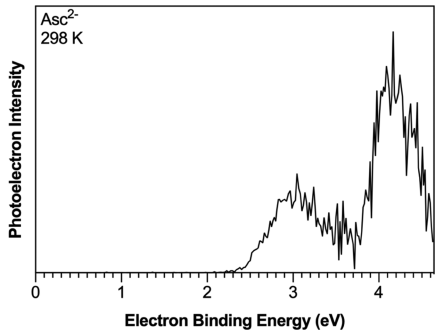

Figure 6. Photoelectron spectrum of deprotonated ascorbate taken at room temperature using the fourth harmonic of a Nd:YAG laser (266 nm, 4.66 eV).

was fitted to be 2.97 eV in the fourth harmonic spectrum. The next higher EBE feature exhibits an onset at an EBE of ~3.75 eV.

DISCUSSION

The vertical detachment energy (VDE) is equal to the energy difference between the electronic ground state configuration of the anion and the anion's neutral counterpart in the same configuration. In terms of the ascorbate anion, the anion's neutral counterpart is the ascorbate radical. The VDE value of the ascorbate anion corresponds to its ionization energy, which is also correlated to its efficiency as an antioxidant. Low ionization energy means that the molecule is readily oxidized and can easily donate an electron.[26] In the presence of molecules with various ionization energies, the molecule or compound with the lower ionization energy will more readily give up an electron. When neutralizing an ROS, an antioxidant donates an electron and becomes oxidized.[2,3] A successful antioxidant will more readily donate an electron than the biological target in the body, such as DNA. We observed that the ionization energy of ascorbate, i.e., the VDE of $AscH^-$, to be 3.85 eV, compared to the calculated ionization energy of 3.755 eV.[17] Using the P3 method, Ortiz et al. also calculated the ionization energies and electron affinities of nucleosides, i.e., 2-deoxyadenosine, 2-deoxycytidine, 2-deoxyguanosine, 2-deoxythymidine, and 2-deoxyuridine, and of three common free radicals, e.g., OH, OOH, and OCl. The free radicals had higher electron affinities (0.350−3.549 eV) than the ascorbate anion (−3.507 eV) and the nucleosides (−0.330 to −0.506 eV). The positive electron affinity of the free radicals indicates the willingness of the radicals to accept an electron. Furthermore, at 3.755 eV, the ascorbate anion had a dramatically lower ionization energy than the nucleosides (7.978−9.176 eV).[17] Thus, the reaction of ascorbate with free radicals is more favorable than the radical−nucleoside reaction. This is consistent with the spectroscopic data presented here. The ascorbate anion will donate an electron to the free radical to reduce it and thus become a more stable radical, $AscH^{\bullet}$, itself. It should be noted the gas-phase ionization energy, 3.85 eV, is over 1 eV lower than the ionization energy of $AscH^-$ in an aqueous solution.[16] In either phase, the ascorbate anion will readily donate an electron to free radicals.

Based on the photoelectron spectrum obtained using 355 nm photons, the observed VDE value of Asc^{2-} is 2.68 eV for detachment of its second excess electron. Although the deprotonated ascorbate dianion, Asc^{2-}, is not seen in high concentration in the human body, it is a better antioxidant

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than the ascorbate anion because it has a lower VDE value and will more readily donate an electron. This is supported by the fact that the radical anion, $Asc^{\bullet-}$, formed by removing an electron from deprotonated ascorbate, is more stable than the dianion; the radical anion is calculated to be lower in energy by 3.24 eV in the gas phase.[16] Since $Asc^{\bullet-}$ is lower in energy than Asc^{2-}, the observed Asc^{2-} photodetachment transitions terminate on the excited states of the radical anion. This implies that the highest EBE peak in the fourth harmonic photoelectron spectrum of Asc^{2-}, i.e., the one with an onset at an EBE of ~3.75 eV, is a transition from Asc^{2-} to a still higher energy excited state of $Asc^{\bullet-}$.

As mentioned above, the observed VDE value in the Asc^{2-} spectrum, i.e., due to its lowest EBE peak, shifted to a slightly higher EBE value when measured with higher energy photons; the VDE value obtained when using 4.66 eV photons is 2.97 eV (Figure 6) compared with 2.68 eV when using 3.49 eV photons (Figure 5). Although the 355 nm photons have higher photon energy than 2.97 eV, the shifted peak intensity was not observed in the third harmonic photoelectron spectrum. This shift was likely due to the repulsive Coulomb barrier that led to multiple detachment channels. At higher photon energy, more detachment channels are accessible, leading to different excited-state transitions. This is consistent with the photoelectron spectrum of the doubly charged citric acid anion; the lower EBE photodetachment channel continued to be observed in the higher photon energy photoelectron spectra of the deprotonated ascorbate.[27]

The potential minimum of the dianion corresponds to the equilibrium distance between the two excess electrons. In order for the dianion to be stable, these charges need to have sufficient separation.[27] Previously, it has been proposed that the excess electrons in Asc^{2-} are positioned on an oxygen located on the furan ring and on an alkyl oxygen (Figure 1).[15] In the condensed phase, solvation helps stabilize these charges, whereas in the gas phase, these charges render the dianion metastable. In order for an electron to escape the deprotonated ascorbate dianion resulting in the more stable radical anion, $Asc^{\bullet-}$, energy is needed to overcome the intervening repulsive Coulomb barrier.

CONCLUSIONS

An electrospray ionization source was added to an existing anion photoelectron spectrometer to study the anions related to ascorbic acid. The photoelectron spectra of the ascorbate anion, $C_6H_7O_6^-$, and the deprotonated ascorbate anion, $C_6H_6O_6^{2-}$, are presented. The antioxidant behavior of both anions is confirmed through the photoelectron spectra. The ionization energy of the ascorbate anion is determined to be 3.85 eV, which is lower than other biological molecules found in the body. The ascorbate anion will readily donate an electron to reactive oxygen species and detrimental radicals in the body and, therefore, protect potential biological targets. Although deprotonated ascorbate anion is not present at biological pH, the ionization energy, 2.68 eV, is lower than the ascorbate anion and is a better antioxidant.

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Author Contributions

M.M., Z.Z., R.H., and E.C. performed the experiments. M.M. wrote the manuscript. K.H.B. oversaw the experiments and writing of the manuscript.

Notes

The authors declare no competing financial interest.

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