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Conformation of L-Ascorbic Acid in Solution
2. L-Ascorbic Acid Anion

Mi Suk Kim, Sung Hee Lee, Uoo Tae Chung, and Young Kee Kang*

Department of Pharmacy, Chungbuk National University, Cheongju 360-763
*Department of Chemistry, Chungbuk National University, Cheongju 360-763
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In the unhydrated and hydrated states, conformational free energies of L-ascorbic acid anion (AAA) were computed with an empirical potential function and the hydration shell model (a program CONBIO). The conformational energy was minimized from possible starting conformations expressed with five torsion angles of the molecule. The conformational entropy of each low energy conformation in both states was computed using a harmonic approximation. As found in L-ascorbic acid (AA), intramolecular hydrogen bonds (HBs) are proved to be of significant importance in stabilizing the overall conformations of AAA in both states, and give the folded conformations, which are quite different from those in crystal. There are competitions between HBs and hydration around O3 atom of the lactone ring and hydroxyls of the acyclic side chain. Especially, the whole conformation of AAA is strongly dependent on the water-accessibility of O3 atom. Though there is a significant effect of the hydration on conformational surface, the lowest energy conformation of the unhydrated AAA is conserved. The different patterns of HBs and hydration result in the conformations of AAA in both states being different from those of AA. It can be drawn by several feasible conformations obtained in the hydrated state that there exists an ensemble of several conformations in aqueous solution.

Introduction

L-Ascorbic acid (vitamin C, hereafter abbreviated to AA) was isolated first by Szent-Györgyi in 1928[1] and it became apparent that this molecule takes a part in a number of physiological processes.[2] It is found that L-ascorbic acid anion (hereafter abbreviated to AAA) has the same effect *in vivo* as the vitamin itself, presumably because of the reversible equilibrium between two substances, and that the anion form exists as the single species at physiological pH of AA. Electrochemists[3] found intermediates in the oxidation pathway from AA to dehydroascorbic acid (DHA); one of considerable interest is the radical anion of DHA, and oxalic acid is the final stable product of AA oxidation.

There are some theoretical studies with MO methods on AAA, which include a modified CNDO with CI,[4] INDO,[5] and *ab initio* STO-3G[5,6] calculations, and were based on standard geometry or very limited geometry optimization. The MNDO calculation on AA radicals[7] showed that DHA anion was found to be thermodynamically the most stable species, due to a significant increase in π-electron delocalization in the five-membered ring. Hvoslef confirmed the crystal and molecular structure of AAA by X-ray diffraction analysis.[8] Berger measured the pH dependence of the ^{13}C chemical shifts of AA and interpreted it in terms of protonation sites.[9] The radicals produced in the radiolysis of aqueous solution of AA were examined with the radiolysis-ESR method by Laroff *et al.*[10] However, there are no detailed studies on the conformation of AAA in aqueous solution yet.

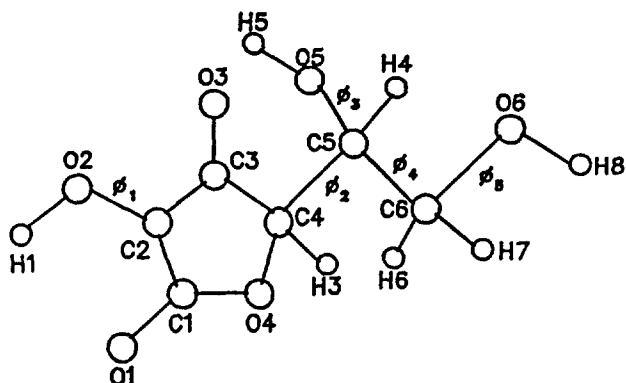In this work, the conformational study of AAA in aqueous

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Figure 1. The chemical structure and torsion angles of L-ascorbic acid anion (AAA).

Table 1. Definition of Torsion Angles of L-Ascorbic Acid Anion[a]

Torsion angle	Sequence of atoms
ϕ_1	H(1)-O(2)-C(2)-C(3)
ϕ_2	C(3)-C(4)-C(5)-C(6)
ϕ_3	C(4)-C(5)-O(5)-H(5)
ϕ_4	C(4)-C(5)-C(6)-O(6)
ϕ_5	C(5)-C(6)-O(6)-H(8)

[a]See Figure 1.

