

Effects of Microsolvation on the Stability of Zwitterionic Valine

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We present calculations for valine (Val) – (H₂O)_n (n = 0-5) to examine the effects of microsolvating water on the relative stability of the zwitterionic vs. canonical forms of Val. We calculate the structures, energies and Gibbs free energies of the conformers at B3LYP/6-311++G(d,p), wB97XD/6-311++G(d,p) and MP2/aug-cc-pvdz level of theory. We find that five water molecules are needed to stabilize the zwitterionic form of Val. By calculating the barriers of the canonical ↔ zwitterionic pathways of Val – (H₂O)₅ conformers, we suggest that both forms of Val – (H₂O)₅ may be observed in low temperature gas phase

Key Words : Valine, Microsolvation, DFT

Introduction

Effects of microsolvation on the structures and reactivity of amino acids, both canonical¹⁻¹² and zwitterionic^{10,13-20} have been under intensive study to address the intriguing question of how many water molecules may stabilize the zwitterionic form of amino acids. Both theoretical and experimental studies carried out to examine the relative stability of the canonical and zwitterionic forms as a function of the number of microsolvating water molecules indicated that the effects of microsolvating water molecules depend highly on the structure of amino acids.¹⁻²² Although most amino acids require 4-5 water to stabilize their zwitterionic conformers molecules,^{1,21,22} those with strongly basic sidechains such as arginine^{8(h)} and lysine⁸⁽ⁱ⁾ may need fewer (less than three) water molecules.

Valine²³⁻²⁶ (Val) is at the opposite side to these latter amino acids in the effects of sidechain. The “isopropyl” sidechain is highly nonpolar, and is neither acidic nor basic. This structural feature of Val allows it to perform some characteristic role as a nonpolar site in the structures and functions of some enzymes such as choline oxidase,²⁷ human muscle creatine kinase,²⁸ and in insulin.²⁹

Because of the hydrophobic nature of the sidechain, the influence of microsolvating water on the stability of the zwitterion of Val is expected to be sluggish. In the present work we examine the Val – (H₂O)_n (n = 0-5) clusters, predicting that zwitterionic Val becomes quasidegenerate with the canonical forms due to the solvating effects of five water molecules. We examine the canonical ↔ zwitterion pathways to examine whether the two lowest energy zwitterionic and canonical conformers of Val – (H₂O)₅ may coexist and be observed in gas phase at low temperature. Since each of the two forms is kinetically correlated with other higher energy conformers, we suggest that both of them may be observed in jet-cooled low temperature gas phase.

Computational Method

We employ the density functional theory (B3LYP^{30,31} and

wB97XD³²) with the 6-311++G* and the MP2/aug-cc-pvdz method, as implemented in the GAUSSIAN 09 set of programs.³³ Structures of Val – water clusters are calculated by employing Monte Carlo technique over extensive configuration space. We find that various initial configurations lead to water molecule bridging the two functional groups, as described below. Stationary structures are confirmed by ascertaining that all the harmonic frequencies are real. Structure of the transition state is obtained by verifying that one and only one of the harmonic frequencies is imaginary, and also by carrying out the intrinsic reaction coordinate analysis along the reaction pathways. Zero point energies (ZPE) are taken into account, and default criteria are used for all optimizations.

Results and Discussion

Val – (H₂O)_n (n = 0-4). Table 1 lists the energies and Gibbs free energies of Val and Val – water clusters studied in this work. Figures 1-6 present the calculated structures and relative Gibbs free energies of zwitterionic and canonical conformers of Val – (H₂O)_n (n = 0-5). We only present the canonical conformers within 3 kcal/mol of the lowest energy structure, whereas the higher lying conformers are given in Supplementary Information. We could not obtain any stationary zwitterionic conformers of Val. The energies of the calculated canonical structures of Val are quite close to one another, with the lowest four conformers lying within 1 kcal/mol. Figure 2 depicts the calculated conformers of Val – (H₂O)₁. Again, the four lowest energy canonical conformers lie within ~1 kcal/mol, with the water molecule bridging the carboxyl group. In some of higher energy conformers ((ca-1-6) and (ca-1-8)), the microsolvating water molecule bridges the –OH and –NH₂ groups. We may find two zwitterionic conformers ((zw-1-1) and (zw-1-2)) of Val – (H₂O)₁, but their Gibbs free energies were quite high (13-15 kcal/mol) relative to lowest energy canonical form. We also find similar structural features for lowest energy canonical structures of Val – (H₂O)₂ depicted in Figure 3. The Gibbs free energy of

