J. Am. Chem. Soc. **2000,** *122,* 2938−2943

Conformation and Hydrogen Bonding in the Alaninamide−Water van der Waals Complex

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Received December 17, 1999. Revised Manuscript Received February 9, 2000

Abstract: Five conformational minima of the alaninamide−water complex were found from MP2/6-31+G** calculations. The structure of the lowest energy conformation has a network of intermolecular hydrogen bonds from the amide to water and from water to the carbonyl oxygen; this structure is also based on the lowest energy conformation of the alaninamide monomer. The amide-to-water and water-to-carbonyl hydrogen bond lengths in the fully optimized structure are 2.061 and 1.914 Å. Rotational spectra have been recorded for four isotopomers of alaninamide−water, and the structure of the complex was fit to the experimental moments of inertia. The intermolecular hydrogen bond lengths in the best-fit structure are 2.042(6) Å (amide-to-water) and 1.931(4) Å (water-to-carbonyl oxygen), and this structure is nearly identical with the lowest energy ab initio structure.

Introduction

The conformational structures of amino acids and peptides are stabilized by a delicate interplay of intramolecular hydrogen bonds; the amine and carboxylic acid groups, as well as any functional groups on the amino acid side chain, can act either as hydrogen bond donors or acceptors. Intermolecular hydrogen bonds, however, may interrupt or weaken the intramolecular hydrogen bonds upon solvation by water.

The conformations of amino acids arise from rotamers about the $C'-C^{\alpha}$ and $C^{\alpha}-N$ bonds, and separate rotational spectra have been found for two conformers of glycine[1−4] and alanine.[5] The lowest energy conformation (conformer I) of both amino acids has the amine and carbonyl groups oriented such that a bifurcated intramolecular hydrogen bond is formed between the hydrogens of the amine and the carbonyl oxygen.[1] A second conformation[2] (conformer II) is stabilized by an intramolecular hydrogen bond from the carboxylic acid to the nitrogen of the amine. Matrix infrared spectroscopy has recently provided experimental evidence for a third conformation of glycine[6] (conformer III). In this conformation the hydrogens of the amine form a bifurcated intramolecular hydrogen bond to the hydroxyl oxygen.

The structures of amino acid−water complexes are complicated by the number of different low-energy conformations available to the amino acid monomer. Each monomer conformation has several binding sites where intermolecular hydrogen bonding can occur. Furthermore, it is possible that the lowest energy monomer conformation is not suited for forming the strongest intermolecular hydrogen bonds in a complex, and that the energy ordering of the monomer conformations changes upon formation of the complex. These same issues may limit the application of isolated molecule studies (gas-phase spectroscopy and ab initio calculations) with respect to the likely conformations in solution. Structural information about amino acid−water van der Waals complexes might help us better understand the relationship between the conformations of isolated amino acids and their conformations in solution.

The conformation and binding sites of amino acid−water complexes have been examined theoretically in an early ab initio study of glycine−water.[7] Five different 1:1 complexes were optimized at the Hartree−Fock level and two generic types of complexes were found. All three glycine monomer conformations are able to form a “terminally bonded” complex (so-called because the water is positioned near the carboxyl terminus), in which the carboxylic acid donates a hydrogen bond to the water oxygen and water donates a hydrogen bond to the carbonyl oxygen. Glycine conformer I is able to form a second type of complex, with the water forming a bridging network from the amine to the carbonyl (i.e. the amine donates a hydrogen bond to the water oxygen, and water donates a hydrogen bond to the carbonyl oxygen). The energetics indicated that the terminally bonded complexes have stronger hydrogen-bonding interactions than the bridging structure. Since the terminal structures are the lowest energy structures of the complex, Basch and Stevens[7] were also able to determine that the energy ordering of the monomer conformations is preserved in the complex (I < III < II, at this level of theory). The energy difference between glycine conformers I and III in the terminally bonded complexes was found to be 5.9 kJ mol^{-1}, a somewhat smaller energy difference than for the same conformations of glycine monomers (8.2 kJ mol^{-1} at this level of theory). The energy difference between the terminally bonded water complexes of conformers

