Conformation of L-Ascorbic Acid in solution. 1. Neutral L-Ascorbic Acid

Young A Shin and Young Kee Kang*

Department of Chemistry, Chungbuk National University, Cheongju 360-763

Received August 24, 1990

Conformational free energy calculations using an empirical potential function and the hydration shell model (a program *CONBIO*) were carried out on the neutral L-ascorbic acid (AA) in the unhydrated and hydrated states. The conformational energy was minimized from starting conformations which included possible conformations of six torsion angles in the molecule. The conformational entropy of each low energy conformation in both states was computed using a harmonic approximation. From the analysis of conformational free energies for AA in both states, intramolecular hydrogen bonds (HBs) are proved to be an essential factor in stabilizing the overall conformations, and cause the conformations in both states to be quite different from those in crystal. In the case of hydrated AA, there is a competition between HBs and hydration, and the hydration around the two hydroxyl groups attached to the acyclic side chain forces the molecule to form less stable HBs. The hydration affects strongly the conformational energy surfaces of AA. Several feasible conformations obtained in this work indicate that there exists an ensemble of several conformations in aqueous solution. The calculated probable conformations for the rotation about the C5-C6 bond of the acyclic side chain are *trans* and *gauche* +, which are in good agreement with results of NMR experiment.

Introduction

L-ascorbic acid (so-called vitamin C, hereafter abbreviated to AA) has been realized to exhibit interesting chemistry and versatile biological activites since its discovery in 1928 by Szent-Györgyi.[1] AA is involved in collagen synthesis where it acts as a hydroxylating agent,[2] in lowering the concentration of cholesterol in tissues,[3] in folic acid metabolism,[4] and in control of vitamin B_{12} levels in food.[5] Also it reduces metal ions such as Cu^{2+} and Hg^{2+},[6] and inhibits the formation of nitrosoamines[7] which are thought to be carcinogenic.

There are some MO calculations on ascorbic acid, which include a modified CNDO with CI,[8] INDO,[9] and *ab initio* STO-3G[9,10] calculations, and were based on standard geometry or very limited geometry optimization. An extensive MINDO/3 and NMDO studies of the structure and energetics of AA tautomers were performed.[11] Hvoslef confirmed the crystal and molecular structure of AA by X-ray diffraction analysis.[12] Recently, Guilleme *et al.*[13] carried out the proton and ^{13}C-NMR spectroscopic study on the conformation of AA in acidic aqueous solution. However, there are no detailed conformational studies on AA in aqueous solution yet.

In this work, the conformational study of AA in aqueous solution is carried out using an empirical potential function[14] and hydration shell model[15] to know its detailed structure and hydration effect as a first step in understanding its biological functions.

Methods

The chemical structure and definition of torsion angles for AA are shown in Figure 1. The bond lengths and bond angles adopted for the molecule were taken from the X-ray crystal results of Hvoslef.[12] 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 are listed in Table 1 and Figure 1.

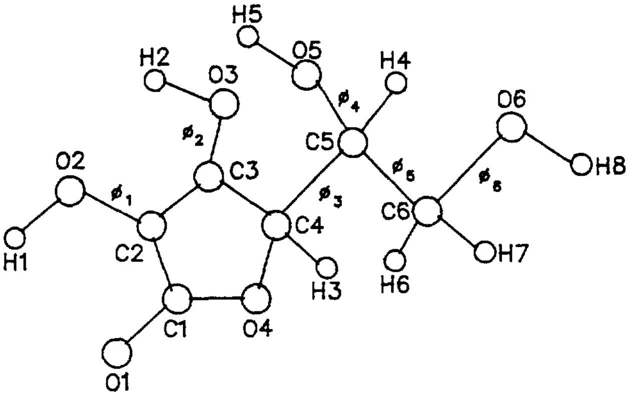

Figure 1. The chemical structure and torsion angles of neutral L-ascorbic acid (AA).

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

Torsion angle	Sequence of atoms
ϕ_1	H(1)-O(2)-C(2)-C(3)
ϕ_2	C(2)-C(3)-O(3)-H(2)
ϕ_3	C(3)-C(4)-C(5)-C(6)
ϕ_4	C(4)-C(5)-O(5)-H(5)
ϕ_5	C(4)-C(5)-C(6)-O(6)
ϕ_6	C(5)-C(6)-O(6)-H(8)

[a]See Figure 1.

The conformational energy computations were carried out with a program *CONBIO* of Kang,[16] in which potential parameters were those described for ECEPP/2 potential[14] and some new parameters were added. The total conformational energy is the sum of the electrostatic, the nonbonded, and

---

* To whom correspondence should be addressed.

the torsional energies. The hydrogen bond energy is included in the nonbonded energy component. The partial atomic charges of the molecule, required for the evaluation of the electrostatic interaction, were determined by the molecular orbital CNDO/2 (ON) method[17] for the fully extended conformation as done in ECEPP/2 calculations.[18] The hydration shell model improved recently[15] was used to compute the hydration free energy of each conformation of the molecules in the hydrated state, where the hydration free energy was obtained as the sum of two contributions from water-accessible volume and polarization. A variable metric algorithm SUMSL[19] was used to minimize the conformational energy and free energy. All the torsion angles of the molecule were allowed to vary during minimization.

