On the Stability of Vitamin C Radicals

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Stability of vitamin C (ascorbic acid – AA) radicals is considered by the semiempirical MNDO method. It is found that $\mathrm{AA}^{\cdot -}$ is thermodynamically the most stable species. Interestingly, the $\mathrm{AA}^{\cdot -}$ radical is more stable than the parent AA. This finding is of great importance in view of omnipresence and versatility of the vitamin C in biochemical processes. The origin of the pronounced stability of $\mathrm{AA}^{\cdot -}$ is elucidated by a particular form of the energy partitioning technique.

The unusually high protective property of ascorbic acid (AA) against free radical damage in biological processes was ascribed to the efficiency of ascorbate as a radical scavenger and to the stability of its radical [1]. It is, therefore, of some interest to examine the stability of AA radical(s) by a reliable theoretical procedure. The method of choice is the MNDO scheme [2] because it is a reasonable compromise between accuracy and computational (financial) feasibility. Although the MNDO method has well known limitations, it is plausible that the errors are the same for closely related compounds and play no role when relative entities are considered. Our previous MNDO calculations on vitamin C tautomers lend support to this conjecture [3]. The four possible AA radical species are shown in Figure 1. The MNDO calculations were carried out by optimizing all independent structural parameters. The resulting heats of formation, ΔH_f, are displayed in Table 1. Earlier results for AA [3] are included for comparison. It appears that the most stable radical is the $\mathrm{AA}^{\cdot -}$ anion, which is compatible with some ESR measurements [4]. Interestingly, $\mathrm{AA}^{\cdot -}$ is thermodynamically more stable than the parent vitamin C itself. This finding sheds some light to the important role of this radical in attenuating free radical damage. The question arises why $\mathrm{AA}^{\cdot -}$ is thermodynamically favourable. Inspection of the structural parameters reveals that there is a significant increase in π-electron delocalization in the five-membered ring relative to the parent (AA) compound. More quantitative data are offerred by the energy partitioning technique. It was observed by Pople [5] that the total SCF energy can be decomposed in two contributions if the ZDO approximation is employed:

$$E_t = \sum_A E_A + \sum_{A<B} E_{AB} \,. \tag{1}$$

The merits of this type of analysis and the corresponding formulas for one- and two-center E_A and E_{AB} terms necessary for various semiempirical methods were discussed by several researchers [6–8]. We shall use the formula (1) in a slightly modified form:

$$E_t = E_1 + E_2 + E_3 \,, \tag{2}$$

where $E_1 = \sum_A E_A$, $E_2 = \sum_{A-B} E(A-B)$ and $E_3 = (1/2) \sum_A E_A^{nb}$. The second term is extended over all directly bonded atoms whereas the last term gives the overall repulsion of nonbonded atoms. It is conveniently written in a form of atomic contributions. The energy partitioning given by (2) is in harmony with the idea of modified atoms in molecules [9, 10]. Indeed, atoms in chemical environment are most perturbed by nearest neighbours as a conse-

Table 1. Decomposition of energy of ascorbic acid and its radicals as obtained by MNDO (in eV) and heats of formation (in kJ mol^{-1}).

	AA	**1**	**2**	**3**	**4**
E_1	−2515.8	−2510.5	−2510.8	−2511.3	−2509.6
E_2	−313.6	−291.6	−303.4	−302.3	−312.8
E_3	14.9	14.9	14.5	14.0	16.4
E_t	−2814.4	−2787.2	−2799.7	−2799.6	−2806.0
Δ_1	9.5	0	7.0	6.6	14.6
Δ_2	6.1	0	2.0	3.2	5.7
Δ_3	−1.3	0	−1.0	−1.5	−1.5
ΔH_f	−1055	−1164	−998	−987	−242
$\Delta(\Delta H_f)$	109	0	166	177	922