Table 1. Energy (hartree), ZPE (kcal/mol), Gibbs free energy G (hartree) at 5 K, and relative Gibbs free energy ΔG (kcal/mol) of Val – (H₂O)_n (n = 0-5) (B3LYP/6-311++G(d,p))

	Conformer	E	ZPE	G _{5K}	ΔG_{5K}
Val	(canonical)				
	ca-1	-402.50368	102.8	-402.34003	0.00
	ca-2	-402.50426	103.3	-402.33992	0.07
	ca-3	-402.50295	102.9	-402.33920	0.52
	ca-4	-402.50218	102.8	-402.33852	0.95
	ca-5	-402.50257	103.3	-402.33821	1.15
	ca-6	-402.50159	102.9	-402.33776	1.43
	ca-7	-402.50153	103.2	-402.33736	1.68
Val-(H ₂ O) ₁	(canonical)				
	ca-1-1	-478.97858	118.6	-478.78985	0.00
	ca-1-2	-478.97785	118.5	-478.78917	0.43
	ca-1-3	-478.97737	118.6	-478.78860	0.79
	ca-1-4	-478.97695	118.6	-478.78816	1.06
	ca-1-5	-478.97384	118.4	-478.78545	2.77
	ca-1-6	-478.97265	118.4	-478.78417	3.57
	ca-1-7	-478.97214	118.1	-478.78416	3.57
Val-(H ₂ O) ₁	(zwitterionic)				
	zw-1-1	-478.95619	118.3	-478.76797	13.73
Val-(H ₂ O) ₂	(canonical)				
	ca-2-1	-555.45561	134.2	-555.24200	0.00
	ca-2-2	-555.45584	134.4	-555.24192	0.05
	ca-2-3	-555.45537	134.4	-555.24146	0.34
	ca-2-4	-555.45419	134.3	-555.24035	1.04
	ca-2-5	-555.44850	134.1	-555.23506	4.36
	ca-2-6	-555.44860	134.2	-555.23497	4.41
	ca-2-7	-555.44786	134.2	-555.23419	4.90
Val-(H ₂ O) ₂	(zwitterionic)				
	zw-2-1	-555.43985	134.6	-555.22559	10.30
	zw-2-2	-555.43818	134.6	-555.22397	11.31
	zw-2-3	-555.43822	134.7	-555.22381	11.42
Val-(H ₂ O) ₃	(canonical)				
	ca-3-1	-631.93063	149.9	-631.69202	0.00
	ca-3-2	-631.92880	150.0	-631.69009	1.21
	ca-3-3	-631.92836	150.3	-631.68902	1.88
	ca-3-4	-631.92613	150.2	-631.68699	3.16
	ca-3-5	-631.92575	150.0	-631.68694	3.19
	ca-3-6	-631.92505	149.7	-631.68678	3.29
	ca-3-7	-631.92636	150.7	-631.68646	3.49
	ca-3-8	-631.92417	150.0	-631.68537	4.18
	ca-3-9	-631.92425	150.1	-631.68534	4.20
Val-(H ₂ O) ₃	(zwitterionic)				
	zw-3-1	-631.91984	150.4	-631.68044	7.27
	zw-3-2	-631.92044	151.1	-631.67996	7.57
	zw-3-3	-631.91855	150.3	-631.67922	8.03
	zw-3-4	-631.91948	151.1	-631.67891	8.23
	zw-3-5	-631.91858	150.6	-631.67880	8.30
Val-(H ₂ O) ₃	(zwitterionic)				
zw-3-6	-631.91946	151.4	-631.67849	8.49	