Table 2. Torsion Angles of Low Free Energy Conformations of Unhydrated L-Ascorbic Acid Anion[a,b]

No.	Conf.[c]	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5
1	$tg^-g^-tg^+$	−180	−56	−49	−176	51
2	$ttg^+g^-g^+$	180	−179	60	−71	62
3	$g^-g^-g^-tg^+$	−59	−56	−49	−176	51
4	$g^+tg^+g^-g^+$	59	−179	61	−71	62
5	$tg^+g^-tg^+$	180	63	−62	−173	50
6	$tg^-g^-g^+g^-$	180	−55	−50	68	−84
7	$g^+tg^-g^-g^+g^-$	59	−55	−50	68	−84
8	$g^+g^+g^-tg^+$	60	63	−61	−173	50
9	$g^-g^-g^-g^+g^-$	−59	−55	−50	69	−84
10	$tg^+g^+g^+g^-$	−180	78	41	75	−53
11	tg^+ttt	−180	59	172	−174	178
12	$ttg^-g^-g^+$	180	−180	−55	−72	60
13	$tg^-g^-g^+g^-$	180	82	−50	74	−55
14	tg^-g^-tt	−180	−57	−49	176	178
15	$tg^+g^+g^-t$	179	61	57	−66	170
16	g^-g^+ttt	−59	59	172	−174	178

[a]Units are in degree. [b]See Figure 1 and Table 1 for definition of torsion angles. [c]Each conformation is defined by conformational letter codes of five torsion angles defined in Figure 1 and Table 1, i.e., $0° \leq g^+ < 120°$, $120° \leq t \leq 180°$ or $-180° \leq t \leq -120°$, and $-120° < g^- < 0°$.

Methods

The bond lengths and bond angles adopted for L-ascorbic acid anion (AAA) in the computation were taken from the X-ray crystal results of Hvoslef.[8] In conformational energy calculations, bond lengths and bond angles were fixed and only the torsion angles for internal rotation were taken as the variables. The definition of torsion angles in AAA is shown in Figure 1, and the sequence of atoms for each torsion angle is listed in Table 1.

All the conformational energy computations in present work were carried out with the CONBIO program of Kang.[13,14] The conformational energy was calculated with an empirical potential function ECEPP/2,[11] and the hydration shell model improved recently[12] was used to compute the hydration free energy of each conformation of the molecules in the hydrated state. The potential functions and their parameters are described in details earlier.[11-14] The conformational energy and free energy were minimized using a variable metric algorithm SUMSL.[15] All the torsion angles of the molecule were allowed to vary during minimization.

From the five combinations of torsion angles ϕ_1(0 and 180°), ϕ_2(± 60 and 180°), ϕ_3(120 and 300°), ϕ_4(± 60 and 180°), and ϕ_5(0 and 180°), the 72 conformations were selected as starting points for energy minimization of AAA in the unhydrated state. These five torsion angles of AAA were allowed to vary during energy minimization in both the unhydrated

solution is carried out using an empirical potential function[11] and hydration shell model[12] to know its detailed structure and hydration effect and to compare with the conformation of AA.[13]

and hydrated states. Each conformation obtained by minimization of the unhydrated AAA was used as a starting conformation for free energy minimization in the hydrated state.

At each energy minimum in the unhydrated and hydrated states, the conformational entropy was computed using a harmonic method of Gō and Scheraga.[16] The elements of a hessian matrix of second derivatives at each minimum were numerically calculated with the step size of each variable equal to 1°.[14d]

The relative total free energy in the hydrated state is given by $\Delta G_{tot} = \Delta G + \Delta\Delta G_{hyd}$, and $\Delta\Delta G_{hyd}$ is the relative hydration free energy. The relative conformational energy is given by ΔE, and the relative entropic contribution to the relative free energy is given by $-T\Delta S$. And $\Delta G = \Delta E - T\Delta S$. The detailed definition for each energy component was described in Part 1.[13] Also the normalized statistical weight of each conformation was computed using an equation of Zimmerman et al.[17] All the thermodynamic quantities have been calculated for $T = 298$ K.

