Conformational Analysis

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Rotational Probes of Six Conformers of Neutral Cysteine**

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The recent emergence of laser ablation molecular beam Fourier transform microwave (LA-MB-FTMW) spectroscopy[1,2] has made possible the gas-phase study of solid biomolecules with high melting points. In this approach, solids are vaporized by a high-energy laser pulse, supersonically expanded into a vacuum chamber, and characterized by their rotational spectrum. Of the biomolecules that have been studied by this technique, aliphatic amino acids have received special attention because of the lack of experimental information and their biological relevance. Amino acids are well known to exist as zwitterions ($NH_3^+CH(R)COO^-$) in the solid state and in aqueous solution,[3] but in the gas phase they are in the canonical neutral form $NH_2CH(R)COOH$. This form represents the best approximation for understanding the inherent properties of these building blocks which are responsible for the specific shape of proteins.[4] The supersonic jet of a LA-MB-FTMW spectrometer provides a collisionless environment where amino acids can be considered isolated, thus allowing the evaluation of the role of intramolecular interactions through the investigation of conformational preferences in the gas phase. Seven different structures were characterized in a recent study on serine.[5] Herein we present our results on the study of cysteine, which is the only coded amino acid with a thiol group in the side chain.

The majority of amino acids (proteogenic[6–13] and non-proteogenic[14–16]) that have been studied so far are α-amino acids with nonpolar groups in their side chains. Their conformational behavior is mainly controlled by the stabilization effects associated with the formation of the three possible intramolecular hydrogen bonds. In configuration I (the notation follows that adopted for other aliphatic α-amino acids),[10] a bifurcated amine-to-carbonyl hydrogen bond (N–H···O=C) and a *cis*-COOH arrangement are established. Configuration II exhibits an intramolecular hydrogen bond between the hydrogen atom of the hydroxy group and the lone pair of electrons on the nitrogen atom of the amino group (N···H–O), which requires a *trans*-COOH configuration. In configuration III, the intramolecular hydrogen bond links the amino group and the oxygen atom of the hydroxy group (N–H···O–H), thereby recovering the *cis*-COOH arrangement. In all cases, the existence of a nonpolar side chain was found to have a negligible influence on the conformational preferences, with the most stable conformer displaying a type I intramolecular hydrogen bond.

Cysteine ($CH_2SHCH(NH_2)COOH$) is a natural amino acid found in high abundance in keratin, the main protein in nails, skin, and hair.[17] A polar side chain in an α-amino acid will bring about a whole new set of interactions with the polar groups (-NH_2, -COOH) in the amino acid backbone. These interactions, along with the great torsional flexibility that is characteristic of amino acids, may give rise to a considerable number of conformational minima relatively close in energy, as previously predicted by ab initio calculations.[18,19] Figure 1 shows the 11 conformers predicted in this work[20] within 1000 cm^{-1} of the global minimum. In the supersonic expansion, the two-body collisions with the carrier gas should cool

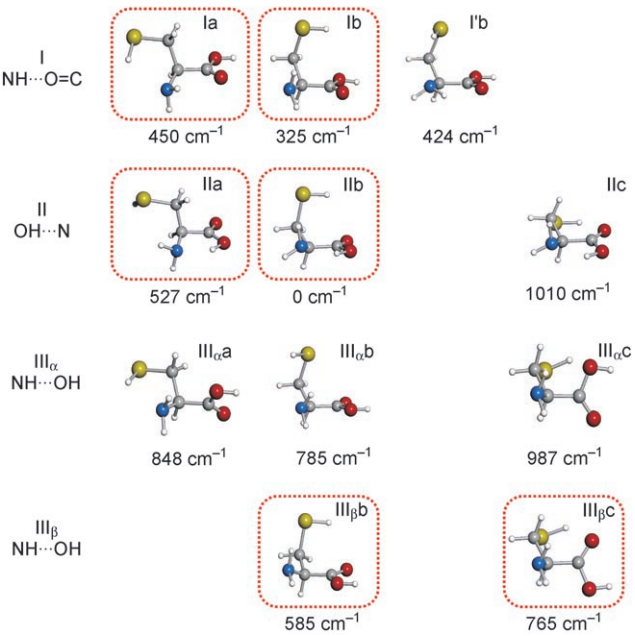

Figure 1. Predicted low-energy conformers of cysteine and relative energies (single-point MP4 calculation on the MP2 structures optimized using the 6-311++G(d,p) basis set) with respect to the global minimum in cm^{-1}. Conformers are classified as I, II, or III depending on the hydrogen bond established between the amino and carboxylic groups (see text), and as a, b, or c depending on the configuration adopted by the -CH_2SH side chain. The detected conformers are circled.