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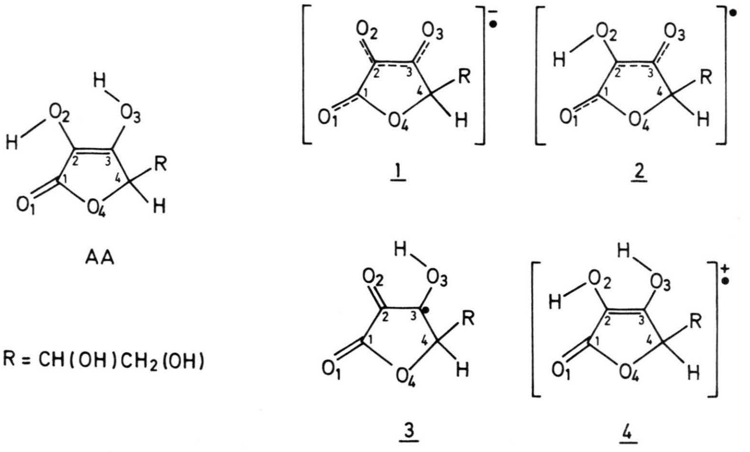

Fig. 1. Schematic representation of Vitamin C and its radical forms.

quence of formation of chemical bonds. The nonbonded repulsions play a secondary role. Intuitively, one can assume that the inequalities $|E_1| \gg |E_2| \gg |E_3|$ hold. This is borne out by the results presented in Table 1. Thus, monocentric terms provide the largest contribution to the total molecular energy whilst interactions of directly bonded atoms are an order of magnitude smaller. Nonbonded repulsions are smaller by about two orders as compared with E_1. We are interested here in heats of formation of AA radical(s). They are defined by

$$\Delta H_f^{mol} = E_t^{mol} - E_f^{pr} + \sum_A \Delta H_f^A , \qquad (3)$$

where $E_f^{pr} = \sum_A E_A^{fa}$ is a sum of electronic energies of free atoms. We shall imagine that the atoms are brought to equilibrium positions by setting their mutual interactions equal zero. Such an ensemble of atoms is termed a promolecule, E_f^{pr} being its total energy. Finally, the last term in (3) is a sum of heats of formation of free atoms. Let us define AA$^{\cdot -}$ as a reference molecule. Then the relative (in)stability of AA can be expressed as

$$\Delta H_f(\mathrm{AA}) - \Delta H_f(\mathbf{1}) = [\Delta_1 + \Delta_2 + \Delta_3]_{ps} + \{\alpha + \beta + \gamma\}_s , \qquad (4)$$

where

$$\Delta_1 = [E_1(\mathrm{AA}) - E_1(\mathrm{H(O_2)}) - E_1(\mathrm{H(O_3)})] - E_1(\mathbf{1}) , \qquad (5a)$$

$$\Delta_2 = [E_2(\mathrm{AA}) - E_2(\mathrm{O_2{-}H}) - E_2(\mathrm{O_3{-}H})] - E_2(\mathbf{1}) , \qquad (5b)$$

$$\Delta_3 = [E_3(\mathrm{AA}) - E_3^{nb}(\mathrm{H(O_2)}) - E_3^{nb}(\mathrm{H(O_3)}) + E_3^{nb}(\mathrm{H(O_2)},\ \mathrm{H(O_3)})] - E_3(\mathbf{1}) \qquad (5c)$$

and

$$\alpha = E_1(\mathrm{H(O_2)}) + E_1(\mathrm{H(O_3)}) + E_2(\mathrm{O_2{-}H}) + E_2(\mathrm{O_3{-}H}) + E_3^{nb}(\mathrm{H(O_2)}) + E_3^{nb}(\mathrm{H(O_3)}) - E_3^{nb}(\mathrm{H(O_2)}, \mathrm{H(O_3)}) , \qquad (6a)$$

$$\beta = -[E_f^{pr}(\mathrm{AA}) - E_f^{pr}(\mathbf{1})] = -2E_H^{fa} = 23.8\ \mathrm{eV} , \qquad (6b)$$

$$\gamma = \sum_B \Delta H_f^B(\mathrm{AA}) - \sum_A \Delta H_f^A(\mathbf{1}) = 2\Delta H_f^H = 4.5\ \mathrm{eV} . \qquad (6c)$$