For AA, the 144 conformations were selected as starting points for energy minimization from the combination of six torsion angles defined in Figure 1 and Table 1. To the torsion angles ϕ_1, ϕ_2, and ϕ_6, 0 and 180° were assigned, the values of ± 60 and 180° were selected for the torsion angles ϕ_3 and ϕ_5, and the values of 120 and 300° for ϕ_4 were chosen. These six torsion angles were allowed to vary during energy minimization of AA in both the unhydrated and hydrated states. Each conformation obtained by minimization of the unhydrated molecule was used as a starting conformation for free energy minimization in the hydrated state.

At each minimum in the unhydrated and hydrated states, the conformational entropy was computed using a harmonic method.[20] 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°.[16b]

The relative total free energy in the hydrated state is given by $\Delta G_{tot} = \Delta G + \Delta\Delta G_{hyd}$, where ΔG is the relative conformational free energy (*i.e.*, $\Delta G = G - G^o$, where G^o is the free energy of the conformation of the lowest free energy), and $\Delta\Delta G_{hyd}$ is the relative hydration free energy (*i.e.*, $\Delta\Delta G_{hyd} = \Delta G_{hyd} - \Delta G_{hyd}{}^o$, where $\Delta G_{hyd}{}^o$ is the hydration free energy of the conformation of the lowest free energy). The relative conformational energy is given by $\Delta E = E - E^o$, where E^o is the conformational energy of the conformation of the lowest free energy. The relative entropic contribution to the relative free energy is given by $-T\Delta S$. And $\Delta G = \Delta E - T\Delta S$. Also the normalized statistical weight of each conformation was computed using an equation of Zimmerman *et al.*[21] 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 less than 1 kcal/mol) of AA 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 components ΔE_{es}, ΔE_{nb}, and ΔE_{tor} of ΔE. For each conformation, a six-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).

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

No.	Conf.[c]	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6
1	$tg^+tg^+g^-g^+$	180	55	171	69	−67	58
2	ttg^+ttt	179	141	74	172	−170	180
3	g^+tg^+ttt	60	141	74	172	−170	180
4	$g^-g^+tg^+g^-g^+$	−66	60	170	69	−67	58
5	$g^-g^-tg^+g^-g^+$	−65	−54	171	69	−67	57
6	$tg^+g^+g^+g^-t$	179	46	59	58	−69	−179
7	$tg^-g^+g^+g^-t$	−178	−52	64	54	−66	−179
8	tg^+tttt	−180	54	178	174	−175	178
9	$g^+g^+g^+g^+g^-t$	64	50	58	61	−70	180
10	tg^+g^+ttt	178	63	64	174	−173	−180
11	$g^-g^-g^+g^+g^-t$	−64	−56	64	54	−66	−179
12	tg^-tttt	−177	−50	179	175	−175	178
13	$g^+g^+g^+ttt$	63	68	65	174	−173	−180
14	$tg^+g^+g^-tg^+$	179	61	66	−65	−173	50
15	$tg^+tg^+g^+t$	179	54	−177	53	64	−180
16	$tg^+g^+g^-g^-t$	180	77	65	−64	−63	−179
17	g^-g^-tttt	−66	−54	178	174	−175	177
18	g^-g^+tttt	−68	58	177	174	−175	178
19	$tg^+tg^+g^-t$	180	54	−179	68	−71	−178
20	$ttg^+tg^+g^-$	179	136	81	−159	72	−58
21	$tg^-tg^+g^+t$	−179	−52	−177	52	64	180
22	$tg^+g^+g^-g^-g^-$	−179	56	61	−66	−72	−57
23	$tg^-tg^+tg^+$	−178	−50	−176	51	−175	51
24	$g^+g^+g^+g^-tg^+$	64	66	66	−66	−173	50
25	$g^+tg^+tg^+g^-$	60	136	81	−159	72	−58

[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 six torsion angles defined in Figure 1 and Table 1, *i.e.*, $0° \le g^+ < 120°$, $120° \le t \le 180°$ or $-180° \le t \le -120°$, and $-120° < g^- < 0°$.

Unhydrated L-Ascorbic Acid. From the 144 starting conformations of AA in the unhydrated state, we obtained the 111 different conformations after minimization and the only 90 conformations have the relative conformational free energy (ΔG) less than 3 kcal/mol. The 22 conformations of them with $\Delta G < 1$ kcal/mol seem to be the most probable conformations of AA in the unhydrated state, which are shown in Tables 2 and 3. However, there are no common characteristics torsion angles of these conformations.

The calculated thermodynamic quantities for the low free energy conformations of AA 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. Except the lowest free energy conformation $tg^+tg^+g^-g^+$ (1), the conformational entropic contribution $-T\Delta S$ is the major factor to stabilize the conformations in the unhydrated state, *e.g.*, though the conformations $g^-g^+tg^+g^-g^+$ (**4**) and $g^-g^-tg^+g^-g^+$ (**5**) have the lower conformational energies than those of the confromations ttg^+ttt (**2**) and g^+tg^+ttt (**3**), the latter have lower conformational free energies than the former due to the more negative contributions of $-T\Delta S$ to ΔG.

