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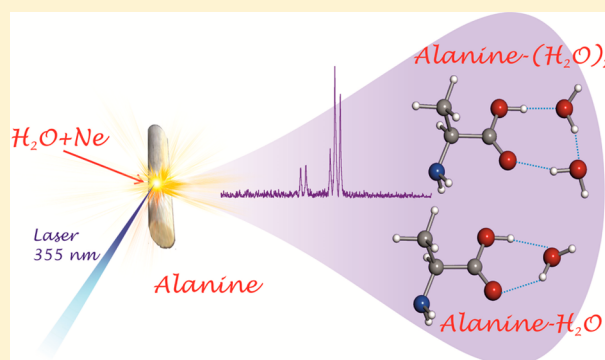
Alanine Water Complexes

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S Supporting Information

ABSTRACT: Two complexes of alanine with water, alanine-(H₂O)_n (*n* = 1,2), have been generated by laser ablation of the amino acid in a supersonic jet containing water vapor and characterized using Fourier transform microwave spectroscopy. In the observed complexes, water molecules bind to the carboxylic group of alanine acting as both proton donors and acceptors. In alanine-H₂O, the water molecule establishes two intermolecular hydrogen bonds forming a six-membered cycle, while in alanine-(H₂O)₂ the two water molecules establish three hydrogen bonds forming an eight-membered ring. In both complexes, the amino acid moiety is in its neutral form and shows the conformation observed to be the most stable for the bare molecule. The microsolvation study of alanine-(H₂O)_n (*n* = 1,2) can be taken as a first step toward understanding bulk properties at a microscopic level.



INTRODUCTION

The role of water as solvent is crucial in many biological processes because solvation may influence the structure and properties of the biomolecules involved by changing their function and reactivity.^{1–4} A relevant example is that of amino acids, which exist as neutral species (NH₂-CH(R)-COOH) when isolated in the gas phase but as doubly charged zwitterions (⁺NH₃-CH(R)-COO[−]) upon solvation. This change in the preferred form of amino acids is driven by the establishment of hydrogen bonds between the functional groups of the amino acid and water molecules. Knowledge of how this phenomenon proceeds at a molecular level is essential to develop a deeper understanding of the influence of water on biological processes such as protein–protein interactions and protein folding, where both water and amino acids participate.^{3,4}

The interactions of amino acids with water are difficult to study in condensed phases because of the interplay between inter- and intramolecular interactions and the dynamical nature of the hydrogen bonds. Isolation of amino acids in the gas phase provides an ideal environment to investigate and control solvent effects because water molecules can be sequentially added (microsolvation), eventually bridging the gap between the gas and solution phases. The number of water molecules necessary to stabilize the zwitterion rather than the neutral form of the amino acid can thus be investigated. In addition, because individual amino acids and their hydrated complexes are observed, inter- and intramolecular interactions (mainly hydrogen bonds) between the different moieties can be revealed.

Rotational spectroscopy is the most incisive tool available for the structural characterization of gas-phase biomolecules. It allows unambiguous identification of different conformers of a given species and determination of molecular structures with extreme accuracy. In our research group, rotational spectroscopy has been coupled to laser ablation and molecular beams (LA-MB-FTMW spectroscopy)^{5–7} to investigate the structures and conformations of biomolecules. The power of this approach is highlighted by recent studies on amino acids,^{8–13} nucleic acid bases,^{14–17} and drugs.¹⁸ Generation of amino acid–water complexes is not favored in the hot plasma created by the laser pulse used to vaporize the amino acid. Until 2006, it was not possible to observe and characterize the 1:1 glycine–water complex.¹⁹ Although gaining experimental insight into the effects of solvation of amino acids remains a challenge, recent improvements in our spectrometers made possible the observation of two water molecules complexed with glycine.²⁰

We further explore the process of microsolvation by investigating the complexes of the amino acid alanine with water. Questions to consider in the study include the determination of the preferred binding sites for water, the type of hydrogen bonds established between water and alanine, and the examination of possible changes in the conformational preferences of alanine upon solvation. These aspects have been taken into account in several theoretical investigations of mono- and dihydrated alanine reported in the literature,^{21–26} where different arrangements for interaction between the monomers

Received: January 24, 2014

Revised: March 7, 2014

Published: March 11, 2014

as well as different conformations of the amino acid are considered. We present here the first experimental investigation on alanine-(H₂O)_{*n*} (*n* = 1,2). To our knowledge, no other experimental studies have been reported on alanine-water complexes to validate those theoretical predictions. Our observations provide a framework for a better understanding of the solvation process on amino acids.