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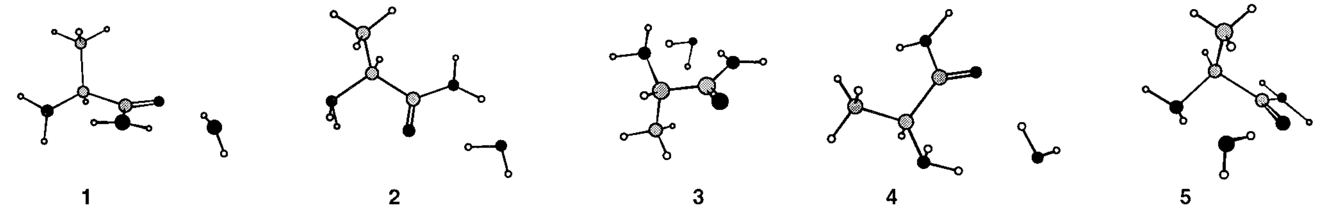

Figure 1. Ab initio model conformations of alaninamide−water. Structure **1** was found to be the lowest energy structure at the MP2/6-31+G** level. See Table 1 for the relative energies of the higher energy conformations.

I and III remained 5.0 kJ mol^{-1} after treating electron correlation at the MP2 level. To date, no experimental structures have been reported for a van der Waals complex of an amino acid with water.

The formamide−water van der Waals complex also has a network of hydrogen bonds, and its structure is equivalent to the terminally bonded complexes of glycine−water.[8−10] Although formamide does not have any conformational flexibility, water donates a hydrogen bond to the carbonyl and accepts one from the amide. This structure was originally proposed by Del Bene[8] on the basis of STO-3G calculations; later calculations[9] with configuration interaction indicate that it is 8−12 kJ mol^{-1} more stable than the other structural minima, which have only one intermolecular hydrogen bonding interaction. The doubly hydrogen-bonded structure of formamide−water has been detected experimentally by rotational spectroscopy.[10] Recent calculations[11] and spectroscopic experiments[12] on formamide−$(H_2O)_2$ indicate that the cyclic network of hydrogen bonds grows to incorporate a second water molecule.

The conformations of amino amides may model the structures of amino acid residues within a peptide or protein, but amide derivatization may also alter the relative stabilities of conformations compared to the amino acid. In a previous study we reported the rotational spectra for seven isotopic species of one conformation of alaninamide.[13] This conformer is stabilized by an intramolecular hydrogen bond from the amide to the amine and is analogous to conformer II of glycine and alanine. Ab initio models (MP2/6-31+G**) indicate that the experimental structure of alaninamide is 6.56 kJ mol^{-1} lower in energy than the conformation with an intramolecular hydrogen bond from the amine to the carbonyl oxygen. The apparent change in the energy ordering of the alaninamide conformations compared to the amino acid was attributed to the presence of both cis and trans hydrogens for the amide; the carboxylic acid of alanine favors the cis hydrogen configuration found in conformer I. Nonetheless, the extensive isotopic data and reliable heavy-atom structure available for alaninamide make its water complex an excellent test of how intermolecular hydrogen bonding affects the relative stabilities of monomer conformations.

Theoretical Modeling

Complete optimizations of the structure of alaninamide−water found five different structural minima at the MP2/6-31+G** level using Gaussian94.[14] The optimizations were begun from the two lowest energy monomer conformations, with either the terminally bonded structure (with intermolecular hydrogen bonds from the amide to the water oxygen and from the water to the carbonyl oxygen) or a structure with water forming a network of hydrogen bonds bridging from the amide to the amine. The optimized structures are listed in Table 1 and illustrated in Figure 1.

