

## Vibrational spectra (FT-IR, Raman and MI-IR) of $\alpha$ - and $\beta$ -alanine

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### Abstract

The vibrational spectra of  $\alpha$ - and  $\beta$ -alanine molecules in both their zwitterionic and neutral forms are studied by FT-IR, Raman and MI-IR spectroscopy. Together with results from theoretical SCF-MO ab initio calculations, the spectroscopic data obtained under the various experimental conditions used in this study (crystalline phase; low temperature matrix isolated molecules) enable to undertake a detailed assignment of the vibrational spectra of the studied compounds. © 1997 Elsevier Science B.V.

*Keywords:* FTIR spectroscopy; Raman spectroscopy; Matrix isolation spectroscopy; Vibrational assignment; Alanine

### 1. Introduction

$\alpha$ -aminoacids are the building blocks of proteins and, for this reason, they have been extensively studied. On the contrary,  $n$ -aminoacids, in spite of their biological importance, have deserved much less attention. In fact, various  $n$ -aminoacids, such as for example GABA ( $\gamma$ -aminobutyric acid) and  $\beta$ -alanine, participate directly, as information carrier molecules, in the neurotransmission processes occurring in the mammalian nervous system [1–3].

Aminoacids are also very interesting systems for fundamental research, since in solution or in the crystalline state they are present as zwitterions, whereas in the gaseous state or isolated in a noble gas matrix at low temperature the neutral forms are much more stable [4]. Besides, the zwitterion and neutral forms

show marked differences regarding their geometries and electronic structures, which are well reflected on their vibrational spectra.

Glycine, simultaneously an  $\alpha$ - and  $n$ -aminoacid,  $\alpha$ -alanine and its structural isomer  $\beta$ -alanine (Fig. 1) are the three simplest aminoacids. While glycine has been the subject of extensive structural and vibrational studies [4], the other two aminoacids, specially  $\beta$ -alanine, which is an  $n$ -aminoacid, have not deserved much attention and, as far as we know, vibrational data on their neutral forms have not yet been reported. Although the most biochemically relevant species correspond to the zwitterionic species, the study of the neutral molecules has attracted an ever growing attention, since its great importance is recognized for identification of interstellar aminoacids, which may provide insight into the formation of large molecules in space.

In this work, FT-IR and Raman spectroscopic measurements were undertaken on crystalline  $\alpha$ - and

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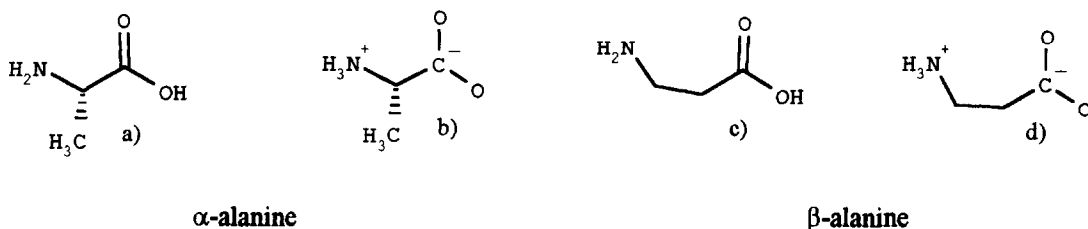


Fig. 1. Neutral and zwitterionic forms of  $\alpha$ - and  $\beta$ -alanine molecules.

$\beta$ -alanine to study the vibrational spectra of these molecules in their zwitterionic forms, and matrix isolation infrared spectroscopy (MI-IR) was used to analyze the vibrational spectra of the neutral forms. A detailed assignment of the spectra, supported by theoretical data obtained by SCF-MO ab initio calculations, is also presented.

## 2. Experimental

FT-IR spectra of the solid compounds were obtained as KBr pellets on a Perkin Elmer 1760 FT-IR spectrometer, equipped with a germanium on CsI beam splitter and a DTGS detector, in the

wavenumber range  $400\text{--}4000\text{ cm}^{-1}$ , with 32 scans and a spectral resolution of  $2\text{ cm}^{-1}$ , at room temperature.