EXPERIMENTAL SECTION

Rotational spectra were recorded in the 4–18 GHz region using LA-MB-FTMW spectroscopy.^{5,6} Two main modifications have significantly improved the original spectrometer setup: the laser ablation nozzle has been modified to a shape closer to a Laval nozzle,²⁷ and picosecond laser technology has been implemented. Parent alanine (Sigma-Aldrich, 99%, mp 315 °C) and the ¹⁵N isotopically substituted species were used. These solid samples were ground and mixed with several drops of a commercial binder before being compacted to form rods of ~6 mm in diameter. Alanine rods were placed in the laser ablation nozzle, where the third (355 nm) harmonic of a 20 ps Nd:YAG laser vaporized the amino acid. A receptacle containing water was attached to the gas line prior to the nozzle.

Complexes are formed in the throat of the nozzle at the onset of the supersonic expansion and are conducted by a Ne flow to the vacuum chamber, where they are macroscopically polarized by a short microwave radiation pulse of 0.3 μs. Once the radiation ceases, molecular relaxation signals are collected in the time domain and Fourier-transformed to the frequency domain. Molecular pulses were typically 0.7 to 1.0 ms. In our setup, the Fabry–Pérot resonator located in the vacuum chamber and the supersonic jet are collinear; therefore, all transitions appear as doublets due to the Doppler effect. The molecular frequency is the arithmetic mean of the Doppler doublets, achieving a frequency accuracy better than 3 kHz.

Geometry optimizations on the lower energy mono- and dihydrated alanine complexes reported in the literature^{16–21} have been performed using ab initio perturbation methods at the MP2/6-311++G(d,p) level using the Gaussian suite of programs²⁸ to predict the molecular parameters relevant to rotational spectroscopic studies. In previous theoretical studies, water interacts with alanine conformers, which are stabilized by type-I (N–H···O=C with a *cis*-COOH arrangement) or type-II (O–H···N with a *trans*-COOH configuration) hydrogen bonds. No interactions between water and conformations of alanine showing a type III hydrogen bond (N–H···O–H with a *cis*-COOH arrangement) were considered. In our calculations, we have also included interactions of water with conformers of alanine stabilized by type-III hydrogen bonds. This choice has been informed by our results on glycine-(H₂O)_{*n*} (*n* = 1,2),^{19,20} where low-energy conformers exhibit water binding to a glycine III conformer. Alanine-(H₂O)_{*n*} (*n* = 1,2) complexes predicted below 1000 cm^{−1} are shown in Figures 1 and 2. The rotational and ¹⁴N-nuclear quadrupole coupling constants together with the values of their dipole moment components along their principal inertial axes are collected in Tables 1 and 2. The dissociation energies of the complexes have also been calculated at the same computational level, including corrections for the basis set superposition error using the counterpoise procedure²⁹ and fragment relaxation terms.³⁰

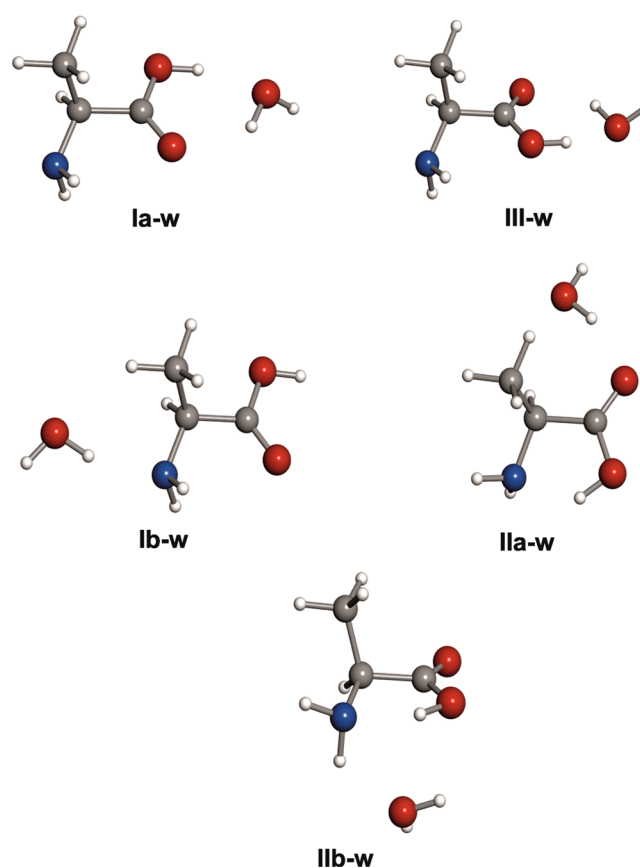


Figure 1. Lowest energy conformers of the neutral alanine-(H₂O) complex computed at the MP2/6-311++G(d,p) level of theory.

