

EXPERIMENTAL AND THEORETICAL INFRA-RED SPECTRAL STUDY OF AMINO ACID ALANINE

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Abstract: Infra-red explorations of amino acid alanine experimentally and theoretically have been performed. The IR frequencies were calculated by semi-empirical methods (PM6 and RM1) and ab initio methods (B3LYP/6-31+G(d)) and were scaled down by factors of 0.925 (RM1), 1.09 (PM6) and 0.89 (B3LYP/6-31+G(d)). The calculated and experimental vibrational frequencies have shown good general agreement.

Keywords: Vibrational studies, Amino Acids, semi-empirical methods (PM6 and RM1), ab initio methods (B3LYP/6-31+G(d)).

Introduction

Amino acids are the molecular building blocks of peptides and proteins. The unit structure adopted by amino acids, on condensation into larger molecules, effectively determines their secondary structure in crystalline samples. Amino acid molecules have a zwitterionic structure when they are in the liquid or solid phase. Indeed, these two cases allow hydrogen bonds to be formed, stabilizing the ionic conformation $R-CH(COO^-)NH_3^+$. On the contrary, the intermolecular bonds are not present in the gas phase and these molecules have a non-zwitterionic structure $R-CH(COOH)NH_2$.

This has been confirmed by experimental studies of the amino acid glycine¹⁻³ and alanine⁴. There are two long-standing problems encountered in the study of zwitterionic amino acids by infrared (IR) absorption spectroscopy and ab initio molecular orbital methods. The first concerns the inability of standard ab initio molecular orbital calculations (for isolated molecules) to provide optimized structures for the monomeric zwitterionic forms of the amino acids. A number of molecular orbital studies (self-consistent field (SCF) methods)⁵⁻⁷ have shown that the zwitterionic amino acids do not exist in the gas phase, as isolated monomers.

This assumption has been established empirically by matrix isolation (MI) studies⁸⁻¹⁰, a procedure in which the amino acids are first vaporized, then trapped and isolated in inert gas matrices at very low temperatures. The MI infrared spectra clearly show that amino acids are present in un-ionized neutral forms after evaporation. For the monomers, the structure optimizations started at the zwitterionic forms, with large basis sets, always converge to the un-ionized neutral molecules. For some smaller basis sets (e.g., 6-31G(d)), convergence to structures with strong intramolecular hydrogen bonding is obtained. These are not expected to be real structures but occur as a consequence of the relatively low theoretical level of the calculations.

Measurement of the IR absorption spectra of the solid (KBr pellet), and solution are not of the monomer but of tiny crystals in the case of KBr pellets, and of strong solute-solvent hydrogen bonding in the case of aqueous solutions. Thus, with the existing standard sampling techniques IR absorption spectra can only be measured when the zwitterions are strongly perturbed, either by neighbouring zwitterions (crystals in KBr pellets), or in solution (strong H-bonding). Other traditional methods involving evaporation of sample (such as MI, molecular beam and gas phase) to obtain monomeric spectra are essentially inapplicable to studying zwitterionic amino acids because of the structural transformation, from zwitterionic to neutral un-ionized form.

Self-consistent reaction field (SCRf) methods [11], which are implemented in Gaussian packages include the solvation effect. These methods model the solvent as a continuum of uniform dielectric constant (ϵ), the reaction field, which can interact with the solute molecules and lead to net stabilization. It should be noted that the solvent used in SCRf calculations is restricted to non-aqueous system [11], because as pointed out by Foresman, where intermolecular interactions are relatively large the continuum model is inappropriate, and specific interactions have also to be included. For aqueous solutions this would require the inclusion of a shell of water molecules inside the cavity in addition to the solute [12]. It was established for glycine in aqueous solution, two water molecules are sufficient to stabilize the glycine zwitterions [13]. Therefore, the use of the continuum model in which no specific intermolecular interactions are included in the calculations excludes comparison with the spectra of aqueous solution and crystal. Moreover, the IR and Raman spectra of amino acids in aqueous solution are usually broad, overlapped or incomplete as a result of strong solvent absorption and solute-solvent interaction. Even in the event that appropriately calculated spectra could be obtained, satisfactory and complete comparisons would be difficult to establish.