Raman spectra were recorded using a SPEX 1403 double monochromator spectrometer (focal distance 0.85 m, aperture  $f/7.8$ ), equipped with holographic gratings with  $1800\text{ grooves mm}^{-1}$  (reference 1800-1SHD). The 514.5 nm argon laser (Spectra-Physics, model 164-05) line, adjusted to provide 220 mW power at the sample, was used as excitation radiation. Detection was effected using a thermoelectrically cooled Hamamatsu R928 photomultiplier. Spectra were recorded using increments of  $1\text{ cm}^{-1}$  and integration times of 1 s. Under these conditions, the estimated errors in wavenumbers are  $\pm 1\text{ cm}^{-1}$ .

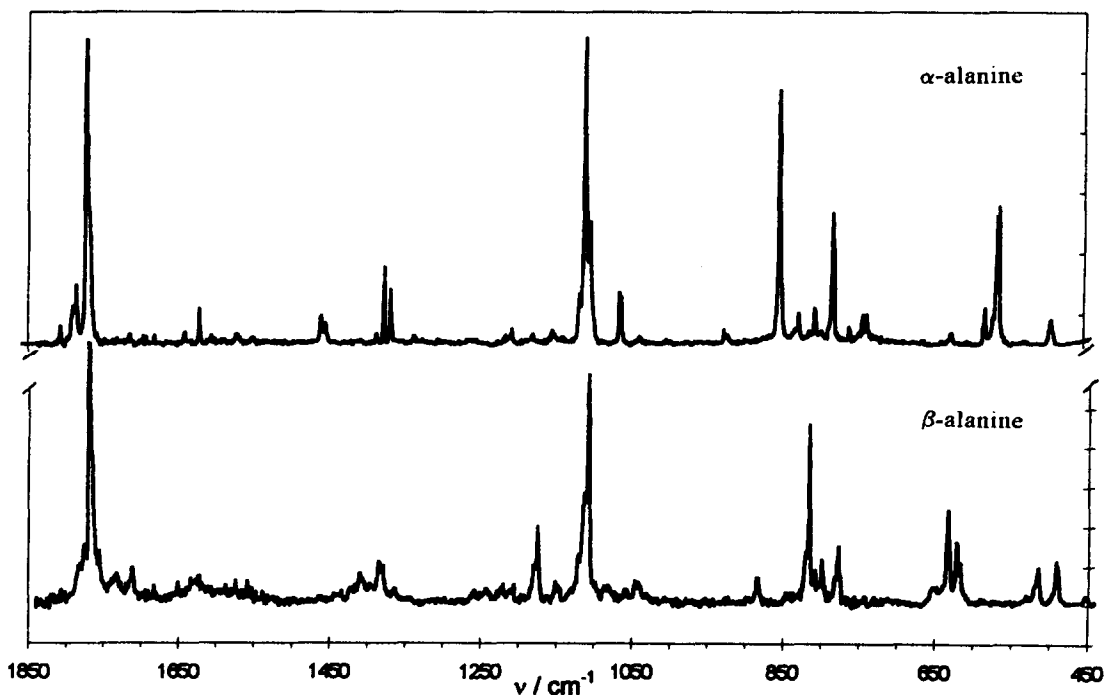


Fig. 2. MI-IR spectra of  $\alpha$ - and  $\beta$ -alanine (Ar matrix;  $T = 17\text{ K}$ ).