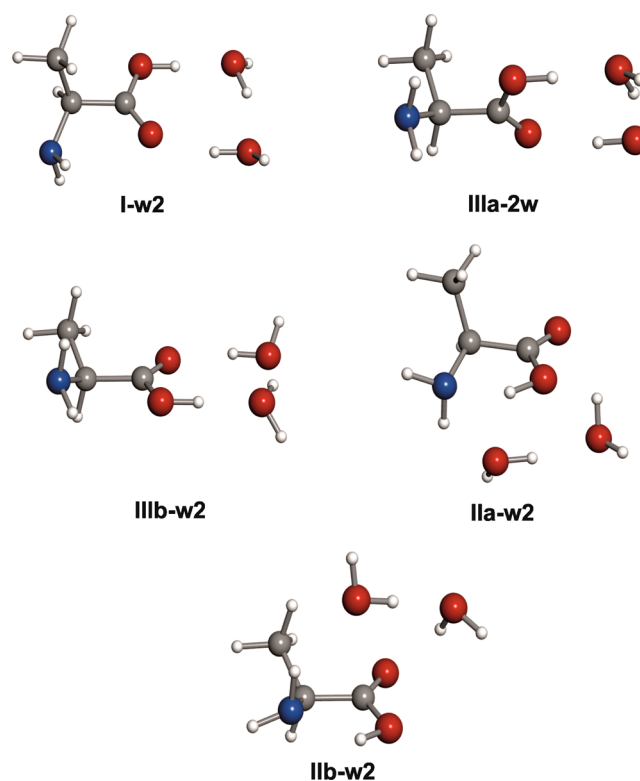


Figure 2. Lowest energy conformers of the neutral alanine-(H₂O)₂ complex computed at the MP2/6-311++G(d,p) level of theory.

Table 1. Theoretical Spectroscopic Parameters Predicted for the Lowest Energy Conformers of ^{14}N Alanine–(H_2O) (below 1000 cm^{-1})

	Ia-w ^a		III-w		Ib-w		IIa-w		IIb-w	
A (MHz) ^b	4844.0	53 ^c	4775.0	60	4519.2	49	2953.1	29	2840.0	43
B (MHz)	1301.7	16	1279.0	15	1211.3	2	1762.4	20	1957.0	1
C (MHz)	1153.2	15	1197.8	15	1079.0	4	1313.6	14	1499.8	12
P_c (uÅ^2)	27.2		39.5		30.3		36.6		49.6	
χ_{aa} (MHz)	−4.21		−4.51		−4.06		−0.30		1.56	
χ_{bb} (MHz)	2.40		2.65		2.34		−0.97		1.91	
χ_{cc} (MHz)	1.80		1.86		1.72		1.28		−3.48	
μ_a (D)	1.7		1.8		1.3		−1.5		0	
μ_b (D)	−0.7		−0.1		−1.0		3.1		3.5	
μ_c (D)	−0.7		0.7		−0.1		1.2		1.0	
ΔE_{MP2} (cm^{-1})	0		259		750		907		967	
D_e (kJ/mol^{-1})	34		30		27		26		24	

^aOptimized structures at the MP2/6-311++G(d,p) level of calculation. ^bA, B, and C are the rotational constants; $P_c = 1/2(I_a + I_b - I_c)$ is the planar moment of inertia, conversion factor: 505379.1 MHz uÅ^2 ; χ_{aa} , χ_{bb} , and χ_{cc} are the ^{14}N nuclear quadrupole coupling parameters; μ_a , μ_b , and μ_c are the electric dipole moment components; ΔE_{MP2} represents relative electronic energies; and D_e is dissociation energy for the complex. ^cIsotopic shift predictions for ^{15}N .