Table 1. Ab Initio Model Structures and Dipole Moments (MP2/6-31+G**) of the Alaninamide−Water van der Waals Complex

structure	description	rel energy/ kJ mol^{-1}	ΔI_{rms}/ amu Å^2	μ_{tot}/ D	μ_a/ D	μ_b/ D	μ_c/ D
1	terminal	0.0	4.4	4.4	3.6	2.2	1.2
2	terminal	7.3	9.0	2.9	0.4	1.5	2.5
3	bridging	14.4	103.2	4.0	2.6	3.0	0.2
4	bridging	15.5	70.7	5.4	3.8	3.1	2.3
5	bridging	17.9	99.5	3.7	3.3	1.1	1.1

Structures **1** and **2**, the two lowest energy structures of the complex, are terminally bonded and are based on the two lowest energy conformations of the alaninamide monomer. The detailed structures of alaninamide within the two lowest energy complexes are nearly identical with the structures of the parent monomer conformations. Bond lengths for alaninamide within a complex differ by less than 0.025 Å from the bond lengths of the corresponding monomers; the bond and torsional angles differ by less than 4°. The energy ordering of the two monomer conformations is preserved in the complex as well. The complex based on alaninamide conformer **2** is 7.3 kJ mol^{-1} higher in energy than the complex based on conformer **1**, so the lowest energy complexes are separated by nearly the same energy as the two lowest monomer conformations (6.6 kJ mol^{-1}, at this level of theory).

Three bridging complexes are approximately 15 kJ mol^{-1} higher in energy than the lowest energy structure, and they are not expected to be significantly populated. The conformation of the alaninamide also distorts within the bridging complexes. While the bond lengths and angles remain close to their monomer values, the ab initio calculations predict that the NCCN torsional angle changes from −13.6° in monomer conformation **1** to −31.1° in alaninamide−water structure **3**; the NCCN angle increases from 151.4° in alaninamide conformer **2** to 173.6° in alaninamide−water structure **4**. Alaninamide−water structure **5** is even more distorted from its parent monomer conformation (monomer conformation **2**), since the NCCN torsional angle has decreased from 151.4° to 114.3°.

The lowest energy ab initio structure of alaninamide−water is stabilized by a network of hydrogen bonds. The amide-to-water and water-to-carbonyl hydrogen bond lengths are predicted

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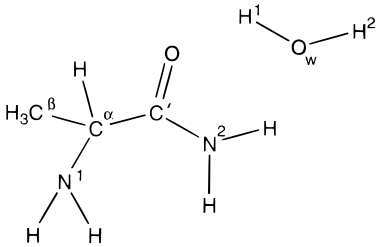

Figure 2. Atomic labels for alaninamide−water.

to be 2.061 and 1.914 Å, respectively; these distances are comparable to lengths of the corresponding hydrogen bonds in the experimental structure[10] of formamide−water (1.99 [(N)H- - -O] and 2.03 Å [(O)H- - -O]). An intramolecular hydrogen bond (2.195 Å [(N)H- - -N]) also stabilizes this conformation of alaninamide.

Structure **1** of alaninamide−water has both intermolecular hydrogen bonds lying in the plane established by the amide group, but the non-hydrogen-bonded hydrogen of the water (H^2 in Figure 2) is rotated 75° below the amide plane. This out-of-plane angle is favored because it directs one of the nonbonding electron pairs on water toward the amide hydrogen; similar torsion angles have been found in the experimental structures of other hydrogen-bonded complexes.[15] The DZP SCF calculations for formamide−water were insensitive to out-of-plane orientations of the non-hydrogen-bonded water hydrogen, and the structure was reported as planar.[9]

Experimental Section

Rotational spectra were recorded for four isotopomers of the alaninamide−water complex using a Balle−Flygare[16] Fourier transform microwave spectrometer (5−18 GHz), which has been described in detail elsewhere.[17] A resonant cavity is established by two 36 cm diameter aluminum mirrors (spherical radius of curvature = 84 cm) which are separated by up to 80 cm. One of the mirrors can be moved to tune the cavity to the desired frequency; the other mirror is fixed. Microwave radiation, generated by a Hewlett-Packard 83711B synthesized frequency generator, is coupled into the cavity by an L-shaped antenna. Molecular emission is detected using the same antenna, frequency reduced by a heterodyne circuit, and digitized by a Keithley-MetraByte DAS-4101 data acquisition board in a personal computer.