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these stable conformers to very low temperatures, thereby trapping them in their energy minima. Those with sufficient population can then be probed in the supersonic expansion by Fourier transform microwave spectroscopy. Since each rotational spectrum is specific for a particular molecular structure, it is possible to identify the conformers conclusively and extract important structural information related to the nature of the intramolecular interactions which take place.

On this basis, we have tackled the challenging problem of determining the exact nature of the conformations of cysteine and their relative abundances. Neutral cysteine molecules were produced by laser ablation of a solid sample (m.p. 300 °C), entrained in several atmospheres of neon, and pulsed through a 1.0 mm nozzle into a vacuum chamber (Fabry–Pérot resonator) to create a supersonic expansion. This process converts what would otherwise be an exceedingly crowded and complex rotational spectrum arising from multiple conformations into a more tractable problem, since highly excited rotational states are depopulated at the low rotational temperatures achieved in the expansion. In spite of this, more than 50 transitions were observed in the range of 5 to 8 GHz. It was relatively straightforward to recognize two sets of a-type R-branch transitions as belonging to rotamers close to the prolate symmetric top limit. All the observed transitions were split into several close hyperfine components that showed the characteristic pattern of a ^{14}N nucleus, which confirms the presence of a single nitrogen nucleus in the observed species. The measured transitions were fitted[21] to the semirigid rotor Hamiltonian of Watson in the A reduction and the I^r representation $H_R^{(A)}$,[22] supplemented with a term to take into account the quadrupole interaction H_Q,[23] namely $H = H_R^{(A)} + H_Q$. The fits yielded a first set of rotational constants that allowed the prediction and measurement of further transitions belonging to the μ_b- and μ_c- types. Finally, the rotational constants and the elements of the quadrupole coupling tensor (χ_{ij}; i, j = a,b,c) listed in the first two columns (L and M) of Table 1 were determined.

A distinguishing feature of rotational spectra is the ability to generate very accurate spectroscopic parameters directly comparable with in vacuo ab initio predictions. A first comparison of the experimental rotational constants of columns L and M with those predicted theoretically (see Table 1) shows that the observed species belong to the "a" family (Ia, IIa, or III_αa conformers). However, the rotamers could not be conclusively identified on this basis alone, as the similar backbone configuration of the "a" forms gives rise to very similar rotational constants. Fortunately, the values of the diagonal elements χ_{aa}, χ_{bb}, and χ_{cc} (see Table 1) clearly discriminate rotamer M and allowed us to conclusively assign it as conformer IIa. The quadrupole coupling constants of the L rotamer match those predicted for conformers Ia and III_αa, which have the same spatial orientation of the -NH_2 group (see Figure 1). Final identification came from the electrical dipole moment components: conformer III_αa should present strong a-, b-, and c-type spectra, while conformer Ia should show a strong a-type spectrum, a weak c-type spectrum, and a very weak or unobservable b-type spectrum. Rotamer L shows strong μ_a-type transitions and weak μ_c-type transitions; the nonobservation of a b-type spectrum is a strong indication of the presence of conformer Ia in the supersonic expansion. μ_a-, μ_b-, and μ_c-type transitions have been detected and measured for the M species, which is in accordance with the predicted values of the dipole moment components for conformer IIa, thus corroborating the assignment based on the quadrupole coupling constants. Furthermore, the optimal microwave power for polarizing the rotational transitions was consistent with the predicted values of the electrical dipole moment components for both conformers.

Further searches were performed in the 8–9 GHz region to detect other low-energy conformers of cysteine. The

Table 1: Experimental spectroscopic constants for the six observed rotamers of cysteine, and ab initio spectroscopic constants for the low-energy conformers shown in Figure 1.