Table 1. Continued

	Conformer	E	ZPE	G _{5K}	ΔG _{5K}
Val-(H ₂ O) ₄	(canonical)				
	ca-4-1	-708.40610	166.4	-708.14114	0.00
	ca-4-2	-708.40318	165.5	-708.13974	0.88
	ca-4-3	-708.40197	165.5	-708.13850	1.66
	ca-4-4	-708.40272	166.4	-708.13785	2.06
	ca-4-5	-708.40032	165.5	-708.13688	2.67
	ca-4-6	-708.40038	165.6	-708.13676	2.75
	ca-4-7	-708.40083	166.0	-708.13662	2.84
	ca-4-8	-708.39983	165.4	-708.13645	2.95
	ca-4-9	-708.40001	165.6	-708.13644	2.95
ca-4-10	-708.39940	165.2	-708.13639	2.98	
Val-(H ₂ O) ₄	(zwitterionic)				
	zw-4-1	-708.39817	167.1	-708.13218	5.63
	zw-4-2	-708.39657	167.2	-708.13035	6.77
	zw-4-3	-708.39544	166.6	-708.13026	6.83
	zw-4-4	-708.39376	165.6	-708.13016	6.89
	zw-4-5	-708.39545	166.8	-708.12996	7.01
	zw-4-6	-708.39374	166.1	-708.12936	7.39
	zw-4-7	-708.39409	166.6	-708.12887	7.70
Val-(H ₂ O) ₅	(canonical)				
	ca-5-1	-784.88204	182.3	-784.59182	0.00 (0.00) ^a (0.00) ^b
	ca-5-2	-784.88110	182.8	-784.59012	1.07
	ca-5-3	-784.87926	181.7	-784.58995	1.17 (0.65) ^a
	ca-5-4	-784.88083	183.1	-784.58934	1.56
	ca-5-5	-784.87714	181.3	-784.58851	2.08
	ca-5-6	-784.87947	182.8	-784.58849	2.09
	ca-5-7	-784.88022	183.2	-784.58847	2.10
	ca-5-8	-784.87811	182.0	-784.58841	2.14
	ca-5-9	-784.87958	182.9	-784.58836	2.17
	ca-5-10	-784.87954	182.9	-784.58828	2.22
	ca-5-a	-784.87393	183.2	-784.58223	6.02 (2.03) ^a
	(zwitterionic)				
	zw-5-1	-784.88060	184.6	-784.58676	3.17 (-1.35) ^a (-1.48) ^b
	zw-5-2	-784.87998	184.2	-784.58671	3.21
	zw-5-3	-784.87889	183.8	-784.58626	3.48
	zw-5-4	-784.87735	183.2	-784.58573	3.82
	zw-5-5	-784.87570	182.7	-784.58481	4.40 (4.01) ^a
zw-5-6	-784.87579	183.2	-784.58419	4.79	
zw-5-7	-784.87317	182.1	-784.58331	5.34	
zw-5-8	-784.87532	183.5	-784.58323	5.39	

^awB97XD/6-311++G(d,p). ^bMP2/aug-cc-pvdz

the most stable zwitterionic form of Val-(H₂O)₂ is ~10 kcal/mol above the lowest energy canonical conformer (ca-2-1).

When more water molecules are allowed to interact with Val, the difference in Gibbs free energies of Val-water clusters seem to increase, as illustrated in Figure 4 and Figure 5 for Val-(H₂O)₃ and Val-(H₂O)₄. Since the Gibbs free energy of one or two canonical conformers is lower by more than 1 kcal/mol than others, it may be much easier to experimentally discern and to assign these conformers at low temperature gas phase. On the other hand, the zwitterionic forms of Val-(H₂O)₃ and Val-(H₂O)₄ are still not stable, because their Gibbs free energies are 5-7 kcal/mol higher than the canonical conformers.

Val-(H₂O)₅. Table 1 indicates that the Gibbs free energies of zwitterionic conformers of Val-(H₂O)₅ become quite close to those of canonical forms, to within ~3 kcal/mol at B3LYP/6-311++G(d,p) level of theory. Because our past experience indicates that the energy gap between these two forms of amino acids tends to widen by using this method, we also employ the MP2/aug-cc-pvdz and wB97XD/6-311++G(d,p) methods that are known to better treat non-covalent weak (such as Van der Waals) interactions. We find that the lowest energy zwitterionic Val-(H₂O)₅ lies slightly lower (by 1.48 [1.35] kcal/mol) than the lowest energy canonical Val-(H₂O)₅ by MP2/aug-cc-pvdz [wB97XD/6-311++G(d,p)] method. This indicates that the two forms of Val-(H₂O)₅ are essentially quasidegenerate. The structures