Here a hydrogen atom attached to an oxygen atom O_n is denoted by $H(O_n)$. The physical meaning of (4) is obvious. The expression in brackets describes perturbation of the common skeleton upon formation of two oxygen-hydrogen bonds ($O_2{-}H$ and $O_3{-}H$). Therefore it carries the subscript ps. It is further partitioned into one-center, two-center bonding and two-center repulsive interactions. The term in curly parenthesis is denoted by "s" because it gives stabilization of the AA system through two additional O−H bonds. Hence, the difference $\Delta H_f(\mathrm{AA}) - \Delta H_f(\mathbf{1})$ is given by interplay of two opposing effects. It appears that perturbations (14.3 eV) prevails over stabilization (−13.2 eV) yielding a difference in stability of 1.1 eV. The change in one-center energies ($\Delta_1 = 9.5$ eV) is largest. Examination of formal atomic charges reveals a rough correlation between electron densities residing on atoms and monocentric energies. Consequently, it follows that one-center terms are more determined by electrostatic attraction between the electron cloud and a nucleus than by intraatomic electron repulsion. This observation is concomitant with the importance of the electrostatic potential in molecular systems [11, 12]. Lower one-center energies in **1** are due to higher electron density spread over the planar part of the radical anion. Two-center bonding interactions are higher in AA by 6.1 eV, the largest portion of which (5.4 eV) belongs to a decrease in π-delocalization over the planar part of the structure, which in turn is highly pronounced in the tricarbonyl system of **1**. This finding lends support to the conjecture made by consideration of bond distances. Nonbonded repulsion is decreased in AA by -1.3 eV. It should be pointed out that the side chain has little influence on the relative stability of **1** against AA. The energy partitioning analysis performed along the same lines shows that the radicals **2, 3** and **4** are less stable predominantly due to changes in monocentric energy and differences in delocalization over the planar parts of the studied molecules. We shall mention **4** as a good illustrative example where the Δ_1 and Δ_2 terms assume values of 14.6 eV and 5.7 eV, respectively. Again, decrease in electron density on atoms belonging to a planar fragment is the main cause of the increase in monoatomic energies. Furthermore, 5.4 eV of the total increase in the bonding bicentric interactions can be ascribed to the planar skeleton as a consequence of the change in delocalization and the concomitant redistribution of bond distances.

The reactivity of the $AA^{\dot{-}}$ radical anion gives an important clue in examining the possible oxidation mechanism of the ascorbic acid [13]. Hence it is of some interest to discuss briefly the composition of the highest singly occupied molecular orbital (SOMO). Neglecting small effects of nonplanarity it reads

$$\psi(\text{SOMO}) \cong -0.21\,\varphi(O_1) + 0.14\,\varphi(C_1) + 0.60\,\varphi(C_2) - 0.49\,\varphi(O_2) + 0.41\,\varphi(C_3) - 0.40\,\varphi(O_3)\,. \quad (7)$$

Here, the π-orbitals are denoted as $\varphi(A)$. The shape of the SOMO is depicted in Figure 2. One observes that SOMO is of π-type, being bonding between carbon atoms C_1, C_2 and C_3 and antibonding in the regions of $C{=}O$ bonds attached to the ring. One can describe SOMO as a linear combination of localized π^* orbitals of $C{=}O$ bonds as could be intuitively anticipated. It should be mentioned, however, that the contribution of the $C_1{=}O_1$ bond is rather small. The highest π-electron density is concentrated at the carbon C_2. We note in passing also that the orbital energy of SOMO (7) is -3.2 eV, being thus much higher than the HOMO orbital energy of -9.4 eV calculated for the parent AA compound. This finding explains at least partly the pronounced reactivity of the $AA^{\dot{-}}$ radical anion. Another point of interest is the distribution of the total spin density in $AA^{\dot{-}}$. It is schematically shown in Figure 3. One observes