Table 2. Theoretical Spectroscopic Parameters for the Lowest-Energy Conformers of ^{15}N -Alanine–(H_2O)₂ (below 1000 cm^{-1})

	I-w2 ^a	IIa-w2	IIb-w2	IIa-w2	IIb-w2
A (MHz) ^b	2834.9	2991.4	2927.4	2304.1	2017.7
B (MHz)	857.0	822.4	868.7	1209.1	1280.1
C (MHz)	722.8	730.9	687.9	972.7	1132.0
P_c (uÅ^2)	34.1	46.0	9.9	58.9	99.4
μ_a (D)	1.6	1.5	−1.5	−2.0	−2.4
μ_b (D)	−0.3	−0.2	−0.2	−2.2	−1.7
μ_c (D)	−0.2	−0.1	−0.3	0	0.6
ΔE_{MP2} (cm^{-1})	0	248	505	806	960
D_e (kJ/mol^{-1})	72	69	66	65	62

^aOptimized structures at the MP2/6-311++G(d,p) level of calculation. ^bA, B, and C are the rotational constants; $P_c = 1/2(I_a + I_b - I_c)$ is the planar moment of inertia, conversion factor: 505379.1 MHz uÅ^2 ; μ_a , μ_b , and μ_c are the electric dipole moment components; ΔE_{MP2} represents relative electronic energies; and D_e is dissociation energy for the complex.

RESULTS AND DISCUSSION

Rotational Spectrum of Alanine– H_2O . Two conformers of alanine have been previously identified by rotational spectroscopy in supersonic jets.^{31,32} The most abundant conformer, labeled conformer I, shows a bifurcated hydrogen bond $\text{N–H}\cdots\text{O}=\text{C}$ with a *cis*-COOH arrangement. The other observed conformer, labeled conformer IIa, exhibits a $\text{O–H}\cdots\text{N}$ hydrogen bond, which forces a *trans* arrangement of the carboxylic group. Another predicted low-energy conformer, III, bearing a $\text{N–H}\cdots\text{O–H}$ hydrogen bond, has not been detected most probably due to collisional relaxation to conformer I in the early stages of the supersonic expansion.^{32–34} It is chemically intuitive to assume that the formation of complexes with water can alter the conformational preferences of bare amino acids. Besides, water molecules can bind to various sites in the amino acid, which further complicates the isomeric space of the hydrated complexes. We have considered water binding to alanine I, II, and III conformations through the carboxylic group, the amino group, and both amino and carboxylic groups. The energies of the alanine– H_2O complexes predicted to lie within 1000 cm^{-1} are shown in Figure 1, and their molecular parameters are collected in Table 1. Our calculations predict

that water preferentially binds to the carboxylic group of alanine in the monohydrated complex, with two conformers, Ia-w and III-w (see Table 1), being predominant. These two conformers differ in the intramolecular interactions within alanine and therefore in the alanine conformation involved in the complex. In Ia-w, alanine is stabilized by a $\text{N–H}\cdots\text{O}=\text{C}$ hydrogen bond (alanine I), while in conformer III-w alanine is stabilized by a $\text{N–H}\cdots\text{O–H}$ hydrogen bond (alanine III).

The lower-energy complexes of alanine–water are predicted to be asymmetric tops (see Table 1) close to the prolate top limit with a sizable component of the dipole moment along the *a* inertial axis (except IIb-w). Hence initial scans were directed to find ^aR-branch transitions, which typically appear to form groups of lines spaced approximately $B+C$ in such asymmetric tops. Before starting the search for monohydrated alanine, it was checked that both alanine and water were present in the supersonic expansion. The presence of alanine was tested by observing the $1_{1,1}\leftarrow 0_{0,0}$ rotational transition of the most abundant conformer I,^{31,32} and the presence of water was confirmed by observing the strong signal of $(\text{H}_2\text{O})_2$.³⁵

After long scans conducted in wide-frequency regions, a set of μ_a R-branch rotational transitions was detected showing the quadrupole hyperfine pattern that can be expected for a molecule with a ^{14}N atom. (See Figure 3.) These transitions no longer appeared in the spectrum when either laser or water was removed, confirming that they arise from a molecule containing alanine and water. Detailed scans were carried out to search for the most intense *b*- and *c*-type transitions (see Table 1), but they were not detected. This is in accordance with the low values of the respective dipole moment components predicted in Table 1. The lines (see Table S1 of the Supporting Information) were fit³⁶ to the semirigid rotor Hamiltonian of Watson in the A reduction and the I' representation $H_{\text{R}}^{(A)}$,³⁷ supplemented with a term, H_{Q} , to take into account the interaction between the nuclear electric quadrupole moment of the ^{14}N with the electric field gradient created at the same ^{14}N nucleus created by the rest of the molecule.³⁸ The final set of rotational and quadrupole coupling constants is listed in Table 3. To further confirm the assignment of the observed spectrum, we proceeded to the analysis of the isotopologue ^{15}N -alanine– H_2O . Measured transitions (see Table S2 of the Supporting Information) for this isotopologue were fitted to the $H_{\text{R}}^{(A)}$