The heated valve (General Valve Series 9) is currently oriented perpendicular to the axis of the microwave cavity. This arrangement results in Doppler splittings of 25 kHz and line widths of 12 kHz for each component when measured with carbonyl sulfide; the frequency resolution of the spectrometer, determined from the number of data points recorded for the free induction decay, was 4 kHz for these measurements. Alaninamide was vaporized into an argon supersonic expansion by heating to 90 °C. The argon carrier gas contained 2% water vapor, and the backing pressure was 1.5 atm.

L-Alaninamide was liberated from the hydrochloride salt (Aldrich) using 1 M NaOH. Isotopically labeled species of alaninamide were prepared from DL-alanine (or ^{15}N-DL-alanine) by a four-step synthesis.[13,18] The first step was to protect the amine by reaction with benzyl chloroformate to form carbobenzoxyalanine. Next, the carboxylic acid was esterified with *p*-nitrophenol with use of the coupling reagent dicylohexylcarbodiimide. The amide was formed by reacting the activated ester with anhydrous ammonia generated in a vacuum line from NH_4Cl (or $^{15}NH_4Cl$) and excess NaOH. Subsequent hydrogenation of the CBZ-alaninamide in cylcohexene with palladium catalyst removed the carbobenzoxy protecting group and resulted in the formation of the desired isotopomer of alaninamide. The intermediate compounds were characterized by FTIR spectroscopy, and the final products were confirmed by microwave spectroscopy. ^{15}N-Alanine and $^{15}NH_4Cl$ were obtained from Cambridge Isotope Labs.

Table 2. Approximate Center Frequencies of the Rotational Transitions of Alaninamide−Water

transition	obsd freq/MHz	obsd − calcd/MHz
$3_{13}-2_{12}$	6905.783	0.019
$3_{03}-2_{02}$	7128.373	0.017
$3_{12}-2_{11}$	7387.103	0.014
$4_{14}-3_{13}$	9201.700	0.019
$4_{04}-3_{03}$	9479.711	−0.026
$4_{13}-3_{12}$	9842.967	0.042
$5_{15}-4_{14}$	11492.746	−0.032
$5_{05}-4_{04}$	11810.766	0.021
$5_{14}-4_{13}$	12292.775	−0.047

Results

Nine *a*-type rotational transitions were measured for the most abundant isotope of the alaninamide−water complex (Table 2). Each rotational transition consists of many hyperfine components arising from the two quadrupolar ^{14}N nuclei. Many of these components overlap, so it was not possible to assign individual components. Instead, the line centers of the rotational transitions were estimated to be near the center of each cluster of hyperfine components; we estimate the uncertainty in the line centers to be 100 kHz. No *b*- or *c*-type transitions were found despite careful searching and good predictions based on the assignment of the *a*-type spectrum. Rotational spectra were measured for the water complexes of $^{15}N^1$-alaninamide, $^{15}N^2$-alaninamide, and $^{15}N^1$,$^{15}N^2$-alaninamide (see Figure 2 for atom labels); transition frequencies for the isotopically labeled alaninamide−water complexes are available in the Supporting Information. Although the hyperfine patterns in the spectra of the two ^{15}N,^{14}N-mixed isotopic species were simpler than those of the most abundant isotopic species, the components still overlapped and could not be assigned. Approximate line centers were again estimated to be near the center of the cluster of components with an uncertainty of approximately 100 kHz.

The rotational constants were obtained by fitting the observed transitions to a rigid-rotor Hamiltonian. The differences between the observed and calculated frequencies were on the order of 30 kHz for the water complexes with alaninamide, $^{15}N^1$-alaninamide, and $^{15}N^2$-alaninamide ($\Delta\nu_{rms}$ = 29, 35, and 26 kHz, respectively). Centrifugal distortion constants were not included in these fits since the deviations were much smaller than the experimental uncertainty. The rotational transitions of the doubly substituted isotopomer ($^{15}N^1$,$^{15}N^2$-alaninamide−water) were fully resolved without nuclear quadrupole hyperfine structure and could be fit to A, B, C, and Δ_J. Spectroscopic constants for all four isotopic species are given in Table 3.