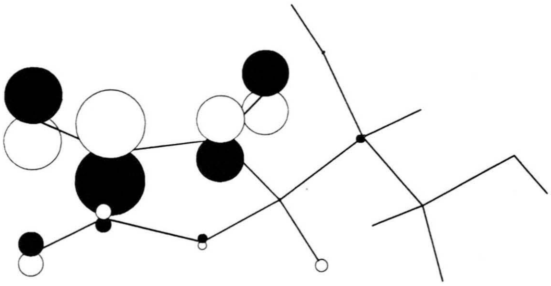

Fig. 2. The singly occupied molecular orbital (SOMO) of the radical anion $AA^{\dot{-}}$.

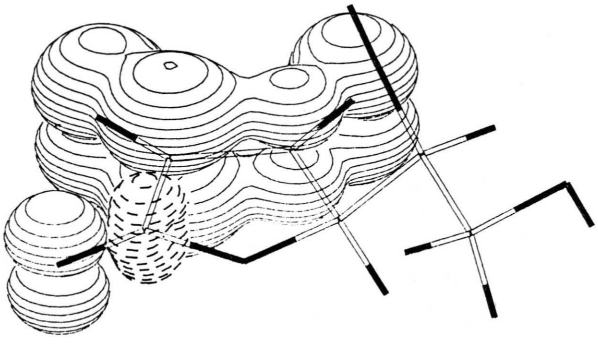

Fig. 3. Spin distribution in $AA^{\dot{-}}$. Full and dashed lines correspond to positive and negative spin densities, respectively. The lines denote the isopycnic curves where the spin density is 10^{-3} (in a.u.).

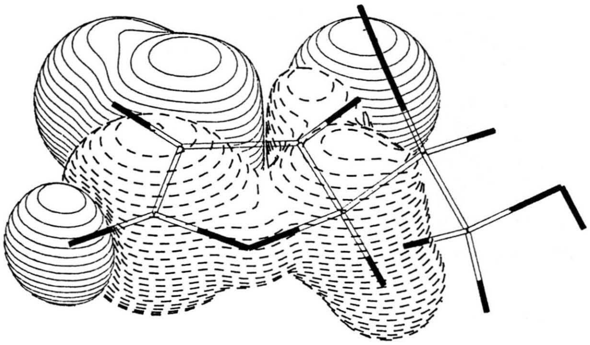

Fig. 4. Spin density distribution of s-orbitals in $AA^{\dot{-}}$. Full and dashed lines represent positive and negative spin densities. The lines are isopycnic curves with the spin density 10^{-3} (in a.u.).

that the peripheral carbonyl oxygens O_1, O_2 and O_3 carry substantial positive (α) density, whilst a relatively small negative (β) concentration is found in the vicinity of the C_1 carbon. It is noteworthy that the positive spin density is distributed over the $O_2-C_2-C_3-O_3$ fragment. The spin distribution arising from the spherically symmetric orbitals, which enter the Fermi contact term, is given in Figure 4. The main difference relative to the total spin density is the increase of the β density at the C_1 site and the appearance of a negative region at the C_3 position. Small negative concentrations are found also at the O_4, C_4 and $H(C_4)$ atoms. Caution is necessary in interpreting these results because it is very difficult to reproduce theoretically the accurate spin density distributions. However, one can safely say that the MNDO method correctly predicts appreciable spin densities at the exposed carbonyl oxygen atoms in accordance with experimental measurements (4).

To summarize, the present calculations support existing evidence that $AA^{\overline{\cdot}}$ is the most stable species in the series AA, **1**−**4.** The origin of the enhanced stability of **1,** which seems to be of great biochemical significance, is elucidated by a particular form of the energy partitioning technique. The latter is based on the explicit estimate of the perturbation of the $AA^{\overline{\cdot}}$ skeleton upon formation of two O−H bond(s) and a stabilizing effect of these two additional O−H bonds. It is a favourable feature of this procedure that the perturbation can be interpreted in simple chemical terms. Although a more sophisticated method may change the numerical values of this analysis, we feel that the main conclusions will remain the same.

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