Table 3. Energetics of Low Free Energy Conformations of Unhydrated Neutral L-Ascorbic Acid^{a,b}

Conf.^{c}	ΔG^{d}	ω^{e}	ΔE^{f}	$-T\Delta S^{g}$	$\Delta E_{es}{}^{h}$	$\Delta E_{nb}{}^{i}$	$\Delta E_{tor}{}^{j}$
1	.00	.077	.00	.00	.00	.00	.00
2	.10	.065	.89	−.79	−.05	.27	.68
3	.11	.064	.89	−.78	.01	.20	.68
4	.37	.041	.38	−.02	.49	−.13	.02
5	.40	.040	.39	.00	.45	−.05	−.00
6	.48	.034	1.43	−.95	−.09	1.60	−.09
7	.54	.031	1.53	−.99	.02	1.68	−.17
8	.55	.030	1.86	−1.31	.31	1.78	−.22
9	.67	.025	1.63	−.95	.22	1.49	−.08
10	.69	.024	2.06	−1.36	.59	1.63	−.16
11	.70	.024	1.61	−.92	.24	1.57	−.19
12	.71	.023	2.08	−1.37	.49	1.80	−.20
13	.75	.022	2.15	−1.40	.75	1.52	1.49
14	.85	.018	2.18	−1.33	.68	1.60	−.10
15	.90	.017	2.01	−1.11	.02	2.21	−2.2
16	.90	.017	2.49	−1.59	.24	2.38	−.12
17	.93	.016	2.24	−1.32	.76	1.70	−.22
18	.93	.016	2.26	−1.33	.81	1.66	−.21
19	.97	.015	2.34	−1.37	.75	1.61	−.03
20	.98	.015	.59	.39	−.14	−.58	1.32
21	.99	.015	2.13	−1.14	.10	2.23	.78
22	.99	.014	2.04	−1.05	.18	1.91	−.04
23	1.01	.014	2.05	−1.04	.59	1.57	−.12
24	1.02	.014	2.37	−1.35	.95	1.50	−.08
25	1.02	.014	.63	.39	−.04	−.65	1.31

^{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 $tg^+tg^-g\ g^-$ ($\Delta G<1.0$ 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; $\Delta G=G\text{-}G^{o}=\Delta E\text{-}T\Delta S$, $G^{o}=1.518$ kcal/mol. ^{e}Normalized statistical weight. ^{f}Intramolecular interaction energy change; $\Delta E=E\text{-}E^{o}$; $E^{o}=E_{es}{}^{o}+E_{nb}{}^{o}+E_{tor}{}^{o}=0.454$ kcal/mol. ^{g}Conformational entropic contribution. ^{h}Electrostatic energy change;$\Delta E_{es}=E_{es}\text{-}E_{es}{}^{o}$, $E_{es}{}^{o}=5.749$ kcal/mol. ^{i}Nonbonded energy change; $\Delta E_{nb}=E_{nb}\text{-}E_{nb}E_{nb}{}^{o}$, $E_{nb}{}^{o}=-5.602$ kcal/mol. ^{j}Torsional energy change; $\Delta E_{tor}=E_{tor}\text{-}E_{tor}{}^{o}$, $E_{tor}{}^{o}=0.306$ kcal/mol.

Especially, the strong contributions of $-T\Delta S$ to ΔG can be found in the conformations $ttg^+tg^-g^-$ (**20**) and $g^+tg^+tg^+g^-$ (**25**), whose conformational energies are lower than those of the conformations **2** and **3**, and whose statistical weights are reduced significantly because of the positive value of $-T\Delta S$. The positive $-T\Delta S$ means that there are deeper potential surfaces around these energy minima than those of other energy minima.

Figure 2a shows a potential energy contour map of AA in the unhydrated state as a function of torsion angles ϕ_3 and ϕ_5, with other torsion angles fixed at those of the lowest energy minimum, *i.e.*, the conformation **1**. The conformational energy was calculated at intervals of 5° in torsion angles ϕ_3 and ϕ_5. Contours are drawn at intervals of 1 kcal/mol relative to the conformation **1**, which is the lowest energy (ΔE) conformation. The map shows that there is one deepest conformational energy minimum. This is caused by a strong hydrogen bond (HB) between O4 and H8 atoms (*i.e.*, R(O4···H8) = 1.81 Å) and a moderate HB between H5 and O6 atoms (*i.e.*, R(H5···O6) = 2.26 Å). The role of HBs in stabilization of AA will be discussed next in details.