Alanine is the smallest naturally occurring chiral amino acid. The assignment of its fundamental vibrations is of importance in modeling amino acids and the nature of the mechanism of their inversion.

The infrared spectra of the nonionized alanine have been analyzed and assigned using DFT/B3LYP/aug-cc-pVDZ and MP2/aug-cc-pVDZ for geometry and frequency theoretical calculations. These methods yields vibrational frequencies in excellent agreement with experimental data [14]. The infrared spectra of the alanine molecule also have been studied in solid as well as in aqueous solution [15]. The vibration frequencies for the fundamental modes of alanine in neutral and zwitterionic form have been calculated using AM1, RHF, and DFT method with different basis sets [15]. RHF/6-31G, DFT/6-31G, 6-31+G* and 6-311++G** calculations for vibrational frequencies of both l- and d-alanine and zwitterionic alanine (zala) have been performed in both gas phase and in aqueous solution [15].

Methods of Calculation

Semiempirical Methods

The Semi empirical theoretical approaches RM1 and PM6 were used as implemented in MOPAC2007 package, on a personal computer. In this procedure, we first searched for the energy minima on the potential energy surface of the selected amino acid alanine. The molecular structures corresponding to the global minima in the potential energy surface for the non-zwitterionic amino acids were predicted starting from a structure with standard parameters, which was optimized simultaneously employing very restricted convergence criteria in the last steps of the computations.

The optimized and minimized RM1 and PM6 geometries were used as the reference geometry to estimate the IR frequencies of the amino acid alanine. It is possible to find a local minimum of the potential energy surface of the amino acid, where all the calculated frequencies were positive, thus agreeing the comparison with experimental results. A scaling factor of 0.925 for RM1 common to all frequencies is used to adjust the theoretical frequencies before it was compared with experimental¹⁸, and 1.09 factor was used for PM6.

Ab initio Methods

The ab initio M.O calculations were carried out employing the Gaussian 03 program. In this technique we first searched for the energy minima on the PES of the amino acid corresponding to the lowest energy conformer and then calculated infrared frequencies using harmonic approximations. Initially, geometry was optimized using Hartree-Fock level of theory with 6-31+G(d) basis set (RHF/6-31+G(d)) and less effective methods of Density Functional Theory (DFT) at B3LYP/6-31+G(d) which consider electron correlations with three-parameters hybrid functions combined with the Lee, Yang and Parr Correlation function¹⁶. The optimized geometry of amino acid alanine was used to calculate the vibrational frequencies. The calculated frequencies values were scaled down by a single factor of 0.89 before it was compared with experimental data

Results and Discussion

The optimized semiempirical RM1, PM6 and ab initio B3LYP/6-31+G(d) geometries were used as starting point to carry force field calculations for non-zwitterionic alanine. To minimize the systematic errors of these calculations, scaling factors of 0.89, 0.925 and 1.09 were applied for ab initio, RM1 and PM6 respectively. We have listed the scaled calculated vibrational frequencies of non-zwitterionic alanine. We have also proposed assignments for the observed frequencies.

Assignment of alanine

The non-zwitterionic structure of l-alanine is shown in Figure. 1. The assignment for calculated vibrational frequencies and those observed in the IR spectra are depicted. The calculated and experimental vibrational frequencies show a good general agreement. The frequencies observed at 3099 cm⁻¹ is assigned to the asymmetric NH stretching mode.