Results and Discussion

Torsion angles and energetics of low free energy conformations (i.e., relative free energy $\Delta G_{tot} < 1$ kcal/mol) of AAA, in the unhydrated and hydrated states are listed in Tables 2-5, respectively. For each conformation, Tables 3 and 5 contain (1) the conformational letter code, (2) the relative total free energy ΔG_{tot}, (3) the normalized statistical weight ω, (4) the relative conformational energy ΔE, (5) the relative conformational free energy ΔG, (6) the relative entropic contribution to conformational free energy $-T\Delta S$, (7) the relative hydration free energy $\Delta\Delta G_{hyd}$, and (8) the relative energy

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Table 3. Energetics of Low Free Energy Conformations of Unhydrated L-Ascorbic Acid Anion[a,b]

Conf.[c]	ΔG^d	ω^e	ΔE^f	$-T\Delta S^g$	ΔE_{es}^h	ΔE_{nb}^i	ΔE_{tor}^j
1	.00	.098	.00	.00	.00	.00	.00
2	.03	.095	-1.23	1.26	.24	-1.57	.10
3	.11	.081	.15	-.04	.17	-.02	.00
4	.14	.078	-1.09	1.23	.41	-1.60	.10
5	.30	.059	.40	-.10	.71	-.33	.02
6	.31	.059	-.61	.92	-.45	-.46	.29
7	.41	.049	-.47	.88	-.29	-.48	.29
8	.42	.049	.55	-.13	.89	-.36	.02
9	.43	.048	-.45	.88	-.28	-.46	.29
10	.70	.030	-.31	1.01	-.46	-.81	.95
11	.83	.024	1.01	-.18	1.48	-.44	-.04
12	.84	.024	-.22	1.06	.77	-1.11	.13
13	.92	.021	.03	.89	-.07	-.97	1.07
14	.92	.021	1.09	-.17	.75	.39	-.05
15	.93	.021	1.09	-.16	1.56	-.45	-.03
16	.98	.019	1.19	-.21	1.67	-.45	-.04

[a]Energies are in kcal/mol, and free energies and entropic contributions are calculated at 298 K. [b]Only the conformations with the relative total free energy to that of the conformation $tg^-g^-tg^+$ ($\Delta G<1.0$ kcal/mol) are listed. [c]The number of each conformation is the same as that of Table 2. [d]The total free energy of each conformation in the unhydrated state; $\Delta G=G\text{-}G^o=\Delta E-T\Delta S$, $G^o=1.374$ kcal/mol. [e]Normalized statistical weight. [f]Intramolecular interaction energy change; $\Delta E=E-E^o$; $E^o=E_{es}^o+E_{nb}^o+E_{tor}^o=7.689$ kcal/mol. [g]Conformational entropic contribution. [h]Electrostatic energy change; $\Delta E_{es}=E_{es}-E_{es}^o$, $E_{es}^o=10.831$ kcal/mol. [i]Nonbonded energy change; $\Delta E_{nb}=E_{nb}-E_{nb}^o$, $E_{nb}^o=-3.277$ kcal/mol. [j]Torsional energy change; $\Delta E_{tor}=E_{tor}-E_{tor}^o$, $E_{tor}^o=0.135$ kcal/mol.

Table 4. Torsion Angles of Low Free Energy Conformations of Hydrated L-Ascorbic Acid Anion[a,b]

No.	Conf.[c]	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5
1	$tg^-g^-tg^+$	-180	-60	-45	-176	48
2	$g^-g^+g^+tg^+$	-55	64	39	-173	45
3	$g^+g^-g^-tg^+$	0	-60	-45	-176	48
4	$g^+tg^+g^-g^+$	56	-177	58	-71	59
5	$g^+g^+g^+g^+g^-$	54	79	32	75	-52
6	$tg^-g^-g^+g^-$	180	-58	-47	69	-79
7	$ttg^+g^-g^+$	-180	-177	58	-71	61

[a,b,c]See footnotes of Table 2.

components ΔE_{es}, ΔE_{nb}, and ΔE_{tor} of ΔE. For each conformation, a five-letter conformational code is used for torsion angles of the molecule defined in Figure 1 and Table 1 (see footnote c of Table 2 for detailed codes).