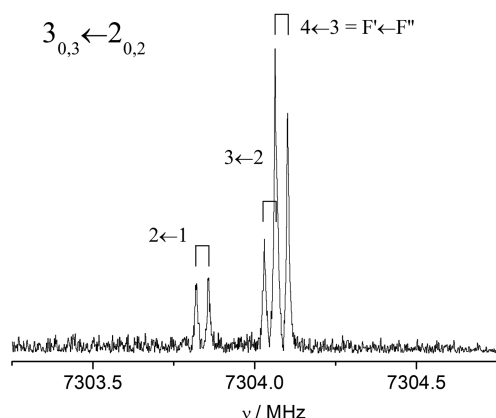


Figure 3. $3_{0,3} \leftarrow 2_{0,2}$ rotational transition of ^{14}N -alanine-(H_2O) showing the nuclear quadrupole hyperfine structure (1000 cycles). Each component labeled as $F' \leftarrow F''$ is observed as a doublet due to the Doppler effect. The molecular frequency is the arithmetic mean of the Doppler doublets.

Hamiltonian (see previous)³⁶ to give the rotational constants listed in the last column of Table 3.

The values of nuclear quadrupole coupling constants χ_{aa} , χ_{bb} , and χ_{cc} inform on the electronic environment at ^{14}N and on the orientation of the amino group in the complex.³⁸ These values are predicted to be very similar for **Ia-w**, **III-w**, and **Ib-w**, which is a consequence of the almost identical orientation of the amino group in these complexes (see Figure 1). Hence, the experimental values χ_{aa} , χ_{bb} , and χ_{cc} are compatible with those predicted for **Ia-w**, **III-w**, and **Ib-w** complexes and do not allow discrimination between them. Furthermore, the frequency shifts predicted (see Table 1) for the structures of **Ia-w** and **III-w** were found to be consistent with those experimentally observed (see Table 3). Fortunately, the values of the experimental rotational constants are only consistent with those predicted for the **Ia-w** complex. The values of the planar moment $P_c = (I_a + I_b - I_c)/2 = \sum m_i r_{ci}^2$, a measure of the mass distribution outside the *ab* inertial plane, near which the heavy atoms of the complex lie, also indicates that the rotamer observed is **Ia-w**.

Additional searches were performed to find the rotational spectrum of **III-w**, predicted to lie at ca. 250 cm^{-1} over **Ia-w**. These spectral searches were conducted using samples of ^{15}N -

alanine because the lack of quadrupole hyperfine structure (^{15}N does not have a quadrupole moment) results in more intense transitions.^{14,15} Unfortunately, no transitions that could be attributed to **III-w** were identified. This absence might be associated with the collisional relaxation of type-III conformers to the most stable conformer $\text{I}^{31,32,39-42}$ in the supersonic expansion, which precludes the formation of complexes involving alanine **III**. Other conformers of alanine- H_2O are predicted to lie at considerably higher energy (ca. 700 cm^{-1} above the global minimum); therefore, they were not expected to have enough population to be detected in a supersonic expansion.