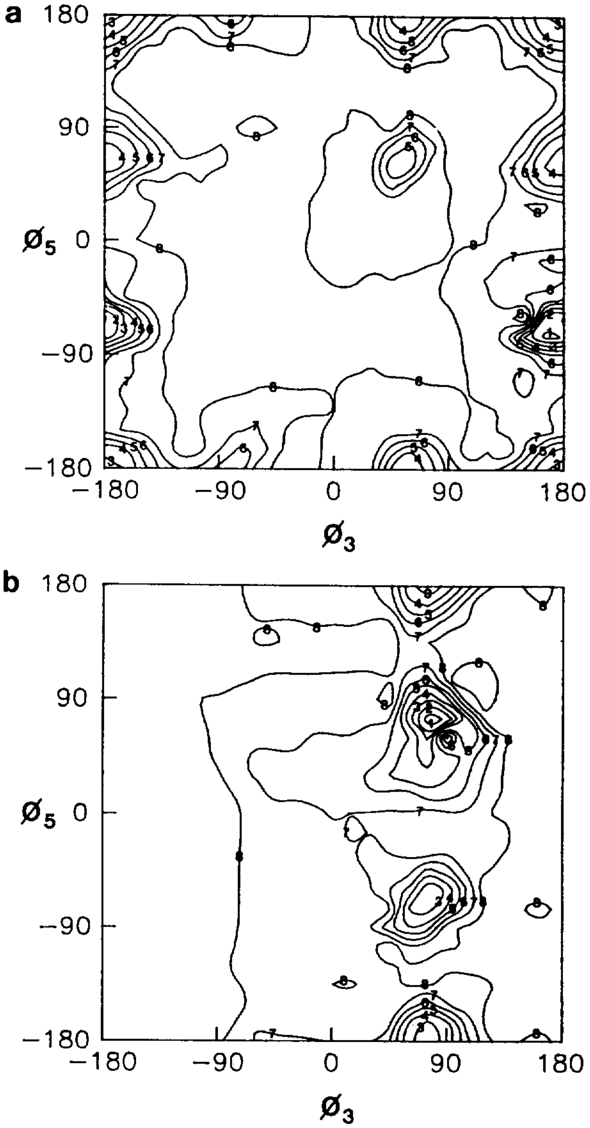

Figure 2. Potential energy contour maps of L-ascorbic acid (AA) as a function of torsion angles ϕ_3 and ϕ_5, with other torsion angles fixed at each lowest energy minimum. Contours are drawn at intervals of 1 kcal/mol: (a) the unhydrated AA relative to the conformation $tg^+tg^+g^-g^+$ and (b) the hydrated AA relative to the conformation $ttg^+tg^+g^-$.

The first 5 low free energy conformations of AA in the unhydrated state are drawn in Figure 3. There are interesting features in torsion angles ϕ_3 to ϕ_6, *i.e.*, the conformations **1**, **4**, and **5** are $tg^+g^-g^+$ for these torsion angles and have a strong intramolecular HB between O4 and H8 atoms with average R(O4···H8) = 1.80 Å and a moderate intramolecular HB between H5 and O6 atoms with R(H5···O6) = 2.26 Å, whereas the conformations **2** and **3** are g^+ttt and have a strong HB between H2 and O5 atoms (R(H2···O5) = 1.85 Å) and a moderate HB between H5 and O6 atoms (R(H5···O6) =

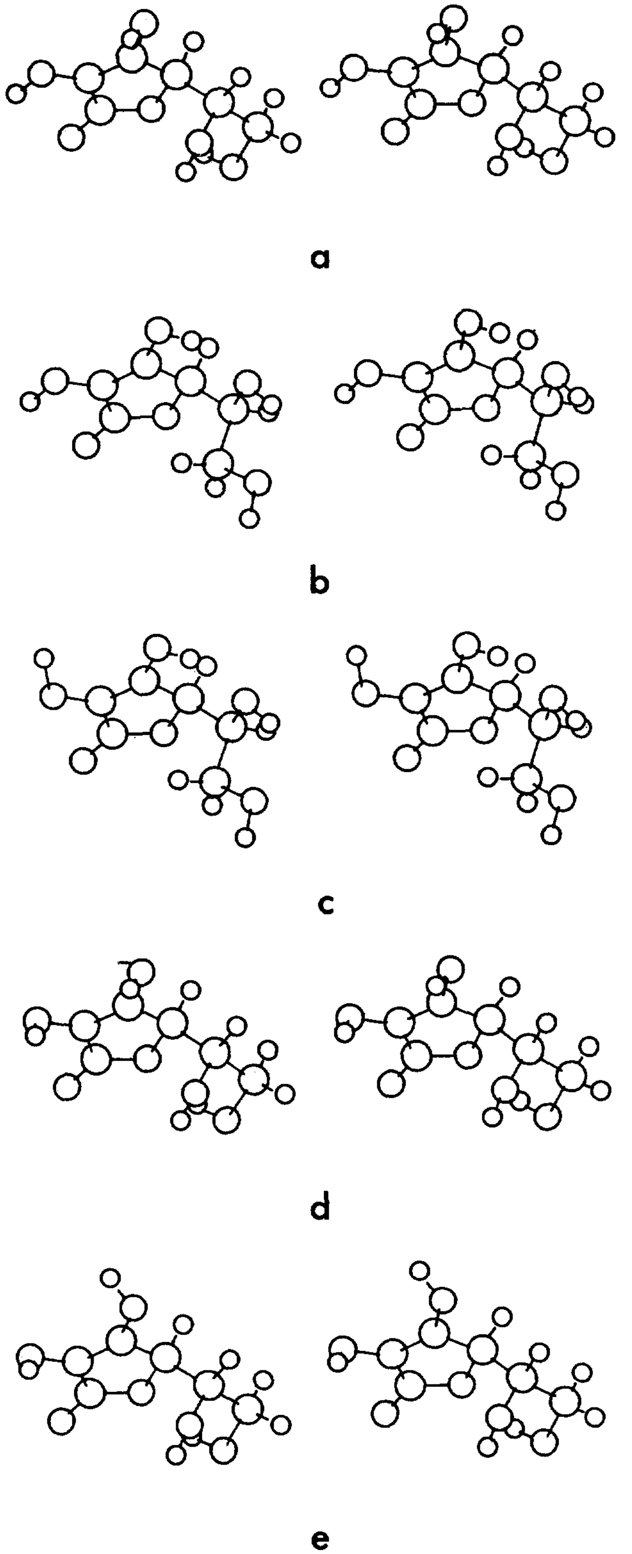

Figure 3. Stereoviews of the low free energy conformations of L-ascorbic acid (AA) in the unhydrated state: (a) $tg^+tg^+g^-g^+$, (b) ttg^+ttt, (c) g^+tg^+ttt, (d) $g^-g^+tg^+g^-g^+$, and (e) $g^-g^-tg^+g^-g^+$.

2.12 Å). The calculated results indicate that the conformational energies do not strongly depend on torsion angles ϕ_1 and ϕ_2, and that the two hydroxyl groups attached to the lactone ring are more flexible than other hydroxyl gorups.