The exact description of the experimental system used to undertake the MI-IR studies has been reported elsewhere [5]. Essentially, the set up used is formed by a dispersive Specord IR75 (updated) infrared spectrometer and an open cycle liquid He cryostat. The samples were deposited in argon matrices over an optical subtract of CsI windows at 17 K. The sample:matrix ratio (1:750 for  $\alpha$ -alanine and 1:500 for  $\beta$ -alanine) was controlled by quartz

microbalances, for a fine adjustment of the flux rate of the deposition of both sample and matrix materials. Because aminoacids tend to decompose at melting temperatures, special care was taken in choosing the adequate temperatures in the Knudsen cells from where the samples were evaporated (429 K for  $\alpha$ -alanine and 422 K for  $\beta$ -alanine). After deposition at 17 K, the aminoacids retained the conformations and the same distribution present at the evaporation

Table 1  
Vibrational frequencies of neutral forms of  $\alpha$ - and  $\beta$ -alanine <sup>a</sup>

$\alpha$ -alanine			$\beta$ -alanine		
Assignment	Calc. (RHF/6-31G*)	Exp. <sup>b</sup> (MI-IR)	Assignment	Calc. (RHF/6-31G*)	Exp. <sup>b</sup> (MI-IR)
$\nu$ OH	3605	3560, <b>3555</b> ,3546	$\nu$ OH	3607	3569, <b>3559</b> ,3547
$\nu_{as}$ NH <sub>2</sub>	3392	n.o.	$\nu_{as}$ NH <sub>2</sub>	3392	3408
$\nu_s$ NH <sub>2</sub>	3321	n.o.	$\nu_s$ NH <sub>2</sub>	3316	n.o.
$\nu_{as}$ CH <sub>3</sub>	2949	2999, <b>2981</b>	$\nu_{as}$ CH <sub>2</sub>	2932	2958
$\nu_s$ CH <sub>3</sub>	2925	2940	$\nu_s$ CH <sub>2</sub>	2893	2925
$\nu$ CH	2886	2886	$\nu_{as}$ CH <sub>2</sub>	2885	2890
$\nu_s$ CH <sub>3</sub>	2859	n.o.	$\nu_s$ CH <sub>2</sub>	2858	2867
$\nu$ C=O	1805	1791,1787 <b>1774</b> ,1771	$\nu$ C=O	1807	1782,1776, <b>1770</b> 1767,1761,1757
$\delta$ NH <sub>2</sub>	1646	1642, <b>1622</b>	$\delta$ NH <sub>2</sub>	1636	<b>1634</b> ,1623
$\delta_{as}$ CH <sub>3</sub>	1463	1460	$\delta$ CH <sub>2</sub>	1461	1463
$\delta_{as}$ CH <sub>3</sub>	1459	1454	$\delta$ CH <sub>2</sub>	1434	1445,1441, <b>1435</b>
$\delta$ CH	1401	1408	$\omega$ CH <sub>2</sub>	1409	<b>1411</b> ,1407
$\delta_s$ CH <sub>3</sub>	1378	1386, <b>1376</b>	twCH <sub>2</sub>	1347	<b>1386</b> ,1380
$\delta$ CH	1349	1368, <b>1335</b>	$\omega$ CH <sub>2</sub>	1341	1365
twNH <sub>2</sub>	1275	n.o.	twCH <sub>2</sub>	1271	1259, <b>1244</b>
$\delta$ COH	1247	1215, <b>1206</b>	$\delta$ COH	1267	<b>1221</b> ,1207
$\delta$ C–O	1164	1153	nC–O	1159	1181, <b>1176</b>
$\nu$ CN	1124	1117, <b>1110</b> ,1105	twNH <sub>2</sub> + $\rho$ CH <sub>2</sub>	1126	<b>1152</b> ,1146
$\rho$ CH <sub>3</sub>	1060	1064	nCN	1050	1113, <b>1109</b>
$\rho$ CH <sub>3</sub>	990	<b>1037</b> ,1002	$\nu$ H <sub>2</sub> C–CH <sub>2</sub>	1018	1088, <b>1082</b>
$\omega$ NH <sub>2</sub>	915	925, <b>920</b>	$\rho$ CH <sub>2</sub> + twNH <sub>2</sub>	946	1047, <b>1042</b>
$\nu$ C–CH <sub>3</sub>	894	<b>852</b> ,826	$\nu$ C–COOH	871	885
$\nu$ C–COOH	777	805, <b>782</b>	$\omega$ NH <sub>2</sub>	828	822, <b>817</b> ,800
$\omega$ COO	730	741, <b>736</b>	$\rho$ CH <sub>2</sub>	752	783, <b>779</b>
$\delta$ COO	615	623	$\tau$ C–O	630	654, <b>634</b>
$\tau$ C–O	566	580, <b>562</b>	$\delta$ COO	620	<b>623</b> ,617
$\delta$ CCO	477	493	$\omega$ COO	503	516, <b>492</b>
$\delta$ CCN	367		$\delta$ CCO	434	<b>453</b> ,451
$\delta$ CCN	297		$\delta$ CCN	348	
$\delta$ CCC	238		$\tau$ NH <sub>2</sub>	278	
$\tau$ NH <sub>2</sub>	227		$\delta$ C–C–C	174	
$\tau$ CH <sub>3</sub>	216		$\tau$ H <sub>2</sub> C–CH <sub>2</sub>	102	
$\tau$ COOH	51		$\tau$ COOH	47	