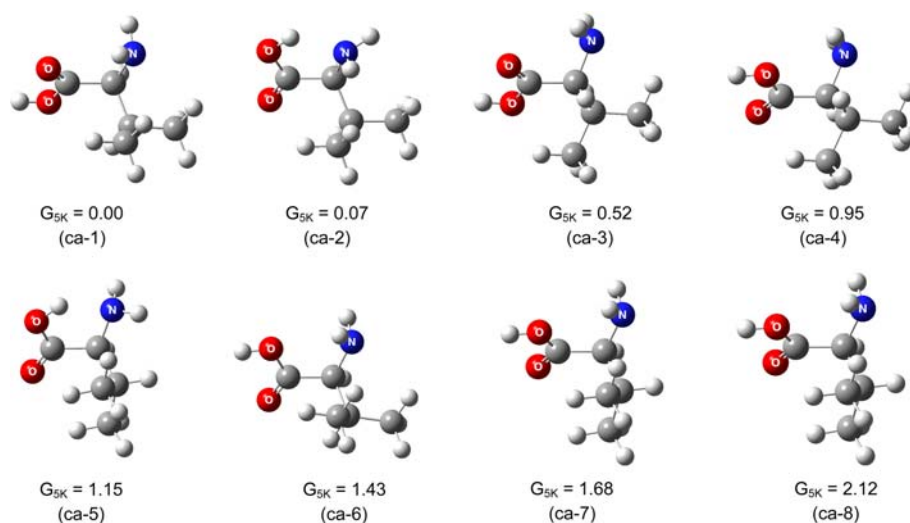


Figure 1. Structures of the lowest energy conformers of Val (relative Gibbs free energy at 5 K in kcal/mol).

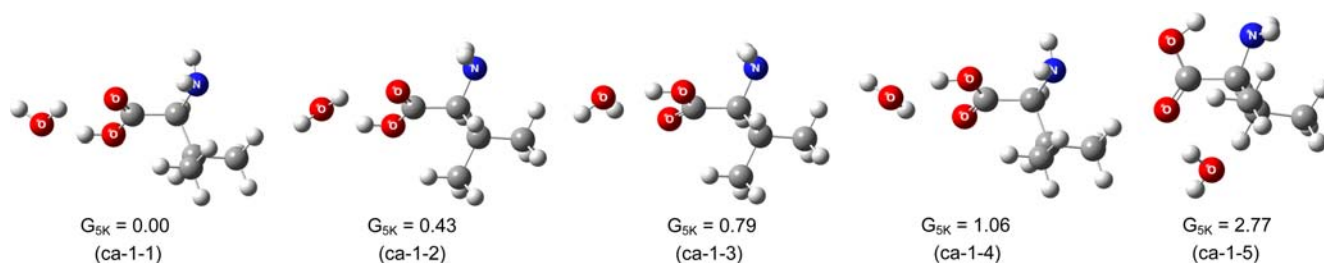


Figure 2. Structures of the lowest energy conformers of Val - (H₂O)₁ (relative Gibbs free energy at 5 K in kcal/mol).

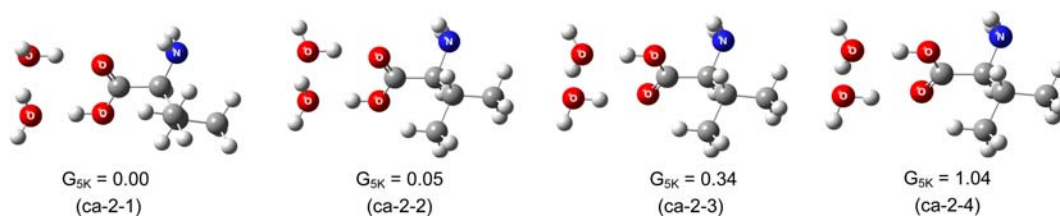


Figure 3. Structures of the lowest energy conformers of Val - (H₂O)₂ (relative Gibbs free energy at 5 K in kcal/mol).