Structure and Discussion

Root-mean-square averages of the differences between the experimental moments of inertia and those calculated from the ab initio structures are included in Table 1: ΔI_{rms} where $\Delta I = I_x(\exp) - I_x(\text{calcd})$ and $x = a$, b, and c for each isotopomer. The bridging complexes (structures **3**−**5**) all have very large ΔI_{rms} values and are inconsistent with the experimental spectra. ΔI_{rms} = 4.4 and 9.0 amu Å^2 for the two terminally bonded complexes (structures **1** and **2**), and the structures of these two complexes were fit to the moments of inertia.

Ab initio structures of complexes **1** and **2** were used as starting structures for the least-squares fits of the molecular

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Table 3. Spectroscopic Constants for the Isotopic Species of Alaninamide−Water

	alaninamide−water	$^{15}N^1$-alaninamide−water	$^{15}N^2$-alaninamide−water	$^{15}N^1$,$^{15}N^2$-alaninamide−water
A/MHz	4789.5 (16)	4736.5 (19)	4729.4 (15)	4680.0 (3)
B/MHz	1271.870 (4)	1258.028 (5)	1270.643 (4)	1256.791 (1)
C/MHz	1111.391 (4)	1098.049 (5)	1107.937 (4)	1094.693 (1)
Δ_J/kHz				0.22 (2)
N^a	9	9	9	9
$\Delta\nu_{rms}{}^b$/kHz	28.5	34.5	26.3	5.2

[a] Number of transitions included in the fit. [b] $\Delta\nu_{rms} = (\nu_{obsd} - \nu_{calcd})_{rms}$.

Table 4. Bond Lengths (Å), Bond Angles (deg), and Torsional Angles (deg) of the Alaninamide Monomer Structures in the Least-Squares Fits of the Complex

	structure **1**	structure **2**
N^1−H	1.017	1.019
N^2−H	1.012	1.012
C^α−H	1.101	1.099
C^β−H	1.095	1.095
N^1−C^α	1.467	1.495
C^α−C′	1.530	1.533
C^α−C^β	1.529	1.532
C′−O	1.235	1.235
C′−N^2	1.349	1.355
H−N^1−H	105.8	103.9
H−N^2−H	123.3	120.3
H−C^β−C^α	110.1	110.2
H−C^β−H	108.8	108.7
C^β−C^α−H	108.5	109.2
N^1−C^α−C′	111.0	112.8
N^1−C^α−C^β	109.6	109.1
C^α−C′−O	120.0	120.4
C^α−C′−N^2	115.1	116.4
N^1−C^α−C′−N^2	−17.1	150.0
N^1−C^α−C′−O	164.5	−31.2
C^β−C^α−C′−N^2	103.1	−89.2

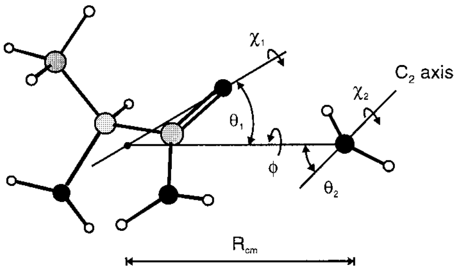

Figure 3. Geometrical parameters used to define the structure of alaninamide−water.

geometry to the experimental moments of inertia; the bond lengths, angles, and torsion angles that define the two alaninamide monomer conformations are given in Table 4. The ab initio structure for water, from the optimized structures of the complex, was also used in the fitting process: [O_w−H^1] = 0.971 Å, [O_w−H^2] = 0.959 Å, and [H^1−O_w−H^2] = 102.6°. The structures of water and alaninamide were assumed to be unchanged and were not fit; this approximation can be made because the conformations of alaninamide in the two complexes are nearly identical with the parent monomer conformations. Six additional structural parameters, shown in Figure 3, are required to describe the relative orientation of the two monomers. The center-of-mass separation and the alaninamide tilt angle, θ_1, were fit to the 12 moments of inertia using the program STRFITQ.[19] The remaining parameters (θ_2, χ_1, χ_2, and ϕ) were insensitive to the moments of inertia of the ^{15}N isotopomers and were left fixed at their ab initio values (Table 5).