<sup>a</sup> Frequencies in cm<sup>-1</sup>;  $\nu$ , stretching;  $\delta$ , bending;  $\omega$ , wagging; tw, twisting;  $\rho$ , rocking;  $\tau$ , torsion; n.o., not observed; s, symmetric; as, asymmetric.

<sup>b</sup> The most intense band of a group of bands assigned to the same vibration is shown in bold.

temperature, remaining in the neutral form after instantaneous sublimation on the cold matrix. All spectra were recorded using a spectral resolution of  $0.2 \text{ cm}^{-1}$ .

The ab initio molecular orbital calculations were performed with a PC equipped with a Am486DX4/100 MHz processor, using GAUSSIAN 92 for Windows (Revision G-3) [6]. All calculations (structure optimizations and frequencies) were carried out at the Hartree–Fock level of theory with the 6-31G\* basis set [7]. The calculated frequency values were scaled down by a single factor of 0.89, and refer to the most stable conformers found in the HF/6-31G\* molecular potential energy surfaces by systematic scanning of the molecular configurational space (structural data are available from the corresponding author).

### 3. Results and discussion

Fig. 2 displays the  $1800\text{--}350 \text{ cm}^{-1}$  spectral region of the MI-IR spectra of the neutral forms of  $\alpha$ - and  $\beta$ -alanine. The corresponding theoretical (ab initio

RHF/6-31G\*) frequencies and the band assignments are presented in Table 1.

The calculated and experimental results show a good general agreement, with exceptions for some of the higher frequency modes (e.g.  $\nu\text{O-H}$ ,  $\nu\text{CH}$ ) and  $\nu\text{C=O}$ . In the case of the  $\nu\text{CH}$  vibrations, the predicted (scaled) frequencies are somewhat lower than those experimentally observed, probably due to the involvement of the  $\nu\text{CH}$  fundamentals in Fermi resonance interactions, as suggested by the appearance of a number of additional lower intensity bands in the  $\nu\text{CH}$  spectral region. On the other hand, the overestimation observed in the 6-31G\* predicted frequencies of  $\nu\text{O-H}$  and  $\nu\text{C=O}$  vibrations when compared to the MI-IR data follows the pattern previously found for similar molecules [8] and may then be attributed to matrix/solute interaction effects. It is important to note that, for both molecules, most of the bands have a well observable structure (in particular  $\nu\text{C=O}$ ) caused by both matrix splitting and the presence of different conformers. This result follows the trend previously observed for glycine [5] and a detailed study of these features is presently being carried out in our laboratories.