Unhydrated L-Ascorbic Acid Anion. From the 72 starting conformations of the unhydrated AAA, we obtained the 62 different conformations after minimization and the only 31 conformations have the relative conformational free energy (ΔG) less than 3 kcal/mol. The 16 conformations of them with $\Delta G<1$ kcal/mol seem to be most probable conformations of AAA in the unhydrated state, which are shown

Table 5. Energetics of Low Free Energy Conformations of Hydrated L-Ascorbic Acid Anion[a,b]

Conf.[c]	ΔG_{tot}^d	ω^e	ΔE^f	ΔG^k	$-T\Delta S^h$	$\Delta\Delta G_{hyd}^i$	ΔE_{es}^j	ΔE_{nb}^k	ΔE_{tor}^l
1	.00	.334	.00	.00	.00	.00	.00	.00	.00
2	.31	.200	-.16	-.68	-.52	.99	.09	-.47	.22
3	.76	.094	.74	.64	-.10	.12	.02	.13	.60
4	.84	.082	-1.10	-.34	.76	1.18	.43	-1.60	.07
5	.90	.074	-.15	.44	.59	.46	-.39	-.90	1.13
6	1.11	.052	-.61	-.08	.53	1.19	-.46	-.35	.20
7	1.33	.036	-1.27	.41	1.68	.92	.29	-1.60	.05

[a-c,e-h,j-l]See footnotes of Table 3. [c]The number of each conformation is the same as that of Table 4. [d]The total free energy of each conformation in the hydrated state; $\Delta G_{tot}=G_{tot}-G_{tot}^o$, $G_{tot}^o=0.649$ kcal/mol. [f]$E^o=7.744$ kcal/mol. [i]Hydration free energy of each conformation; $\Delta\Delta G_{hyd}=\Delta G_{hyd}-\Delta G_{hyd}^o$, $\Delta G_{hyd}^o=-52.460$ kcal/mol. [j]$E_{es}^o=10.760$ kcal/mol. [k]$E_{nb}^o=-3.195$ kcal/mol. [l]$E_{tor}^o=0.178$ kcal/mol.

in Tables 2 and 3. There are no common characteristics in torsion angles of these low free energy conformations of AAA.

The calculated thermodynamic quantities for the low free energy conformations of AAA in the unhydrated state are listed in Table 3. From the analysis of total free energies of the conformations, the conformational energy and entropy are both the major contributions to the total free energy. Though the conformations $ttg^+g^-g^+$ (2) and $g^+tg^+g^-g^+$ (4) have the lower conformational energies than that of the conformation $tg^-g^-tg^+$ (1), the latter has lower conformational free energy than the former due to the more negative contribution of $-T\Delta S$ to ΔG. It corresponds the increasing conformational entropy and indicates that there are deeper potential surfaces around the energy minima of the conformations 2 and 4 than that of the conformation 1. The similar analysis on the contribution of $-T\Delta S$ to ΔG can be found for the conformations $tg^-g^-g^+g^-$ (6), $g^+g^-g^-g^+g^-$ (7), $g^-g^-g^-g^+g^-$ (9), $tg^+g^+g^+g^-$ (10), and $ttg^-g^-g^+$ (12).

Figure 2a shows a potential energy contour map of AAA in the unhydrated state as a function of torsion angles ϕ_2 and ϕ_4, with other torsion angles fixed at those of the lowest energy minimum, i.e., the conformation 2. The conformational energy was calculated at intervals of 5° in torsion angles ϕ_2 and ϕ_4. Contours are drawn at intervals of 1 kcal/mol relative to the conformation 2, which is the lowest energy (ΔE) conformation. The map shows that there is one deepest conformational energy minimum. This is caused by a very strong intramolecular hydrogen bond (HB) between O4 and H6 atoms with $R(O4\cdots H6)=1.76$ Å (each atom is designated in Figure 1). The role of HBs in stabilizing conformational energies of AAA will be discussed in the following paragraph in details.