Experimental	L	M	N	O	P	Q
A[a] [MHz]	4235.63210(58)[b]	4359.22320(77)	2889.44652(93)	3071.1437(15)	3216.218(26)	3004.1689(90)
B [MHz]	1187.27897(20)	1178.27610(13)	1622.99829(32)	1606.53664(36)	1572.74943(63)	1527.40718(53)
C [MHz]	1003.10663(23)	1015.27433(13)	1367.83448(26)	1331.80185(34)	1276.79135(55)	1210.70722(46)
χ_{aa} [MHz]	−4.263(11)	−0.4060(9)	−0.1465(36)	−3.1200(53)	[0.0][c]	0.505(10)
χ_{bb} [MHz]	2.776(11)	2.2314(43)	0.4419(43)	2.4418(61)	−0.449(25)	−1.991(20)
χ_{cc} [MHz]	1.488(11)	−1.8254(43)	−0.2954(43)	0.6782(61)	0.449(25)	1.486(20)

MP2/6-311++G(d,p)	Ia	IIa	III_αa	Ib	I′b	IIb	III_αb	III_βb	IIc	III_αc	III_βc
A [MHz]	4221	4346	4277	2855	2834	3040	2934	3114	3526	2813	2934
B [MHz]	1185	1181	1143	1664	1717	1623	1606	1607	1452	1613	1559
C [MHz])	1013	1023	1043	1386	1425	1347	1372	1307	1084	1278	1235
χ_{aa} [MHz]	−4.67	−0.32	−4.62	−0.01	−3.13	−3.14	−1.88	−0.03	−3.15	0.73	0.61
χ_{bb} [MHz]	2.86	2.25	2.87	0.25	2.86	2.59	1.13	−0.33	1.82	−2.33	−2.19
χ_{cc} [MHz]	1.80	−1.93	1.76	−0.24	0.28	0.55	0.75	0.37	1.33	1.60	1.58
ΔE_{MP4}[d] [cm^{-1}]	450	527	848	325	424	0	785	585	1010	987	765
μ_a [D]	1.9	3.1	2.0	−1.1	−1.2	1.6	−1.3	−2.0	0.5	1.4	2.3
μ_b [D]	0.2	−2.3	−1.8	1.5	−0.9	−4.0	−1.7	0.0	−4.8	−0.2	0.1
μ_c [D]	0.5	−1.5	−0.9	−1.2	−2.4	−1.6	0.3	0.1	−0.2	−2.1	0.0
μ_{total} [D]	2.0	4.2	2.9	2.2	2.8	4.6	2.2	2.0	4.9	2.5	2.3

[a] A, B, and C represent the rotational constants; χ_{aa}, χ_{bb}, and χ_{cc} are elements of the ^{14}N nuclear quadrupole coupling tensor; μ_a, μ_b, and μ_c are the electrical dipole moment components. [b] Standard errors are shown in parentheses in units of the last digit. [c] Parameters in square brackets were kept fixed in the fit. [d] Relative energies from single-point MP4 calculations on the MP2 structures using the 6-311++G(d,p) basis set.

manifold of lines originating from conformers Ia and IIa could be easily identified and excluded, thus allowing the assignment of four additional rotamers. Measured transitions were fitted following the same procedure as described above, which led to the spectroscopic parameters collected in columns N, O, P, and Q of Table 1. The magnitude of the rotational constants made them consistent only with the values predicted for conformers of the "b" and "c" families. A detailed comparison between the experimental and theoretical values of the rotational and quadrupole coupling constants in Table 1 led to the following identification: N corresponds to Ib, O to IIb, P to III_βb, and Q to III_βc. It is noteworthy that the observation of nuclear quadrupole coupling arising from the presence of a ^{14}N nucleus, native to all amino acids, constitutes a tool for the identification of conformers, and allows the orientation of the $-NH_2$ group to be established. As an example, the $3_{0,3}\leftarrow2_{0,2}$ rotational transition is shown in Figure 2 for the six detected conformers. The measured experimental frequencies of all the observed conformers are collected in Tables S1–S6 of the Supporting Information, together with a complete set of spectroscopic constants in Table S7.