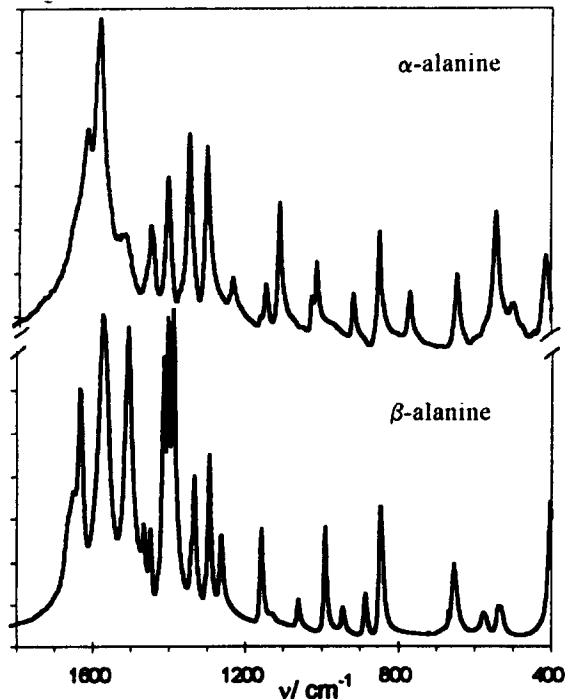


Fig. 3. FT-IR spectra of  $\alpha$ - and  $\beta$ -alanine.

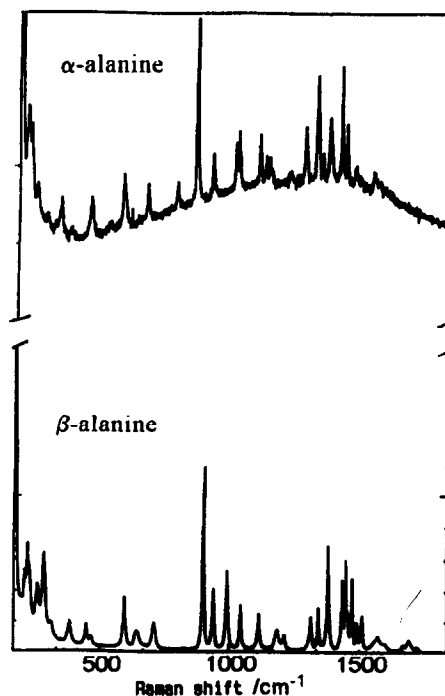


Fig. 4. Raman spectra of  $\alpha$ - and  $\beta$ -alanine.

The FT-IR spectra of the  $\alpha$ - and  $\beta$ -alanine zwitterions are presented in Fig. 3 and their Raman spectra are shown in Fig. 4. These spectra can be compared with the corresponding theoretical vibrational frequencies in Table 2. As in the case of the neutral forms of the studied molecules, the experimental and theoretical results obtained for the zwitterions agree quite well, the main exceptions to this general

rule occurring in vibrations which are similar to those that are also less well predicted for the neutral species (e.g.  $\nu_{\text{as}}\text{COO-H}$ ,  $\nu\text{CH}$ ).

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This study has been carried out under the PRAXIS

Table 2  
Vibrational frequencies of zwitterionic forms of  $\alpha$ - and  $\beta$ -alanine <sup>a</sup>