Table 5. : Structural Parameters[a] Used To Describe the Relative Orientation of Alaninamide and Water

	structure **1**	planar least squares	structure **2**
θ_2	103.5°	85.3°	106.2°
χ_1	−6.0°	0.0°	11.5°
χ_2	40.1°	0.0°	−36.4°
ϕ	221.0°	180.0°	140.8°

[a] $\theta_2 = O_w-X_w-X_a$, $\chi_1 = X_w-X_a-O-H$, $\chi_2 = H^1-O_w-X_w-X_a$, $\phi = O_w-X_w-X_a-O$; X_a = alaninamide center of mass; X_w = water center of mass.

Least-squares fits of complex **2** did not improve agreement with the experimental data. The best fit of structure **2** still has ΔI_{rms} = 8.189 amu Å^2 and is much worse than structure **1**; structure **2** will not be considered further. The least-squares fit of structure **1** changes the center-of-mass separation only slightly: from 3.936 Å to 3.968(2) Å. The tilt angle changes substantially from 40.2° to 33(2)°, and ΔI_{rms} improves to 0.628 amu Å^2. The smaller tilt angle moves the water closer to the carbonyl oxygen and farther from amide nitrogen. The amide-to-water hydrogen bond increases to 2.395(70) Å and the water-to-carbonyl hydrogen bond length decreases to 1.750(37) Å; these hydrogen bond lengths have changed by 0.334 and −0.164 Å from the corresponding hydrogen bonds in the lowest energy ab initio structure. Least-squares fits of the water position, however, may be contaminated by vibrational averaging effects or the large uncertainties in the rotational constants. These effects might be particularly pronounced for the *b* and *c* coordinates of the water oxygen (or the water center of mass), which are small and are determined by fitting the tilt angle θ_1.

The least-squares fit of structure **1** places water hydrogen H^2 below the amide plane because θ_2, χ_1, χ_2, and ϕ are fixed at the ab initio values. We also fit R_{cm} and θ_1 for the "planar" form of complex **1** (a structure of the complex in which H^1, O_w, and H^2 all lie in the plane of the amide group). The initial structure of the planar complex was obtained from ab initio structure **1** by rotating the position of H^2 about the O_w−H^1 bond into the plane established by the amide; θ_2, χ_1, χ_2, and ϕ for this structure are given in Table 5. The best-fit planar structure reproduces the experimental moments of inertia very well: ΔI_{rms} = 0.094 amu Å^2. The fit values for R_{cm} and θ_1 of 3.972(1) Å and 40.2(1)° are very close to the initial values (3.957 Å and 40.1°); aside from the location of H^2, this structure is nearly identical with ab initio structure **1**. The intermolecular hydrogen bond lengths are 2.042(6) Å (amide to water) and 1.931(4) Å (water to carbonyl oxygen).

Kraitchman's equations for isotopic substitution[20,21] were used with the moments of inertia for the ^{15}N-labeled species to independently calculate nitrogen atomic positions. The Kraitchman analysis yields absolute values of the *a*, *b*, and *c* principal axes coordinates; Table 6 compares the Kraitchman coordinates to the atomic coordinates from the least-squares fit, planar least-

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Table 6. Atomic Coordinates (Å) of the Nitrogen Atoms from Kraitchman Analysis, Least-Squares Fitting, Planar Least-Squares Fitting, and the Initial ab Initio Structure