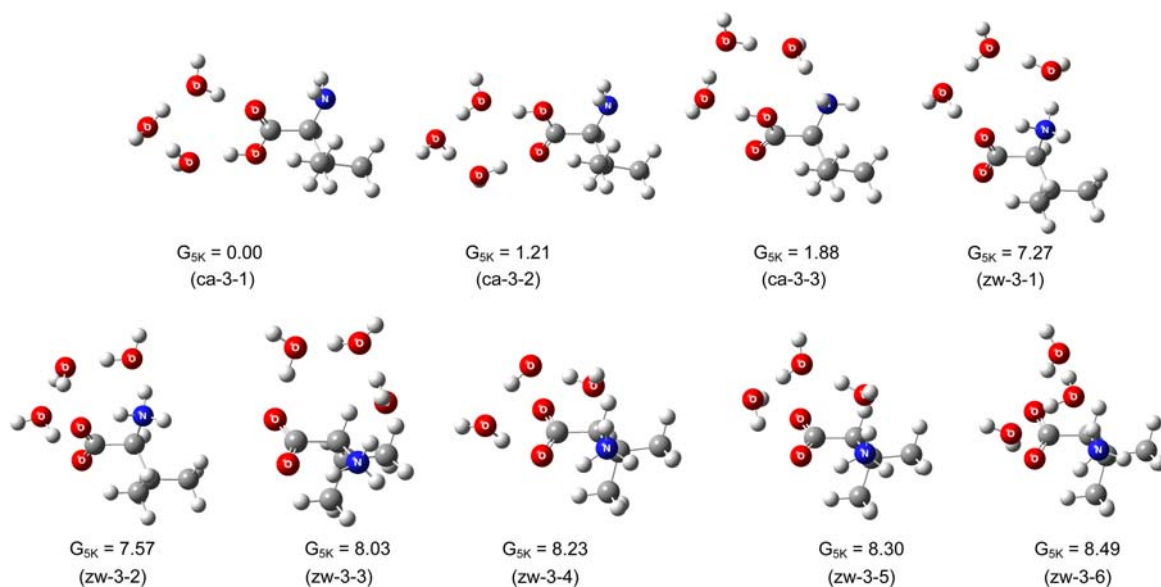


Figure 4. Structures of the lowest energy conformers of Val - (H₂O)₃ (relative Gibbs free energy at 5 K in kcal/mol).

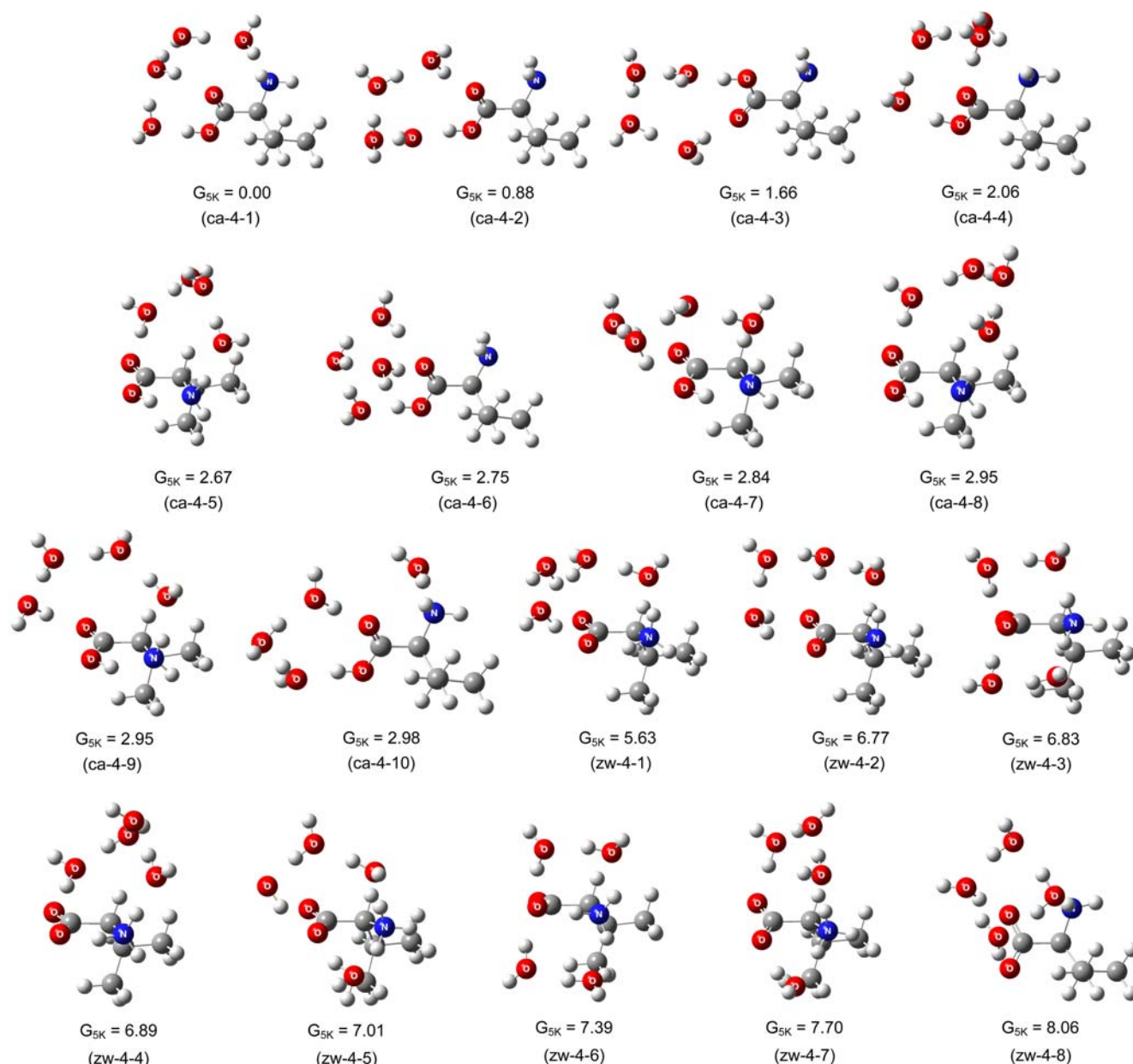


Figure 5. Structures of the lowest energy conformers of Val - (H₂O)₄ (relative Gibbs free energy at 5 K in kcal/mol).