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

No.	Conf.[c]	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6
1	$ttg^+g^-tg^+$	179	136	72	−90	−171	48
2	$g^+tg^+tg^+g^-$	60	136	81	−159	71	−58
3	ttg^+ttt	179	141	75	174	−172	179
4	$g^+tg^+g^-tg^+$	59	137	72	−90	−171	47
5	$ttg^+tg^+g^-$	179	137	81	−159	71	−57
6	g^-tg^+ttt	61	141	75	177	−173	177
7	$tg^-tg^+tg^+$	−178	−47	−173	51	−175	48
8	$g^+tg^+g^-g^+g^-$	60	131	79	−96	72	−56
9	$tg^-tg^-tg^+$	−178	−50	179	−79	−177	52
10	$g^-g^-tg^+tg^-$	−67	−55	−174	51	−175	49
11	$tg^-tg^+g^-g^+$	179	57	171	70	−67	56

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

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

Conf.[c]	ΔG_{tot}[d]	ω[e]	ΔE[f]	ΔG[g]	$-T\Delta S$[h]	$\Delta\Delta G_{hyd}$[i]	ΔE_{es}[j]	ΔE_{nb}[k]	ΔE_{tor}[l]
1	.00	.136	.00	.00	.00	.00	.00	.00	.00
2	.04	.127	−1.27	.22	1.49	−.18	−.53	−1.12	.37
3	.05	.125	−1.00	−.59	.40	.65	−.54	−.16	−.30
4	.06	.125	.08	.09	.01	−.04	.14	−.05	−.02
5	.07	.122	−1.31	.22	1.52	−1.6	−.63	−1.04	.36
6	.60	.049	−.97	.02	.98	.59	−.47	−.19	−.32
7	.87	.031	.18	.08	−.11	.80	.08	1.09	−.98
8	.91	.030	−.79	.97	1.75	−.07	−.31	−1.01	.53
9	1.22	.017	.52	.26	−.27	.97	.22	1.35	−1.04
10	1.36	.014	.34	.38	.04	.98	.39	1.00	−1.04
11	1.36	.014	−1.89	.00	1.89	1.37	−.48	−.42	−.99

[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; $\Delta G_{tot}=G_{tot}-G_{tot}^{\circ}$, $G_{tot}^{\circ}=1.183$ kcal/mol. [f]$E^{\circ}=2.353$ kcal/mol. [i]Hydration free energy of each conformation; $\Delta\Delta G_{hyd}=\Delta G_{hyd}-\Delta G_{hyd}^{\circ}$, $\Delta G_{hyd}^{\circ}=-22.736$ kcal/mol. [j]$E_{es}^{\circ}=6.224$ kcal/mol. [k]$E_{nb}^{\circ}=-5.140$ kcal/mol. [l]$E_{tor}^{\circ}=1.268$ kcal/mol.

The diverse of torsion angles ϕ_2 and ϕ_3 shows that there is a strong correlation between the two torsion angles. The relative values of $-T\Delta S$ indicate that the HB between H2 and O5 atoms allows the molecule to be more flexible than the HB between O4 and H8 atoms (see Table 3). These intramolecular HBs give the overall conformations to be folded. Therefore, these low free energy conformations quite differ from those of crystal.[12] in which *all* hydroxyl groups are involved in *intermolecular* HBs and so the overall crystal conformations are nearly *all trans*.

Hydrated L-Ascorbic Acid. In the hydrated state, the only 58 conformations of AA obtained from the 144 minimized conformations in the unhydrated state were found to have the relative total free energy (ΔG_{tot}, a sum of ΔG and $\Delta\Delta G_{hyd}$) less than 3 kcal/mol. The 8 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 may show