The first 6 low free energy conformations of AAA in the unhydrated state with $\Delta G<0.5$ kcal/mol are drawn in Figure 3. There are interesting features in torsion angles ϕ_2 and ϕ_5, i.e., the conformations 1 and 3 are $g^-g^-tg^+$ for these torsion angles and have two weak intramolecular HBs between O4 and H5 atoms and between O5 and H8 atoms with $R(O4\cdots H5)=2.43$ Å and $R(O5\cdots H8)=2.21$ Å. The conforma-
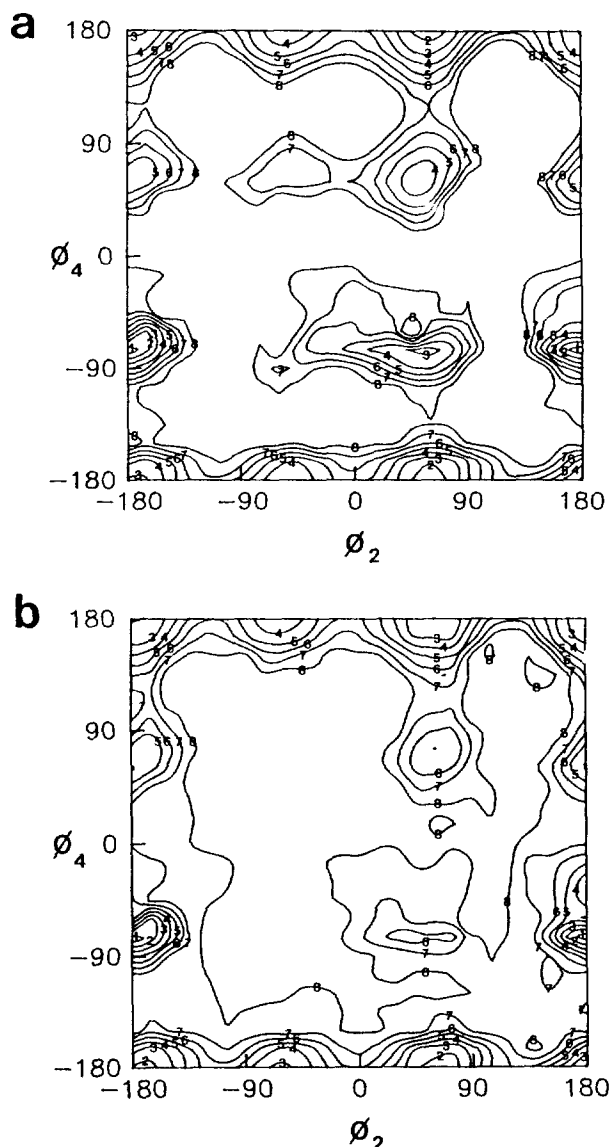
a

b

Figure 2. Potential energy contour maps of L-ascorbic acid anion (AAA) as a function of torsion angles ϕ_2 and ϕ_4, with other torsion angles fixed at each lowest energy minimum. Contours are drawn at intervals of 1 kcal/mol: (a) the unhydrated AAA relative to the conformation $ttg^+g^-g^+$ and (b) the hydrated AAA relative to the conformation $ttg^+g^-g^+$.

tions **2** and **4** are $tg^+g^-g^+$ for these torsion angles and have one strong HB between O4 and H6 atoms with a short interacting distance as described in the previous paragraph. Although this strong HB gives a deepest well in the potential surface of AAA shown in Figure 2a, the conformations **2** and **4** are less stable than the conformations **1** and **3** due to the lower conformational entropies. The conformation **5** has one moderate intramolecular HB between O5 and H8 atoms with $R(O5\cdots H8)=2.17$ Å, and the conformation **8** has also the same HB. The conformation **6** as well as **7** and **9** has one strong HB between O3 and H6 atoms $(R(O3\cdots H8)$ $=1.90$ Å) and one weak HB between O4 and H5 atoms $(R(O$ $4\cdots H5)=2.44$ Å). The stronger HB between O3 and H6 atoms of the conformations **6**, **7**, and **9** than that between O5 and H6 atoms of the conformations **1** and **3** is believed
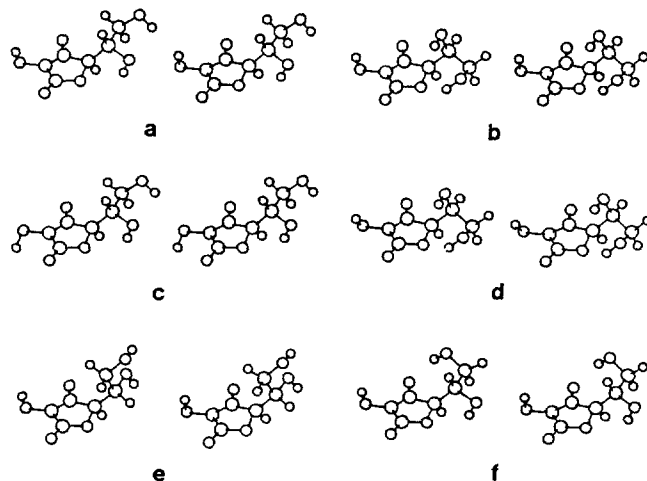