of Val - (H₂O)₅ are depicted in Figure 6. In lowest energy canonical and zwitterionic conformers of Val - (H₂O)₅ all water molecules are located away from the nonpolar side-chain in Val, bridging the carboxyl and the amino, and the carboxylate and the ammonium groups, respectively. This near degeneracy may raise a question of whether the two forms of Val - (H₂O)₅ would coexist at low temperature gas phase environment. If the barrier of canonical ↔ zwitterion transformation is not large enough, then the thermodynamically more favorable form would only be observed.

Figure 7 illustrates the dynamic pathways of transformation from the lowest energy canonical (ca-5-1) and from the lowest energy zwitterionic form (zw-5-1). We find that the canonical form (ca-5-1) may transform to other higher energy conformer via considerable barrier (~8 kcal/mol).

Moreover, this canonical conformer is not kinetically connected to the lower lying (zw-5-1). Thus, although this canonical conformer is slightly less favorable in thermodynamic sense than the zwitterionic form (zw-5-1), it may still be stable kinetically. Figure 7(b) describes that the lowest energy zwitterionic conformer (zw-5-1) may transform by double proton transfer mediated by water molecules to a higher lying (by ~3.4 kcal/mol) canonical form via an activation barrier of ~6 kcal/mol. Therefore, we suggest that both the lowest energy zwitterionic (zw-5-1) and canonical (ca-5-1) conformer of Val - (H₂O)₅ may be observed in low temperature (~5 K) gas phase.

Figure 8 depicts the infrared (IR) spectra (B3LYP/6-311++G(d,p)) frequencies scaled by a factor of 0.9613 of (zw-5-1) and (ca-5-1) that may help to discern the two forms