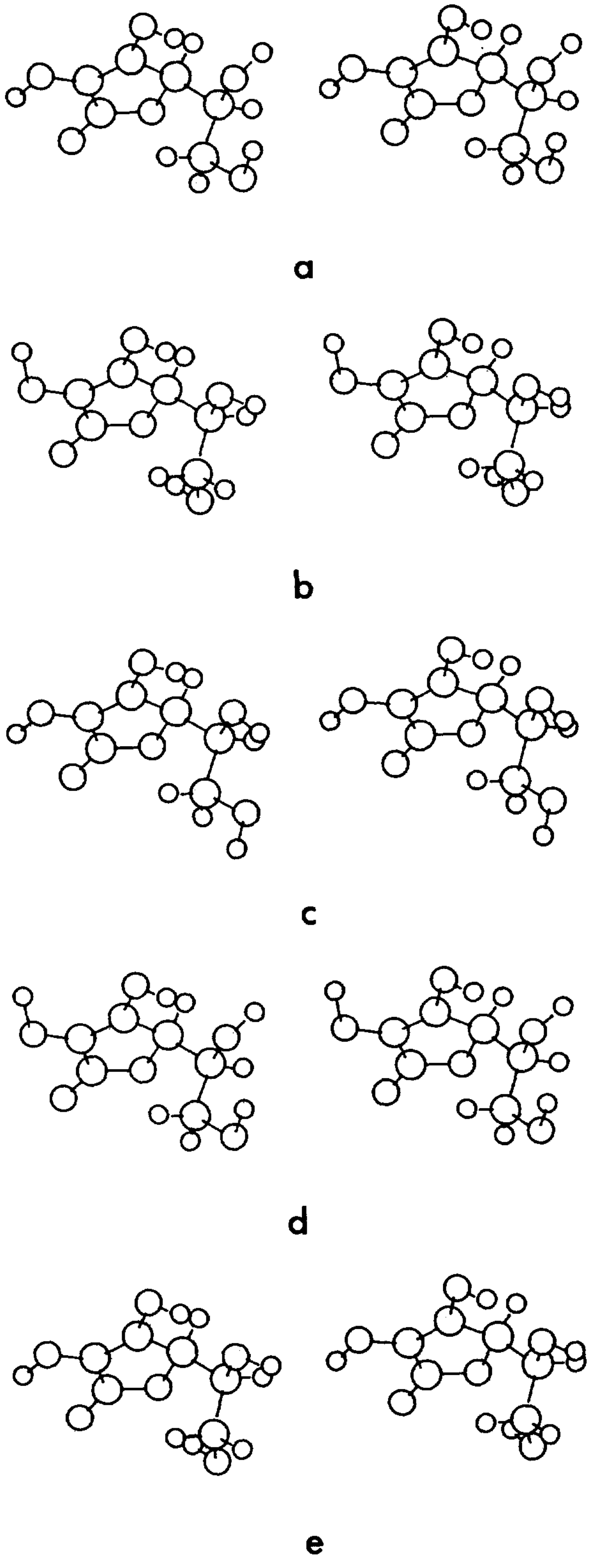

Figure 4. Stereoviews of the low free energy conformations of L-ascorbic acid (AA) in the hydrated state: (a) $ttg^+g^-tg^+$, (b) $g^+tg^+tg^+g^-$, (c) ttg^+ttt, (d) $g^+tg^+g^-tg^+$, and (e) $ttg^+tg^+g^-$.

the significance of the hydration effect on the conformations of AA in the hydrated state. The anlaysis of energetics listed in Table 5 tells us that the hydration does not directly affect the hydration free energy ($\Delta\Delta G_{hyd}$) of each conformation, but contributes to alter the potential surface around each free energy minimum (*i.e.*, $-T\Delta S$). It is interesting that in general, the value of $-T\Delta S$ of each confromation relative to that of the lowest free energy conformation contributes to the total free energy (ΔG_{tot}) *positively* in the hydrated state, whereas *negatively* in the unhydrated state (see Tables 3 and 5). As found in the unhydrated state, there are no common characteristics in torsion angles of low free energy conformations.

Figure 2b shows a potential free energy contour map of AA in the hydrated state as a function of torsion angles ϕ_3 and ϕ_5, with other torsion angles fixed at those of the lowest free energy minimum, *i.e.*, the conformation $ttg^+tg^+g^-$ (**5**). 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_3 and ϕ_5. Contours are drawn at intervals of 1 kcal/mol relative to the conformation **5**. The map shows that local energy minima are localized along ϕ_3 equal to g^+, while those in the unhydrated state are dispersed at ϕ_3 equal to t and g^+ (cf. Figure 2a).

The first 5 low free energy conformations of AA in the hydrated state are drawn in Figure 4. As found in the case of unhydrated state, there are interesting correlations between torsion angles and intramolecular HBs, *i.e.*, all the 5 conformations have a common HB between H2 and O5 atoms (the R(H2···O5) varies from 1.82 to 1.88 Å) and this HB is brought by torsion angles of ϕ_2 and ϕ_3 equal to t and g^+, respectively. According to torsion angles ϕ_4 to ϕ_6, these conformations have another different HB, *i.e.*, the conformations **1** and **4** have a HB between O5 and H8 atoms (R(O5···H8)=2.14 and 2.13 Å, respectively) with torsion angles ϕ_4, ϕ_5, and ϕ_6 to be g^-, t, and g^+, respectively, whereas the conformations **2** and **5** have a HB between O4 and H8 atoms (R(O4···H8)=1.78 Å for both) with torsion angles ϕ_4, ϕ_5, and ϕ_6 equal to t, g^+, and g^-, respectively. The conformation **3** has a HB between H5 and O6 atoms (R(H5···O6)=2.15 Å) with torsion angles ϕ_4, ϕ_5, and ϕ_6 to be all t. The calculated results indicate that the contribution from the conformational entropy is essential in stabilizing the conformation **1** (needless to say, it seems to be caused by the hydration as mentioned previously), though the strength of HB between O5 and H8 atoms is weaker than that between O4 and H8 atoms or between H5 and O6 atoms (see Table 5). The flexibility of the hydroxyl group attached to C2 atom of the lactone ring is also found as the same as in the unhydrated state. Intramolecular HBs force the overall conformations to be folded, though different from those in the unhydrated state. Hence, our calculated conformations in the hydrated state quite differ from those of crystal.[12]

Hydration free energy of each group of low free energy conformations of AA is listed in Table 6. The total hydration free energy of each group is expressed in terms of two components coming from water-accessible volume and polarization (see ref. 15(b) for details). From comparing the conformational energies (ΔE) and hydration free energies (ΔG_{hyd}) of the lowest free energy confromations in the hydrated and unhydrated states (*i.e.*, the conformations **1** and **11** in Table 5), it is found that there is a competition between intramolecular HBs and hydration. The conformations **11** can form more stable HBs than the conformation **1**, but the hydration