Figure 3. Stereoviews of the low free energy conformations of L-ascorbic acid anion (AAA) in the unhydrated state: (a) $tg^-g^-tg^+$, (b) $ttg^+g^-g^+$, (c) $g^-g^-g^-tg^+$, (d) $g^+tg^+g^-g^+$, (e) $tg^+g^-tg^+$, and (f) $tg^-g^-g^+g^-$.

to be caused by the more negative charges on the O3 atom than those of the O5 atom. However, the deeper potential wells coming from the stronger HBs result in the conformations **6**, **7**, and **9** to have the larger positive contributions of $-T\Delta S$ to ΔG than the conformations **1** and **3**.

In all the first 9 low free energy conformations (shown in Tables 2 and 3), there is an interesting feature in torsion angle ϕ_1, *i.e.*, the *trans* conformation is lower in conformational energy by about 0.2 kcal/mol than the *gauche* conformation. It was found that the conformation of ϕ_1 does not perturb the HBs as found in the unhydrated AA of Part 1.[13] Anyway, the intramolecular HBs are proved to be essential in stabilizing the overall conformations of AAA to be folded as the same as AA in the unhydrated state.

From comparison of the pattern of HBs of AAA and AA[13] with conformational energies less than 0.5 kcal/mol in the unhydrated state, it is found that HBs between O4 and H6 atoms and between O5 and H8 atoms can be still conserved, but that HB between hydroxyl hydrogen attached to C3 atom and O5 atom is replaced by another HBs between O4 and H5 atoms and between O3 and H8 atoms. This is believed to be brought by protonation and more negative charge on the hydroxyl attached to C3 atom. However, the conformation of torsion angle ϕ_1 is not dependent on the protonation from O3 atom.

As the same as in AA, intramolecular HBs of AAA give the overall conformations to be folded, and these low free energy conformations quite differ from those of crystal,[8] in which *all* hydroxyl groups are involved in *intermolecular* HBs and so the overall crystal conformations are nearly *all trans*.

Hydrated L-Ascorbic Acid Anion. In the hydrated state, the only 19 conformations of AAA obtained from the 72 minimized conformations in the unhydrated state were found to have the relative total free energy $(\Delta G_{tot}$, a sum of ΔG and $\Delta\Delta G_{hyd})$ less than 3 kcal/mol. The 5 conformations of them with $\Delta G_{tot}<1$ kcal/mol are believed to be most feasible in the hydrated state, and are shown in Tables 4 and 5. The reduced number of low free energy conformations in the hydrated state than those in the unhydrated state
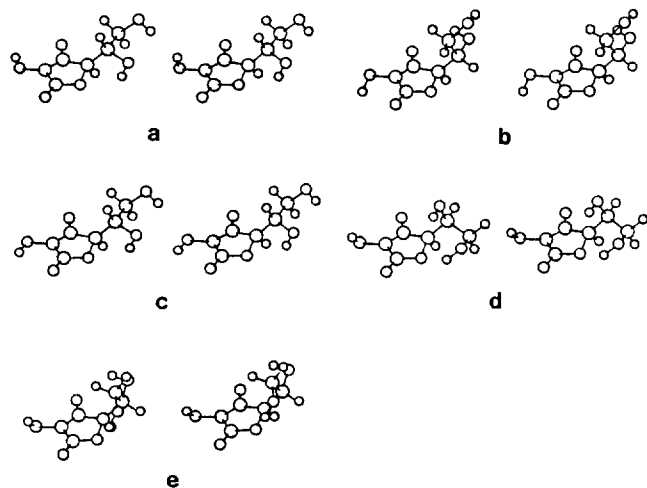
Figure 4. Stereoviews of the low free energy conformations of L-ascorbic acid anion (AAA) in the hydrated state: (a) $tg^-g^-tg^+$, (b) $g^-g^+g^+tg^+$, (c) $g^+g^-g^-tg^+$, (d) $g^+tg^+g^-g^+$, and (e) $g^+g^+g^+g^+g^+$