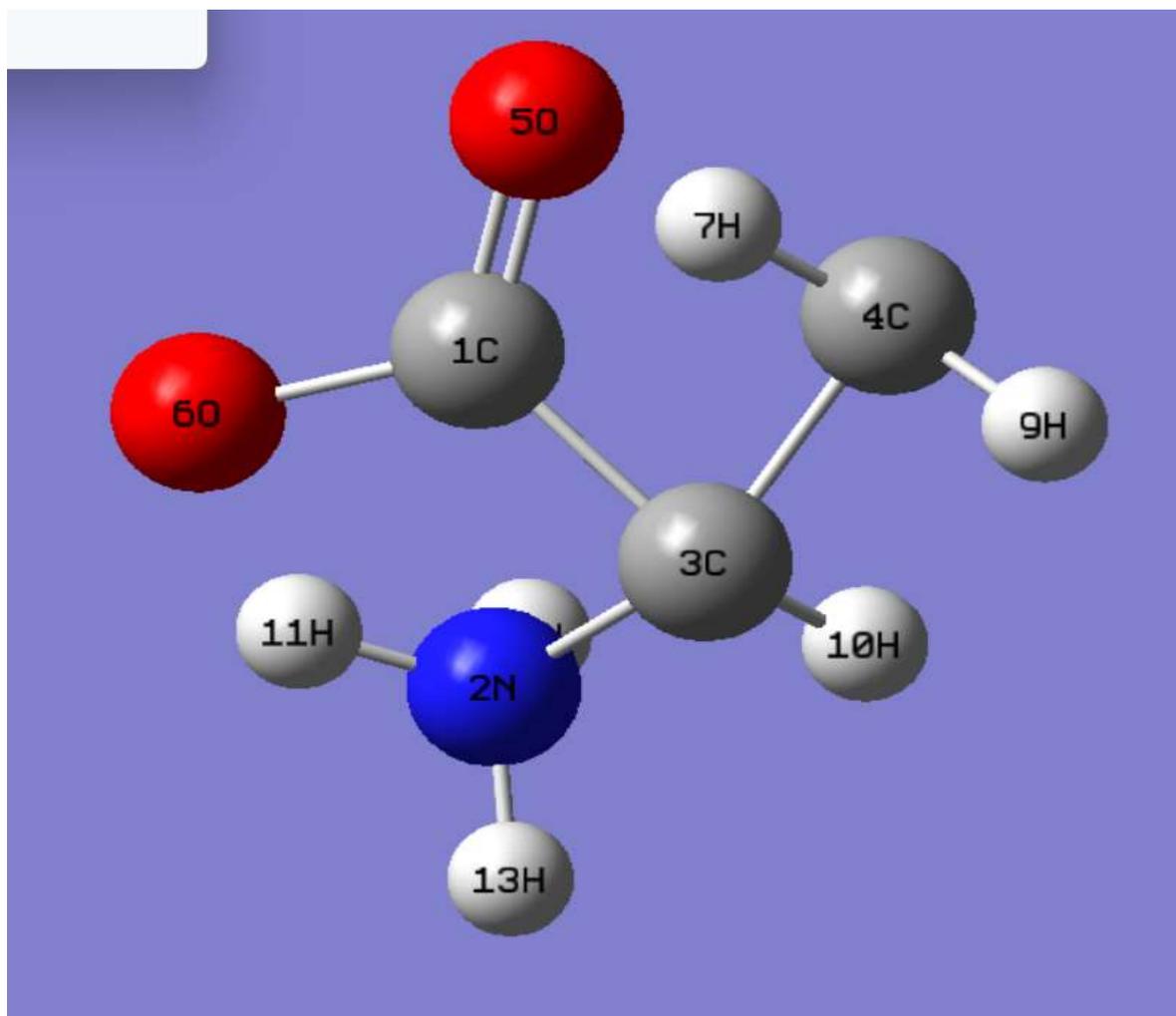


Fig. 1. Molecular Structure of Alanine as projected by the ab initio method, with the atomic numbering used in this work.

Relative Stability of Amino Acid (ΔE) at Different Semiempirical Methods RM1 and PM6.