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

Corf.[d]	Lactone ring[e]			CHOH[f]			CH_2OH[g]		
	$\Delta\Delta G_h$	$\Delta\Delta G_p$	$\Delta\Delta G_{hyd}$	$\Delta\Delta G_h$	$\Delta\Delta G_p$	$\Delta\Delta G_{hyd}$	$\Delta\Delta G_h$	$\Delta\Delta G_p$	$\Delta\Delta G_{hyd}$
1	.00	.00	.00	.00	.00	.00	.00	.00	.00
2	.05	−.29	−.24	−.06	−.01	−.08	.25	−.11	.13
3	−.06	−.07	−.13	−.02	.43	.41	−.06	.42	.36
4	.00	−.05	−.03	.00	−.01	−.00	.00	.00	.00
5	.05	−.26	−.21	−.07	−.01	−.08	.25	−.12	.13
6	−.06	−.10	−.16	−.02	.42	.41	−.07	.41	.34
7	−.13	.26	.12	.21	.45	.66	−.11	.13	.00
8	.08	−.25	−.17	−.06	.04	−.02	.24	−.12	.12
9	−.07	.31	.24	.19	.49	.68	−.11	.15	.03
10	−.10	.43	.33	.23	.40	.63	−.11	.13	.00
11	.06	.00	.06	.21	.55	.77	.24	.29	.53

[a] Energies are in kcal/mol. [b] Relative energies to the conformation **1** ($ttg^+g^- tg^+$). [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. 15 for details. [d] The number of each conformation is the same as that of Table 4. [e] $\Delta G_h^o = -14.60$ kcal/mol, $\Delta G_p^o = 0.05$ kcal/mol, and $\Delta G_{hyd}^o = -14.55$ kcal/mol. [f] $\Delta G_p^o = -4.27$. kcal/mol, $\Delta G_p^o = -0.13$ kcal/mol, and $\Delta G_{hyd}^o = -4.40$ kcal/mol. [g] $\Delta G_h^o = -4.18$ kcal/mol, $\Delta G_p^o = 0.40$ kcal/mol and $\Delta G_{hyd}^o = -3.78$ kcal/mol.

around the two hydroxyl groups attached to the acyclic side chains forces the molecule to form less stable HBs, *i.e.*, stronger HBs in the unhydrated state between O4 and H8 atoms and between H5 and O6 atoms are switched to weaker HBs between H2 and O5 atoms and between O5 and H8 atoms (see 1st and 11th rows in Table 6).

Several feasible conformations obtained from conformational free energy calculation in the hydrated state indicate that there exists an ensemble of several conformations in aqueous solution, rather than a single dominant conformation.

Guilleme *et al.*[13] reported the populations for rotamers about the C4-C5 and C5-C6 bonds of the acyclic side chain of AA determined by NMR experiments. They determined the populations from vicinal H-H and C-H coupling constants without considering the orientation of a hydroxyl group attached to C3 atom of the lactone ring. So their feasible conformations of torsion angles ϕ_3 for the C4-C5 bond can not be compared directly with our low free energy conformations, because there is a strong correlation between the adjacent torsion angles ϕ_2 and ϕ_3 (see Table 4). Their results show that dominant conformations for the rotation about the C5-C6 band are t and g^+, which are in good agreement with our calculated low free energy conformations for torsion angles ϕ_5 (see Table 4).

Conclusions

From the analysis of conformational free energies for L-ascorbic acid (AA) in the unhydrated and hydrated states, intramolecular hydrogen bonds (HBs) are proved to be an essential factor in stabilizing the overall conformations in both states. These HBs cause the conformations in both states to be quite different from those in crystal.

In the case hydrated AA, there is a competition between HBs and hydration, and the hydration around the two hydroxyl groups attached to the acyclic side chain forces the molecule to form less stable HBs. Several feasible conformations obtained in this work indicate that there exists an ensemble of several conformations in aqueous solution. The calculated probable conformations for the rotation about the C5-C6 bond of the acyclic side chain are t and g^+, which are in good agreement with results of NMR experiment.

The results obtained from conformational studies of AA in both states in this work may bee helpful in understanding its structure and biological functions.

References

1. A. Szent-Györgyi, *Biochem. J.*, **22**, 1387 (1929).
2. M. J. Barnes and E. Kodicek, in *Vitamins and Hormones*, Vol. 30, Academic, New York, p. 1, 1972.
3. E. Ginter, P. Bobek, J. Babala, F. Kubec, D. Urbanova, and O. Černa, *Adv. Physiol. Sci.*, **12**, 79 (1981).
4. F. Smith, in *The Vitamins*, Vol. 1, W. H. Sebrell, Jr. and R. S. Harris, eds., Academic, New York, p. 241, 1954.
5. V. Herbert and E. Jacob, *J. Am. Med. Assoc.*, **230**, 241 (1974).
6. E. V. Shtamm, A. Purmalis, and Y. Shurlatov, *Zh. Fiz. Khim.*, **48**, 2233 (1974).
7. M. Archer, S. Tannenbaum, T. Fan, and M. Weisman, *J. Natl. Cancer Inst.*, **54**, 1203 (1975).
8. E. Flood and P. N. Skancke, *Acta Chem. Scand.*, **27**, 3069 (1973).
9. C. Thomson, *J. Mol. Struct.*, **67**, 133 (1980).
10. C. L. Carlsson, H. Lable, and L. C. Pedersen, *Chem. Phys. Lett.*, **38**, 75 (1976).
11. (a) P. Bischof, M. Eckert-Maksić, and Z. B. Maksić, *Z. Naturforsch., A*, **36**, 502 (1981); (b) M. Eckert-Maksić, Z. B. Maksić, and P. Bischof, in *Modeling of Structure and Properties of Molecules*, Z. B. Maksić, ed., Ellis Horwood, Chichester, p. 315, 1987.
12. J. Hvoslef, *Acta Crystallogr., Sec. B*, **24**, 23 (1968).
13. J. Guilleme, E. Diez, M. M. Gomez, M. Secundino, and L. Garrigos, *J. Mol. Struct.*, **142**, 387 (1986).