$\alpha$ -alanine				$\beta$ -alanine			
Assignment	Calc. (RHF/6-31G*)	Exp. <sup>b,c</sup> (IR-crystal)	Exp. <sup>b</sup> (Raman-crystal)	Assignment	Calc. (RHF/6-31G*)	Exp. <sup>b,c</sup> (IR-crystal)	Exp. <sup>b</sup> (Raman-crystal)
$\nu_{\text{as}}\text{NH}_3$	3388			$\nu_{\text{as}}\text{NH}_3$	3350		
$\nu_{\text{as}}\text{NH}_3$	3315			$\nu_{\text{as}}\text{NH}_3$	3350		
$\nu_{\text{as}}\text{CH}_3$	2966	3002	3011	$\nu_{\text{s}}\text{NH}_3$	3248		
$\nu\text{CH}$	2931	2982	2991	$\nu_{\text{as}}\text{CH}_2$	3028	3017	3016
$\nu_{\text{as}}\text{CH}_3$	2899	n.o.	2960	$\nu_{\text{s}}\text{CH}_2$	2965	2955	2980
$\nu_{\text{s}}\text{CH}_3$	2851	n.o.	2897	$\nu_{\text{as}}\text{CH}_2$	2883	n.o.	2936
$\nu_{\text{s}}\text{NH}_3$	2509	2597	n.o.	$\nu_{\text{s}}\text{CH}_2$	2847	n.o.	2917
$\nu_{\text{as}}\text{COO}$	1775	1623	n.o.	$\nu_{\text{as}}\text{COO}$	1751	1633	1630
$\delta_{\text{as}}\text{NH}_3$	1621	1592	1604	$\delta_{\text{as}}\text{NH}_3$	1632	1653	1686, <b>1655</b>
$\delta_{\text{as}}\text{NH}_3$	1592	1523	1527	$\delta_{\text{as}}\text{NH}_3$	1626	1573	1556, <b>1533</b>
$\delta_{\text{as}}\text{CH}_3$	1459	n.o.	1491	$\delta_{\text{s}}\text{NH}_3$	1454	1508	1519
$\delta_{\text{as}}\text{CH}_3$	1458	1452	1469	$\delta\text{CH}_2$	1442	1466	1473
$\delta_{\text{s}}\text{CH}_3$	1396	1408	1421	$\delta\text{CH}_2$	1441	1447	1452, <b>1434</b>
$\nu_{\text{s}}\text{COO}$	1354	1354	1390, <b>1368</b>	$\nu_{\text{s}}\text{COO}$	1344	1413, <b>1403</b>	1409, <b>1395</b>
$\delta_{\text{s}}\text{NH}_3$	1335	1307	1316	$\omega\text{CH}_2$	1299	1333	1338
$\nu\text{C-CH}_3$	1288	n.o.	n.o.	tw $\text{CH}_2$	1261	1294	1300
$\delta\text{CH}$	1241	1235	1250	tw $\text{CH}_2$	1229	1263	1270
$\rho\text{NH}_3$	1169	1149	<b>1166,1151</b>	$\omega\text{CH}_2$	1219	n.o.	n.o.
$\rho\text{CH}_3$	1071	1114	1125	$\nu\text{H}_2\text{C-CH}_2$	1073	<b>1158,1138</b>	1167, <b>1138</b>
$\delta\text{CH}$	1035	1027	1036	$\rho\text{NH}_3$	1033	1061	1068
$\rho\text{CH}_3$	960	1014	1024	$\rho\text{NH}_3$	904	991	998
$\rho\text{NH}_3$	943	919	930	$\rho\text{CH}_2$	863	945	945
$\nu\text{C-COO}$	838	851	862	$\nu\text{C-COO}$	825	886	893
$\nu\text{CN}$	783	769	781	$\rho\text{CH}_2$	757	n.o.	n.o.
$\omega\text{COO}$	745	n.o.	n.o.	nC-N	745	847	853
$\delta\text{COO}$	604	646	654	$\delta\text{COO}$	648	654	659
$\delta\text{CCO}$	500	<b>544,498</b>	<b>553,499</b>	$\omega\text{COO}$	564	<b>576,538</b>	589, <b>541</b>
$\delta\text{CCN}$	367	412	418	$\delta\text{CCO}$	443	403	410, <b>392</b>
$\delta\text{CCN}$	315		335	$\delta\text{CCN}$	318		327
$\tau\text{NH}_3$	270		<b>293,270</b>	$\tau\text{NH}_3$	219		<b>224,199</b>
$\delta\text{C-C-C}$	246		235	$\delta\text{C-C-C}$	187		167, <b>160</b>
$\tau\text{CH}_3$	226		194	$\tau\text{H}_2\text{C-CH}_2$	94		112
$\tau\text{COO}$	49			$\tau\text{COO}$	51		

<sup>a</sup> Frequencies in  $\text{cm}^{-1}$ ;  $\nu$ , stretching;  $\delta$ , bending;  $\omega$ , wagging; tw, twisting;  $\rho$ , rocking;  $\tau$ , torsion; n.o., not observed; s, symmetric; as, asymmetric.

<sup>b</sup> The most intense band of a group of bands assigned to the same vibration is shown in bold.

<sup>c</sup> The  $\text{nNH}_3$  vibrations give rise to very broad overlapping bands in the  $3400\text{--}2400\text{ cm}^{-1}$  spectral region.

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