Amino acid	Heat of formation in Kcal/mol	
	PM6	RM1
Alanine	-89.28	-91.94

The corresponding theoretical values at B3LYP/6-31+G(d), RM1 and the PM6 level are 3310 cm^{-1} , 3085 cm^{-1} and 3082 cm^{-1} , respectively, literature work at 3389 cm^{-1} (ab initio¹⁵), 3085 cm^{-1} (AM1¹⁵), which shows semi-empirical methods give closed value to the experimental methods than ab initio. The peak calculated at 3167 cm^{-1} , 3071 cm^{-1} and 3041 cm^{-1} having no counterpart in the IR spectrum refers to the CH stretching mode. The peak observed at 2926 cm^{-1} in the IR is attributed to the asymmetric CH stretching in the CH₃ group, since the corresponding theoretical values are 3096 cm^{-1} , 3065 cm^{-1} and 3035 cm^{-1} for ab initio, RM1 and PM6 respectively, in the literature 2898 cm^{-1} by ab initio¹⁵ and 2758 cm^{-1} for Am1¹⁵. The peak observed at 2604 cm^{-1} in the IR spectrum is assigned to the NH stretching in the NH₃ group. The corresponding calculated frequencies are 2691 cm^{-1} , 2633 cm^{-1} and 2748 cm^{-1} by ab initio, RM1 and PM6 respectively. The frequency of 2631 cm^{-1} was calculated by ab initio¹⁵. The calculated peaks at 1643 cm^{-1} , 1809 cm^{-1} and 1979 cm^{-1} for the asym. COO⁻ stretching coupled with a NH bending mode, does not appear in the IR spectrum. Barthes et. al.¹⁶ have reported this frequency at 1630 cm^{-1} in IR spectrum. The peak at 1595 cm^{-1} in the IR spectrum is attributed to the NH₂ scissoring mode, according to calculated values at 1522 cm^{-1} , 1514 cm^{-1} and 1755 cm^{-1} . The calculation of this peak was reported at 1620 cm^{-1} (ab initio¹⁴) and 1464 cm^{-1} (Am1¹⁵). Other vibrations in the finger print region ($400\text{-}1354\text{ cm}^{-1}$) and the corresponding calculated frequencies by ab initio, RM1 and PM6 are listed in Table. 1.

Table.1. Calculated Vibrational Frequencies cm^{-1} for Alanine Compared with Experimental.

Mode	Obs.	RM1	PM6	Ab initio	Assignment
V1	3099	3085	3082	3310	Asym. NH_2 stretching
V2		3071	3041	3167	CH stretching in CH_3
V3	2926	3065	3035	3096	Asm. CH stretching in CH_3
V4		2853	2936	2796	Puckering of CH_3
V5		2801	2924	2774	CH stretching
V6		2798	2903	2717	CH stretching in CH_3 group
V7	2604	2633	2748	2691	NH stretching NH_2
V8		1809	1979	1643	Asym. COO^- stretching + NH bend
V9	1595	1514	1755	1522	NH_2 bending
V10	1354	1410	1475	1354	NH_2 scissoring
V11	1307	1256	1328	1343	NH_3 puckering
V12		1245	1285	1276	CH_3 deformation
V13		1226	1248	1262	CH_3 deformation
V14		1198	1246	1195	CH_3 puckering
V15		1194	1230	1150	Mixed Vibration (NH_2 puck + CH_3 def)
V16		1182	1184	1133	CH bend + NH bend
V17	1149	1104	1176	1044	CH_2 bend + CH bend. + NH_2 bend
V18	1114	1062	1165	1005	C-C Stretch + CH bend + NH bend
V19	1014	985	1073	966	H-N-C-C bend.
V20		965	984	913	H-C-C-H bend + NH_2 def
V21	918 930	935		821	CNH bend
V22	850 826	893	816		CN stretch. + C-C stretching
V23	769 677	840	706		HNC + CCH bending
V24	646	657	676	654	CCC + COO Bending
V25	543	570	558	569	OCOH + NH_2 bending
V26	412	486	472	441	OH bending
V27		378	426	432	NCC bending + CCCO

Conclusion

The infra-red spectral assignments for amino acid alanine have been performed experimentally and theoretically. New parameters of semi-empirical methods in MOPAC2007 MP6 and RM1 are used. Comparison of scaled theoretical and experimental vibrational frequencies exhibit good correlation confirming the reliability of the method employed here.

Conflict of Interest: The authors of this manuscript do hereby declare conflict of no interest whatsoever.

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