Rotational Spectrum of Alanine-(H_2O)₂. Three conformations of alanine-(H_2O)₂ (see Figure 2), **I-w2**, **IIIa-w2**, and **IIIb-w2**, arising from the interaction of two water molecules with the carboxylic group of either alanine **I** or alanine **III**, are predicted to be lowest in energy (below 500 cm^{-1} , see Table 2). All three are predicted to be near-prolate asymmetric rotors with the largest dipole moment component oriented along the *a* inertial axis. We could anticipate a very weak spectrum for the dihydrated species because of a lower probability of multiple-body collision in the throat of the nozzle and its proximities. Hence, a sample of ^{15}N -alanine was employed to maximize spectral intensity. Spectral searches were carried out to find the recognizable pattern of R-branch transitions in prolate asymmetric tops. Long scans were undertaken, adding a large number of spectral acquisitions. Three transitions $4_{14} \leftarrow 3_{13}$, $4_{04} \leftarrow 3_{03}$, and $4_{13} \leftarrow 3_{12}$, which showed a pattern consistent with that expected from a complex of alanine-(H_2O)₂, were assigned in the survey. On this basis, additional transitions (see Table S3 of the Supporting Information) were measured and finally fitted to the semirigid rotor Hamiltonian $H_{\text{R}}^{(A)}$ of Watson to determine the rotational constants listed in Table 3. These values are only consistent with those predicted for the dihydrate **I-w2**. The experimental value of the planar moment P_c confirms the assignment to **I-w2**.

Structural Considerations. A quantitative comparison between experimental and theoretical rotational constants of both **Ia-w** and **I-w2** indicates that the theoretical value of the rotational constant *C* shows the largest deviation from the experimental value, of only $\sim 1\%$. Predicted rotational constants *A* and *B* have maximum deviations from experimental values of 0.4% . As a consequence of this excellent agreement, the

Table 3. Experimental Parameters Determined for the Observed Monohydrated ^{14}N - and ^{15}N -Alanine and Dihydrated ^{15}N -Alanine

	$^{14}\text{N-Ala}\cdots\text{H}_2\text{O}$	$^{15}\text{N-Ala}\cdots\text{H}_2\text{O}$		$^{15}\text{N-Ala}\cdots(\text{H}_2\text{O})_2$
<i>A</i> (MHz) ^a	4861.72(12) ^b	4806.32(33)	55.4 ^c	2830.910(25)
<i>B</i> (MHz)	1306.45978(32)	1290.6690(12)	15.8	859.45513(18)
<i>C</i> (MHz)	1136.17570(39)	1121.3755(12)	14.8	716.22912(22)
Δ_J (kHz)	0.296(25)	[0.296] ^d		0.1184(46)
P_c (uÅ ²)	22.9872(14)	23.0173(40)		30.46676(96)
χ_{aa} (MHz)	-3.9288(69)	- ^e		-
χ_{bb} (MHz)	2.472(41)	-		-
χ_{cc} (MHz)	1.456(41)	-		-
σ (kHz) ^f	1.8	5.3		1.2
<i>N</i> ^g	17	8		9

^a*A*, *B*, and *C* are the rotational constants; Δ_J is the quartic centrifugal distortion constant; $P_c = 1/2(I_a + I_b - I_c)$ is the planar moment of inertia, conversion factor: 505379.1 MHz uÅ^2 ; χ_{aa} , χ_{bb} , and χ_{cc} are the ^{14}N nuclear quadrupole coupling parameters; μ_a , μ_b , and μ_c are the electric dipole moment components; and D_e is the dissociation energy for the complex. ^bStandard error in parentheses in the units of the last digit. ^cIsotopic shift observations for ^{15}N . ^dFixed to the value of parent alanine- \cdots water. ^e ^{15}N lacks a quadrupole moment and therefore there are no quadrupole coupling interactions. ^frms deviation of the fit. ^gNumber of fitted transitions.

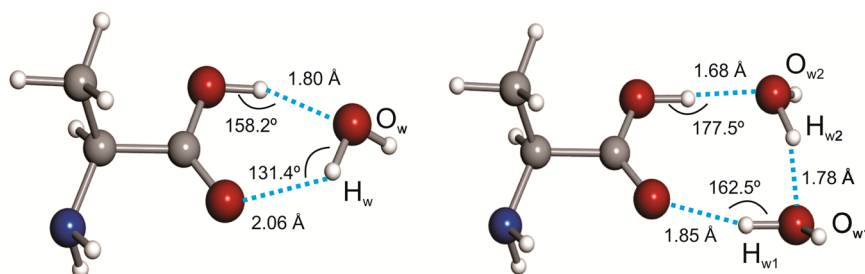


Figure 4. Observed conformers of alanine-(H₂O)_n (*n* = 1, 2) complexes showing structural parameters of the hydrogen bonds obtained from quantum-chemical calculations.