The intramolecular hydrogen bonds that can be established between the three polar groups in cysteine control the preferred cysteine conformers. As can be seen in Figure 2, conformers Ia and Ib are both stabilized by an N–H···O=C bond and a *cis*-COOH interaction similar to those occurring in aliphatic α-amino acids.[5–7,10–13,15,16] The two conformers differ in their side-chain interactions: in conformer Ia there is an S–H···N hydrogen bond while in conformer Ib the hydrogen atom of the S-H group points to the carboxylic region. The available experimental parameters do not allow us to specify the location of the hydrogen atom. Conformers IIa and IIb both possess a *trans*-COOH arrangement, which allows the formation of O–H···N hydrogen bonds, which are also present in α-amino acids without polar side chains, as well as an N–H···S interaction. However, the thiol group in conformer IIb also interacts with the -COOH group and this may account for the higher stability of IIb in comparison to IIa. The two type III conformers detected exhibit an N–H···O–H hydrogen bond and a *cis*-COOH configuration. An additional N–H···S–H interaction seems to take place in conformer III_βb. As in Ib and IIb, the side chain of conformer III_βb points to the -COOH region. In conformer III_βc, the H atom of the -SH group interacts with the carbonyl oxygen atom of the –COOH group.

Type III conformers have not been observed in aliphatic α-amino acids, except in serine.[5] Their nonobservation has been explained in terms of a collisional relaxation to the lowest energy type I conformer. Conformational relaxation (III→I) takes place through rotation of the -COOH group if the energy barriers to interconversion are sufficiently low.[24–26] This could be the case for conformer III_αa of cysteine, which was not detected in the supersonic expansion. Ab initio calculations predict a low interconversion barrier (III_αa→Ia) of about 220 cm^{-1} (see Figure S1 in the Supporting Information), which allows a relaxation process to take place. In contrast, conformers III_βb and III_βc have been observed for cysteine. Conformer III_βc is the lowest energy form belonging to the c family, and thus it cannot relax to any other conformers of this family. Both Ib and III_βb conformers coexist in the supersonic expansion, despite the fact that conformer III_βb is predicted to lie at about 250 cm^{-1} above conformer Ib. The energy barrier to interconversion (III_βb→Ib) is calculated to be approximately 700 cm^{-1} (see Figure S2 in the Supporting Information), which is high enough to hinder relaxation. Experimental measurements using a different carrier gas[24,27] have been performed, but they do not indicate any evidence of relaxation. No signals that could be attributed to conformer I′b were observed in the rotational spectrum of cysteine, despite long and intense searches in which hundreds of cycles were accumulated. This fact could be attributed to a relaxation to the lower energy conformer Ib by a complex pathway involving motion of both the $-NH_2$ and -SH groups in the amino acid.

The post-expansion abundances of the detected conformers can be evaluated by measurement of the relative intensities. The estimated ratios, assuming that the line intensity is proportional to the corresponding electrical dipole moment component (see Table 1 for μ_i, i = a,b,c), are $N_{IIb}/N_{Ia}/N_{Ib}/N_{IIa}/N_{III\beta c}/N_{III\beta b} = 10:10:8:3:3:2$. These values are not in good qualitative agreement with the energies predicted by in vacuo ab initio calculations, which show conformer IIb to be the global minimum. The experimental abundances are more adequately related to the equilibrium conformational

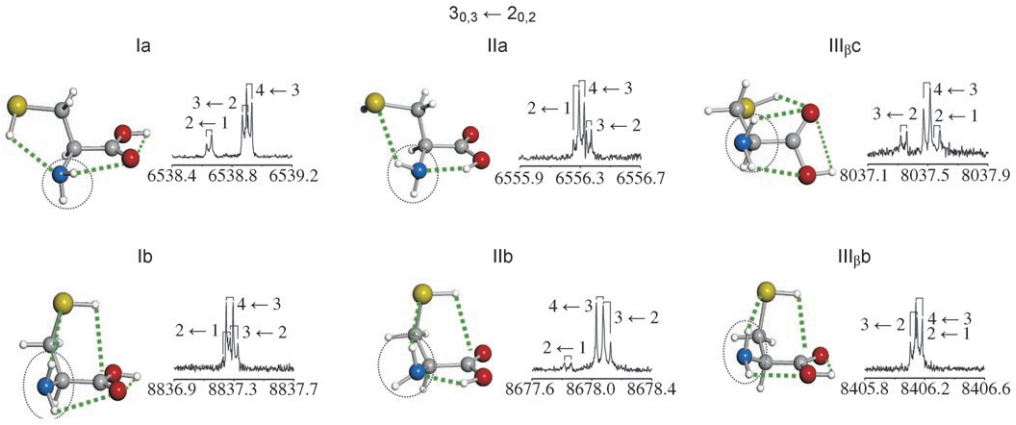

Figure 2. Rotational transition $3_{0,3}\leftarrow2_{0,2}$ for the six observed conformers of cysteine. The hyperfine components (F′←F″) are labeled by the quantum number $F = I + J$. Each component appears as a doublet because of the Doppler effect.