may show the significance of the hydration effect on the conformations of AAA in the hydrated state. The analysis of energetics listed in Table 5 tells us that the hydration does not directly affect the hydration free energy of each conformation, but contributes to alter the potential surface around each free energy minimum. For example, the conformation $tg^-g^-tg^+$ (1) is found to be the lowest total free energy state due to the hydration, though the conformation $ttg^+g^-g^+$ (7) has the lowest free energy given as the sum of conformational energy (ΔE) and hydration free energy ($\Delta\Delta G_{hyd}$) than the conformation 1.

Figure 2b shows a potential free energy contour map of AAA in the hydrated state as a function of torsion angles ϕ_2 and ϕ_4, with other torsion angles fixed at those of the lowest free energy minimum, i.e., the conformation $ttg^+g^-g^+$ (7). The conformational free energy was calculated as the sum of conformational energy (ΔE) and hydration free energy ($\Delta\Delta G_{hyd}$) at intervals of 5° in torsion angles ϕ_2 and ϕ_4. Contours are drawn at intervals of 1 kcal/mol relative to the conformation 7. The map shows that there are large changes in potential surfaces along ϕ_2 equal to g^+ and that energy minima along ϕ_4 equal to t are somewhat lowered through the hydration.

The first 5 low free energy conformations of AAA with $\Delta G_{tot} < 1.0$ kcal/mol in the hydrated state are drawn in Figure 4. There are interesting correlations between torsion angles and intramolecular HBs, i.e., the conformations 1, 2, and 3 have a common moderate HB between O5 and H8 atoms ($R(O5\cdots H8) = 2.19$, 2.13, and 2.19 Å for the three conformations, respectively) resulting from torsion angles ϕ_4 and ϕ_5 equal to t and g^+, respectively. However, the conformations 4 and 5 have a common strong HB ($R(O4\cdots H8) = 1.75$ and 1.82 Å for the two conformations, respectively) causing from the combination of torsion angles ϕ_2, ϕ_4, and ϕ_5. The conformations 1 and 3 have another weak HB between O4 and H5 ($R(O4\cdots H5) = 2.36$ Å) with a pair of torsion angles (ϕ_2, ϕ_3) equal to (g^-, g^-) and (g^+, g^+), respectively, whereas the conformation 4 has the second weak HB between H5 and O6 atoms with $R(H5\cdots O6) = 2.30$ Å and torsion angles

Table 6. Hydration Free Energy of Each Group of Low Free Energy Conformations of L-Ascorbic Acid Anion[a, b, c]

Conf.[d]	Lactone ring[e]			CHOH[f]			CH₂OH[f]		
	$\Delta\Delta G_h$	$\Delta\Delta G_p$	$\Delta\Delta G_{hyd}$	$\Delta\Delta G_h$	$\Delta\Delta G_p$	$\Delta\Delta G_{hyd}$	$\Delta\Delta G_h$	$\Delta\Delta G_p$	$\Delta\Delta G_{hyd}$
1	.00	.00	.00	.00	.00	.00	.00	.00	.00
2	.56	.35	.91	.31	.26	.56	.12	.05	.17
3	.12	.22	.33	.00	.00	.00	.00	.00	.00
4	−.42	.34	−.07	.74	.58	1.31	.86	.48	1.34
5	−.16	.15	−.00	.19	.19	.37	.44	.04	.48
6	.68	−.05	.62	−.10	−0.2	−.11	.61	.27	.87
7	−.69	.05	−.64	.75	.59	1.33	.87	.49	1.36