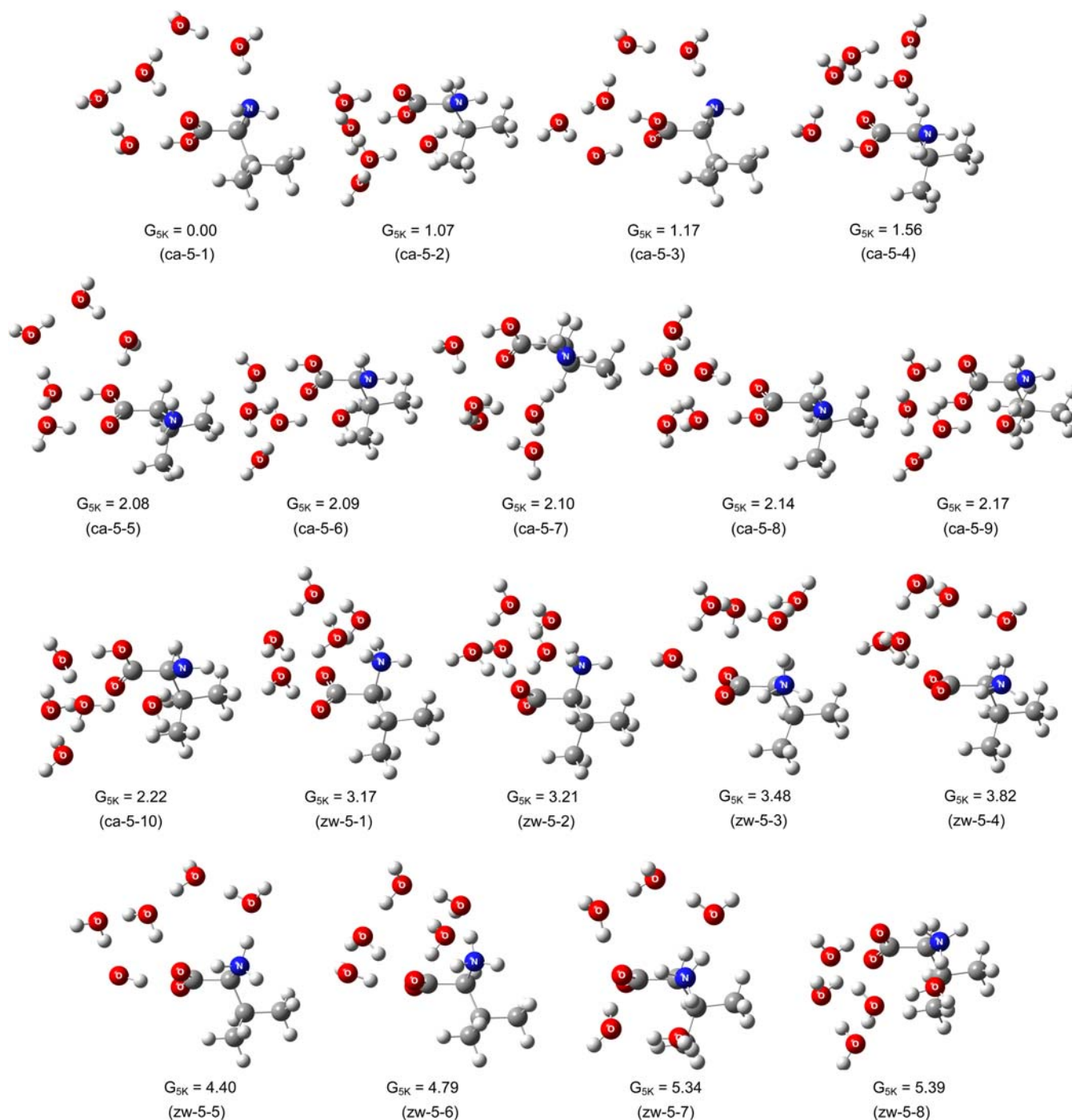


Figure 6. Structures of the lowest energy conformers of Val - (H₂O)₅ (relative Gibbs free energy at 5 K in kcal/mol).

experimentally. The IR spectrum of (ca-5-1) is characterized by a wide window in the 1700–2800 cm⁻¹ region, and by three very strong bands at 2877, 3097 and 3146 cm⁻¹ describing the proton transfer modes between the carbonyl, water molecules and the amino group. On the other hand, the IR absorption of (zw-5-1) is featured by a number of intense bands above 2700 cm⁻¹. It seems that the bands at 2722 and 3010 cm⁻¹, which describe the proton transfer between the ammonium to the microsolvating water molecules, may be useful to assign the IR spectrum of (zw-5-1) experimentally obtained as obtained for the bare Val.^{34,35}

Conclusion

We find that at least five water molecules are required to stabilize Val zwitterion because of nonpolar side chain. Thus, Val is a “normal” amino acid in its behaves towards the effects of microsolvating water molecules, in contrast to other amino acids such as arginine and lysine whose zwitterions were predicted to become stable under the influence of fewer number of water molecules due to side chains of strong basicity. The kinetic paths and activation barriers of canonical \leftrightarrow zwitterion transformation of Val -