14. (a) G. Némethy, M. S. Pottle, and H. A. Scheraga, *J. Phys. Chem.*, **87**, 1883 (1983); (b) M. J. Sippl, G. Némethy, and H. A. Scheraga, *ibid.*, **88**, 6231 (1984).
15. (a) Y. K. Kang, G. Némethy, and H. A. Scheraga, *J. Phys. Chem.*, **91**, 4015 (1987), *erratum, ibid.*, **92**, 1382 (1988); (b) *ibid.*, **91**, 4109 (1987); (c) *ibid.*, **91**, 4118 (1987); (d) Y. K. Kang, K. D. Gibson, G. Némethy, and H. A. Scheraga, *ibid.*, **92**, 4739 (1988).
16. (a) Y. K. Kang, *Conformational Analysis Programs for Biological Molecules (CONBIO)*, KRICT Research Report (1988); (b) Y. K. Kang and D. W. Kim, *Bull. Korean Chem. Soc.*, **11**, 144 (1990); (c) B. S. Kim, S. H. Lee, U. T. Chung, and Y. K. Kang, *Yakhak Hoeji*, **33**, 350 (1989); (d) Y. K. Kang, *Korean Biochem. News*, **10**(1), 17 (1990); (e) S. H. Lee, U. T. Chung, and Y. K. Kang, *Arch. Pharm. Res.*, **13**, 43 (1990).
17. Y. F. Yan, F. A. Momany, and H. A. Scheraga, *J. Phys. Chem.*, **74**, 420 (1970).
18. F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).
19. D. M. Gay, *ACM Trans. Math. Software*, **9**, 503 (1983).
20. (a) N. Gō and H. A. Scheraga, *J. Chem. Phys.*, **51**, 4751 (1969); (b) *Macromolecules*, **9**, 535 (1976).
21. S. S. Zimmerman, M. S. Pottle, G. Némethy, and H. A. Scheraga, *Macromolecules*, **10**, 1 (1977).

Stability of the Pentagon Structure of Water Cluster

Byoung Jip Yoon and Mu Shik Jhon*

Department of Chemistry, Kangreung National University, Kangreung 210-702
**Department of Chemistry, and Center for Molecular Science, Korea Advanced Institute of Science and Technology, Seoul 136-791.* *Received August 27, 1990*

A hexagonal hexamer of water cluster is optimized by *ab initio* method using the 4-31G basis set. At this geometry the nonadditive many-body interactions are calculated. The *ab initio* calculation with large basis set [T. H. Dunning, *J. Chem. Phys.*, **53**, 2823 (1970); **54**, 3958 (1971)] shows that a pentagonal unit is rather stable among several kinds of clustering units of water molecules.

Introduction

Due to the characteristic bent <HOH bond angle and the strong hydrogen bonds (H-bonds), water molecules form various shapes of clusters. The binding energy of H-bonds per molecule increases with the increase of the cluster size. Any structure of unique large water cluster has not been experimentally found in the liquid state of water. However the anomalous properties of water have been explained theoretically assuming small water clusters, especially in order to explain the properties related to the volume of liquid water in the mixture models[1,2]. Among the clusters, five-membered ring structures are proposed as high density species that would have a small molar volume[2]. Molecular dynamics studies indicate pentagon units[3,4]. Speedy and Mezei[5] also considered that the anomalies of water may be related to the self-replicating propensity of pentagons. In the results of Speedy *et al.*, the concentrations of five-membered rings increase as the temperature decreases. On the other hand, we proposed that the concentration-ratio of five- to six-membered rings (as, maybe, fractures of low density ice having large molar volume) increases with the increase of temperature. According to the ring-analysis from the results of molecular dynamic simulation of Belch and Rice[6], the concentration of five-membered ring decreases as the temperature increases, but the rate of decrease is much less than that reported by Speedy *et al.* (only a factor of 1.1 as the temperature drops from 313 to 273 K). However their analysis supports that the ratio of five- to six-membered ring increases with temperature rise. Besides the five- and six-membered rings in the simulation of Belch *et al.*, the concentrations of seven- and eight-membered rings are also high compared with those of six- and five-membered rings. The conformations of local minima of water clusters optimized with two-body plus three- and four- body interactions have many four-membered ring structures which have more H-bonded OH-bonds.[7] In the molecular dynamics simulation of Speedy *et al.*, the total number of heptagon is the largest, but the pentagon and hexagon are dominant as "primitive" polygons[8]. However the result does not show any regular pattern of the temperature dependancy of the ratio of pentagon to hexagon. A different analysis was done by Geiger and Stanley[9] from the results of molecular dynamic simulations of Stillinger and Rahman[10] using ST2 potential. According to their analysis, the hydrogen-bonded network includes tiny spatially correlated patches of four-bonded molecules, and the local density near a patch is lower than the global density. The four-bonded patches are essentially tetrahedraly bonded and do not like a ring shape. The hydrogen-bonded structures depend very much on the potential functions. The structure and stability of water pentamers were also investigated using molecular dynamics of small clusters[11].

It is, however, difficult to determine the shapes and binding energies of clusters in the liquid state, and thus calcula-