	Kraitchman	least-squares fit	planar least squares	ab initio
N^1				
a	±2.096(7)	2.162(1)	2.038(1)	2.069
b	±1.095(13)	−1.013(1)	−1.151(1)	−1.121
c	±0.12(12)	0.221(8)	0.243(6)	0.194
N^2				
a	±0.481(26)	−0.410(4)	−0.545(3)	−0.538
b	±1.095(11)	−1.180(1)	−1.039(1)	−1.074
c	±0.399(33)	−0.437(3)	−0.385(4)	−0.334

squares fit, and ab initio structures. Kraitchman substitution coordinates are less affected by vibrational averaging than zero-point coordinates (from least-squares fits),[21] so the large differences between the least-squares fit coordinates and the Kraitchman coordinates are one indication that vibrational averaging is significant. The least-squares fit of structure **1**, therefore, is probably unreliable because it attempts to compensate for the effects of vibration by reducing the tilt angle, θ_1, and moving the water closer to the carbonyl. The Kraitchman coordinates are in better agreement with the nitrogen coordinates from the ab initio structure and the planar fit, and these structures are more representative of the position of the water. Uncertainties given for the Kraitchman coordinates in Table 6 were determined by propagating the uncertainties in the moments of inertia; uncertainties for the least-squares fit coordinates were estimated from Costain's rule.[21] Because of extensive vibrational averaging (see below), these uncertainties underestimate the true uncertainties, and the ab initio structure (including hydrogen bond lengths) is probably the most reliable.

The ^{15}N isotopic data are insufficient to determine whether H^2 lies in the amide plane or not. By analogy with other hydrogen-bonded complexes, it seems likely that this hydrogen is undergoing large-amplitude motion. For example, there is no significant barrier to rotation of the off-axis water hydrogen about the intermolecular hydrogen bond in the trimethylamine−water complex[22] (and of H_2S in trimethylamine−hydrogen sulfide[17]). The non-hydrogen-bonded water hydrogen of dimethylamine−water is also subject to a large-amplitude torsional motion.[23] A low barrier torsion about the O_w-H^1 bond might be expected to have a planar arrangement for H^2 on average, even if the minimum of the potential energy surface is nonplanar. We calculated single-point energies at the MP2/6-31+G** level for a series of structures with the O_w-H^2 bond out of the amide plane. The structure of alaninamide and the location and orientation of the O_w-H^1 bond were kept fixed at the minimum energy configuration while the out-of-plane angle was varied. Two minima are observed in the plot of relative energy versus out-of-plane angle (Figure 4). The minimum with H^2 below the amide plane (on the opposite side from the methyl group) is 90 cm^{-1} lower in energy than the minimum with H^2 above the amide plane, and there is a barrrier of approximately 240 cm^{-1} that separates the two minima. The shallow wells indicate large-amplitude vibrational motion along this coordinate; a complete geometry relaxation of the planar configuration would further reduce the barrier between the wells.

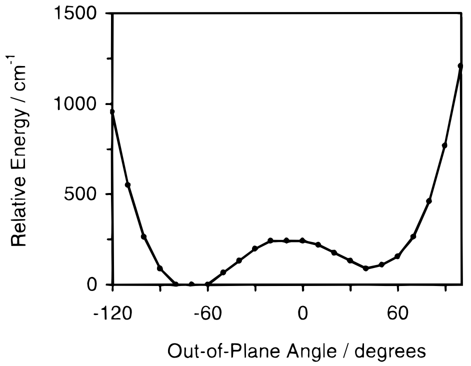

Figure 4. Relative energy (MP2/6-31+G**) as a function of the H^2 out-of-plane angle. Negative values for the out-of-plane angle refer to H^2 below the amide plane (on the opposite side from the methyl group).

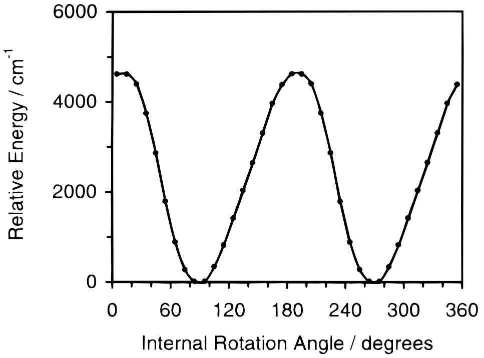

Figure 5. Relative energy (MP2/6-31+G**) as a function of water internal rotation angle.