distribution at the carrier gas temperature.[11] Therefore, the equilibrium populations of the lowest energy conformers of cysteine at 298 K have been calculated from the ab initio Gibbs energies as $N_{IIb}/N_{Ia}/N_{Ib}/N_{I'b}/N_{IIa}/N_{III\beta c}/N_{III\beta b}/N_{III\alpha a} = 32:19:18:12:3:3:5:3$. However, if we take into account that the relaxation processes I′b→Ib and III_αa→Ia take place, we obtain a corrected prediction for the populations of the observed conformers $N_{IIb}/N_{Ia}/N_{Ib}/N_{IIa}/N_{III\beta c}/N_{III\beta b} = 32:22:30:3:3:5$. These results show the same trend as those found in the experimental observations of three fairly abundant conformers and three other forms with significantly lower populations. Conformers Ia and IIb are similarly abundant in our molecular beam, but it should be taken into account that the experimental abundance estimated for conformer Ia has a contribution from conformer III_αa. This situation points to a higher stability of IIb, which can be considered the global minimum. This result is in contrast with the behavior of aliphatic α-amino acids investigated to date,[5–7,10–13,15,16] where a type I conformer is observed as a global minimum. In conformer IIb of cysteine, three sequential intramolecular hydrogen bonds seem to close a ring, which is particularly stable[28] and can be responsible for the enhanced stability of conformer IIb with respect to α-amino acids with nonpolar side chains.

The presence of a polar side chain allows the observation of forms not observed before in α-amino acids with nonpolar side chains. In fact, the interaction of the -CH_2SH group with the -COOH group increases the energy barrier for the III↔I interconversion or reverses the conformational stability of forms III and I, thus making possible the existence of conformers III in the supersonic jet. The same behavior has been observed for serine,[5] which has a polar -CH_2OH side chain.

The six observed conformers of cysteine are the consequence not only of their relative energies but also of the energy barriers for interconversion between them. They constitute the conformational basis to understand the structure of cysteine in the gas phase. Our results can be contrasted with those recently reported in IR matrices, in which from three IR bands in the N-H, O-H, and S-H regions the authors speculated on the existence of three to six conformers of cysteine.[19] The quality of the information provided by LA-MB-FTMW spectroscopy and the fact that it is directly comparable with ab initio computations provide an unmatched means to conclusively identify low-energy conformers.

LA-MB-FTMW spectroscopy remains a developing technique, yet it has matured sufficiently to make an impact on a variety of problems in regard to biomolecular structure. In addition to amino acids, studies on nitrogen bases[29,30] and neurotransmitters have been, and are currently being, carried out. Continued advances are still needed to expand the scope of problems that this technique can address: namely microsolvation, dipeptides, or larger biomolecules. Further studies on amino acids are also needed to collect a sufficient number of experimental data that will improve our understanding of the interplay of intramolecular interactions and how they influence conformational preferences.

Experimental Section

The rotational spectrum of cysteine was investigated by using a LA-MB-FTMW spectrometer[1,2] which works in the 5–18 GHz frequency region. Solid samples of cysteine (99%, Aldrich) were vaporized by ablation with the second harmonics of a Nd:YAG laser (ca. 50 mJ per pulse). The amino acid molecules were seeded in a Ne flow at 5.5 bar and expanded adiabatically into a Fabry–Pérot resonator to form a molecular beam. A short microwave radiation pulse (0.3 μs) was then applied to macroscopically polarize the molecules in the beam. The subsequent molecular de-excitation signal was collected and Fourier transformed to obtain the frequency spectrum. In our setup, the microwave radiation travels parallel to the axis of the resonator, and consequently all transitions appear as doublets because of the Doppler effect (see Figure 2). The estimated accuracy of the frequency measurements is greater than 3 kHz.

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