[a]Energies are in kcal/mol. [b]Relative energies to the conformation 1 ($tg^-g^-tg^+$). [c]$\Delta\Delta G_h$ and $\Delta\Delta G_p$ correspond to hydration free energies due to water-accessible volume and polarization relative to the conformation 1, and $\Delta\Delta G_{hyd}$ is the sum of $\Delta\Delta G_h$ and $\Delta\Delta G_p$. See ref. 12 for details. [d]The number of each conformation is the same as that of Table 4. [e]$\Delta G_h^o = -45.18$ kcal/mol, $\Delta G_p^o = -0.25$ kcal/mol, and $\Delta G_{hyd}^o = -45.43$ kcal/mol. [f]$\Delta G_h^o = -3.60$ kcal/mol, $\Delta G_p^o = 0.77$ kcal/mol, and $\Delta G_{hyd}^o = -2.83$ kcal/mol. [g]$\Delta G_h^o = -3.68$ kcal/mol, $\Delta G_p^o = 0.65$ kcal/mol, and $\Delta G_{hyd}^o = -3.03$ kcal/mol.

ϕ_3 and ϕ_4 equal to t and g^-, respectively. The calculated results indicate that the contributions from the hydration and conformational entropy are essential in stabilizing the conformation 1, though this conformation has two weaker HBs than the conformations 4, 5, or 7. The hydroxyl group attached to C2 atom of the lactone ring is found to be less flexible than that in the unhydrated state (e.g., compare the ΔE's of the conformations 1 and 3 in Tables 3 and 5). However, the lowest free energy conformation $tg^-g^-tg^+$ is conserved in the hydrated state as well as in the unhydrated state, and this is one of significant features in the conformations of AAA, compared with those of AA of Part 1.[13] Intramolecular HBs force the overall conformations to be folded, though different from those in the unhydrated state. The calculated conformations in the hydrated state quite differ from those of crystal.[8]

Hydration free energy of each group of low free energy conformations of AAA is listed in Table 6. The total hydration free energy of each group is computed as the sum of two components coming from water-accessible volume and polarization (see ref. 12b for details). As found in the case of AA of Part 1, there are competitions between intramolecular HBs and hydration. For example, comparing with the conformation 1, the conformation 6 ($tg^-g^-g^+g^-$) forms a HB between O3 and H8 atoms, but hydration free energies (ΔG_h) of the lactone ring and the CH₂OH group are increased positively due to the excluded water-accessible volumes of O3 atom and hydroxyl of the CH₂OH through the HBs (see Table 6). The same kind of competition between HBs and hydration of hydroxyls of the CHOH and CH₂OH groups can be found in the conformations 1 and 4. Therefore, the hydration around O3 atom of the lactone ring and the hydroxyls attached to the acyclic side chain forces the molecule to form less stable HBs.

Several feasible conformations obtained from conformational free energy calculations in the hydrated state indicate

that there exists an ensemble of several conformations in aqueous solution, rather than a single dominant conformation. However, the number of low free energy conformations of the hydrated AAA was significantly reduced than that of AA,[13] and this fact can be considered as causing from the highly negative hydration free energy of charged oxygen atom (see Table 1 of ref. 12c). The NMR experiments on AAA can supply useful informations to check the calculated results in this work.

Conclusions

Intramolecular hydrogen bonds (HBs) are proved to be of significant importance in stabilizing the overall conformations of L-ascorbic acid anion (AAA) in both the unhydrated and hydrated states from the analysis of conformational free energies. The folded conformations caused from these HBs in both states are quite different from those in crystal and of L-ascorbic acid (AA).[13]

There are competition between HBs and hydration in the hydrated AAA, and the hydration around O3 atom (protonated site in the lactone ring) and hydroxyls of the acyclic side chain forces the molecule to form less stable HBs. Several feasible conformations obtained in this work account for that there exists an ensemble of several conformations in aqueous solution, though the number of conformations is reduced than that of AA. However, the lowest free energy conformation of AAA in the unhydrated state is conserved in the hydrated state. The different patterns of HBs and hydration result in the conformations of AAA in both states being far from those of AA. The conformational study by NMR experiments can be served to check the results of this theoretical work.

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