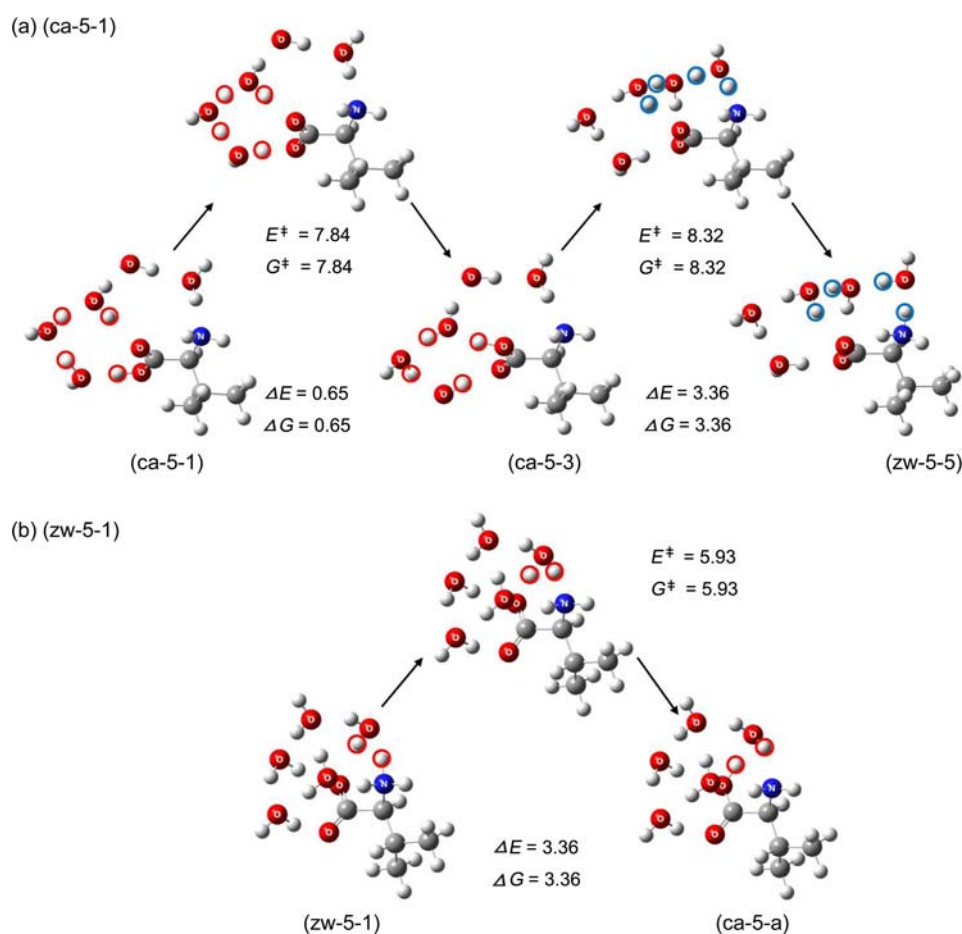


Figure 7. Transformation from the lowest energy zwitterionic (zw-5-1) and canonical (ca-5-1) conformer of Val-(H₂O)₅ (relative energy in kcal/mol, ZPE included; wb97XD/6-311++g**))

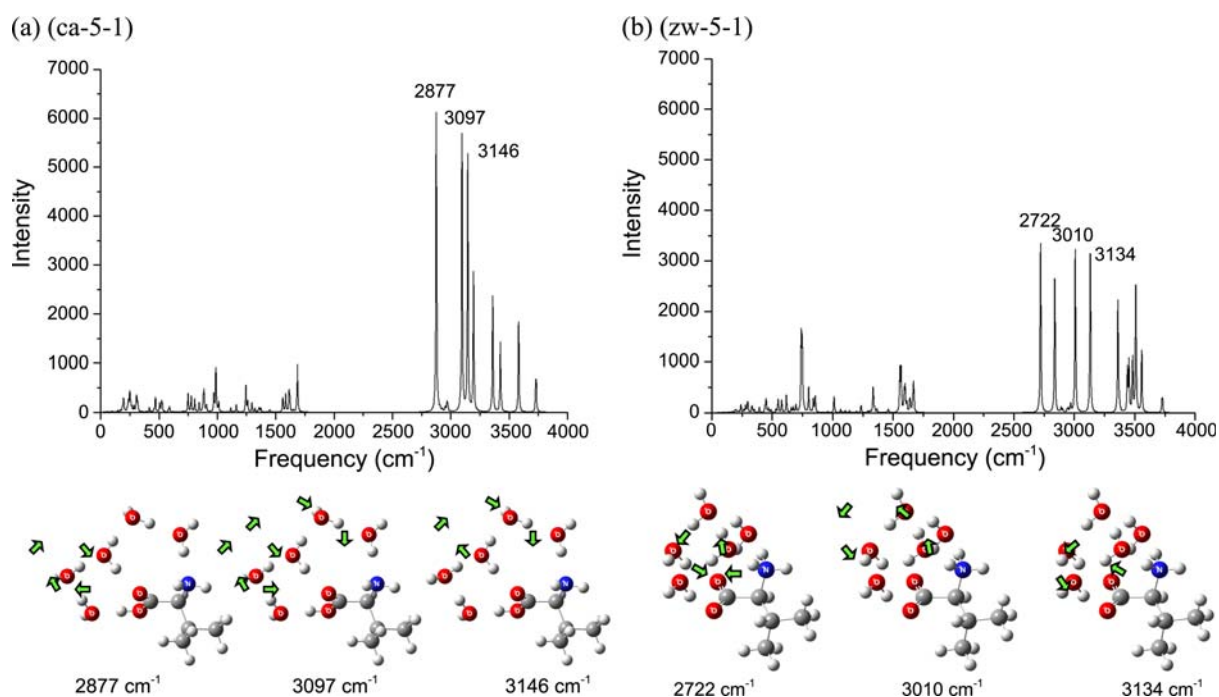


Figure 8. IR spectra of the lowest energy and normal modes of the most intense bands of (a) canonical (ca-5-1) and (b) zwitterionic (zw-5-1) conformers of Val - (H₂O)₅ (B3LYP/6-311++G** with a scaling factor of 0.9613).

(H₂O)₅ indicate that both (canonical, zwitterionic) forms may be observed in jet-cooled low temperature gas phase. Experimental study such as carried out by Bowen and co-workers¹ on this intriguing Val–water system would be highly desirable.

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