A doubling of rotational transitions is the usual indicator of a low barrier internal motion that exchanges equivalent hydrogens. The doubling arises from ortho and para nuclear spin states which must combine with the tunneling and rotational wave functions so that the overall wave function is antisymmetric. Tunneling doublets will not arise from the torsional motion about the O_w-H^1 bond because the two minima are not the same depth. The water protons H^1 and H^2, however, are exchanged by the internal rotation of water about its C_2 axis. We estimated the barrier for C_2 rotation of water by calculating single-point energies for a series of structures that varied the degree of water internal rotation, keeping alaninamide and the water oxygen fixed. The 4600 cm^{-1} barrier to C_2 tunneling (shown in Figure 5) is high enough to quench the appearance of tunneling doublets in the rotational spectrum, and no features were found in the experimental spectrum that could be assigned to another tunneling state. The spectrum of alaninamide−water is similar to that of formamide−water,[10] which does not exhibit tunneling splittings in its microwave spectrum, despite a similar structure and higher symmetry. Tunneling splittings due to methyl internal rotation are also quenched by a high barrier; single-point calculations estimate this barrier to be 1600 cm^{-1}. The quenching of methyl internal rotation is not surprising, however, because no tunneling doublets were observed in the rotational spectra of the alanine and alaninamide monomers.

The moments of inertia of alaninamide−water are consistent only with structure **1** of the complex. This structure is based on the lowest energy conformation of the alaninamide monomer. From the energy ordering of the ab initio complexes, we conclude that the terminal binding site is stronger than any of the bridging sites. When water binds to the amide in the terminal configuration, it does not interrupt the amide-to-amine intramolecular hydrogen bond that stabilizes the lowest energy alaninamide monomer conformation.

No rotational transitions have been found for any of the higher energy structures of alaninamide−water. The ab initio calcula-

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(22) Tubergen, M. J.; Kuczkowski, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 9263−9266.

(23) Tubergen, M. J.; Kuczkowski, R. L. *J. Mol. Struct.* **1995**, *352*, 335−344.

tions predict that structure **2** of the complex is 7.3 kJ mol^{-1} higher in energy than structure **1**, so structures **1** and **2** should be populated in the ratio 11:1. Moreover, the ab initio calculations predict very different values for the dipole moments and their projections onto the principal axes; see Table 1. The strongest transitions of structure **1** are *a*-type, while structure **2** should exhibit a strong *c*-type spectrum. The intensities of rotational transitions are proportional to the squares of these dipole projections; the strongest *c*-type transitions of structure **2**, therefore, will only be half as intense as the strongest *a*-type transitions of structure **1** (i.e. for transitions originating from the same rotational state and with comparable direction cosine matrix elements). Together, the differences in populations and dipole moments will cause the spectrum of structure **2** to be approximately 20 times weaker than that of structure **1**.

Conclusions

Five conformational minima of the alaninamide−water van der Waals complex were found from calculations at the MP2/6-31+G** level. Water binds preferentially to the amide group, forming a network of hydrogen bonds from the amide nitrogen to the water oxygen and from water to the carbonyl oxygen. The lowest energy structures of the alaninamide−water complex are based on the two lowest energy conformations of the alaninamide monomer; the relative energies of the two complexes are very close to those of the parent monomer conformations. Rotational spectra consisting of nine *a*-type transitions were found for four isotopomers of the lowest energy alaninamide−water complex. The structure of the complex was fit to the moments of inertia derived from the spectra: $R_{cm} = 3.972(1)$ Å and $\theta_1 = 40.2(1)°$. Single-point energy calculations were used to estimate the barriers along the water out-of-plane flapping coordinate (240 cm^{-1}) and along the C_2 water internal rotation coordinate (4600 cm^{-1}).

Acknowledgment. This work was supported by a grant from the National Science Foundation (Grant CHE-9700833).

Supporting Information Available: Tables of transition frequencies for the isotopic species of alaninamide−water. This material is available free of charge via the Internet at http://pubs.acs.org.

JA9944150