theoretical structures of **Ia-w** and **I-w2** can be considered validated by the experimental data. On that account, the theoretical structural parameters characterizing the intermolecular hydrogen bonds can be derived and are shown in Figure 4. In both mono- and dihydrated alanine, the O_w⋯H–O_{Ala} bond is the shortest intermolecular hydrogen bond, and the angle ∠O_wHO_{Ala} is closer to 180°, which indicates that the O_w⋯H–O_{Ala} bond is the strongest of the intermolecular hydrogen bonds in the complexes. This implies that the acidic character of the hydroxyl group of the –COOH of alanine dominates the interaction between water and alanine. The strong character of water as proton acceptor has also been reported for glycine–H₂O¹⁹ and formamide–H₂O.⁴³

Hydrogen bond cooperativity has been described as the strengthening of hydrogen bonds occurring in chains or cycles when the molecules involved act simultaneously as hydrogen bond donors and acceptors,^{44,45} as is the case of **I-w2**. The hydrogen-bond distances O_{w2}⋯H–O_{Ala} and O_{w1}–H⋯O=C in **I-w2** are considerably shorter than the corresponding ones in **Ia-w** (see Figure 4) as corresponds to a reinforcement of these hydrogen bonds due to cooperativity.

Examination of the dissociation energies of the complexes provides another measure of cooperativity. A value of 72.5 kJ mol^{–1} was obtained for **I-w2** in excellent agreement with that reported by Mullin et al.²⁴ If there were no cooperative effects, this value should correspond to the sum of the dissociation energies necessary to break each of the intermolecular hydrogen bonds established within the complex. Calculation of the dissociation energies for the 1:1 complexes of alanine with water in the same geometries as they have in **I-w2** yields energies of 13.3 and 24.0 kJ mol^{–1} for the intermolecular hydrogen bonds O_{w1}–H⋯O=C and O_{w2}⋯H–O_{Ala}, respectively. The energy of the O_{w1}⋯H_{w2}–O_{w2} bond has been approximated to that of the water dimer, calculated to be 18.6 kJ mol^{–1}. The sum of these energies is 55.9 kJ mol^{–1} for **I-w2**, considerably lower than the calculated dissociation energy of 72.5 kJ mol^{–1}, providing evidence of the existence of hydrogen-bond cooperativity. Hydrogen-bond cooperative effects have also been reported for dihydrated complexes of other biological molecules.^{20,46–48}

CONCLUSIONS

In the observed alanine–H₂O and alanine–(H₂O)₂ complexes, water molecules bind to the carboxylic group of alanine, acting as both proton donors and acceptors. In alanine–H₂O, the water molecule establishes two hydrogen bonds, forming a six-membered cycle (see Figure 4), while in alanine–(H₂O)₂, the two water molecules establish three intermolecular hydrogen bonds, forming an eight-membered ring (see Figure 4). The conformation of alanine in the complexes corresponds to the

most stable alanine **I** conformer stabilized by a N–H⋯O=C intramolecular hydrogen bond that is not disrupted by the interactions with water molecules.

The results obtained on alanine–(H₂O)_n (*n* = 1, 2) complexes can be compared with those for glycine–(H₂O)_n (*n* = 1, 2).^{15,20} The hydrogen-bond structural parameters and dissociation energies for the observed complexes of glycine or alanine with one water molecule are very similar, as they are those describing the interactions between the detected complexes of glycine or alanine with two water molecules. The difference in structure between glycine (R = –H) and alanine (R = –CH₃) does not seem to affect significantly intermolecular interactions with water at this level of microsolvation.

Future prospects to examine microsolvation of amino acids with polar side chains (e.g., serine, aspartic acid) would be interesting to determine if different polar side chains condition how water binds to the amino acid. Would polar side chains favor different sites for water binding? And even if water preferentially binds to the carboxylic group, would the structural parameters of the hydrogen bond change with respect to those of hydrated complexes of nonpolar amino acids? In addition to exploring these questions, such studies would also further examine the effect of water on amino acid conformational preferences, which may be more dramatic on amino acids with polar side chains because they have a remarkable conformational variety.

ASSOCIATED CONTENT

Supporting Information

Complete ref 28, transition frequencies of the alanine–(H₂O)_n (*n* = 1, 2) complexes, and Cartesian coordinates for the observed conformers. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work has been supported by the Ministerio de Ciencia e Innovación (Grants CTQ2006-05981/BQU, CTQ2010-19008,

and Consolider-Ingenio 2010 CSD2009-00038) and Junta de Castilla y León (Grant VA070A08). C.C. also thanks Junta de Castilla y León for a postdoctoral contract (CIP13/01).

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