Vibrational Spectra and Analysis of Tryptophan

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The Fourier transform infrared and the Fourier transform Raman spectra of tryptophan amino acid were recorded from 4000–400 cm^{-1} and 4000–100 cm^{-1} respectively. The spectra were analysed on the basis of fundamentals and the frequencies were assigned to various modes of vibration. Further, based on the present assignment, a partial vibrational analysis was carried out for this molecule and the results are briefly discussed.

INTRODUCTION

The amino acid tryptophan with aliphatic hydrocarbon R group with two aromatic rings are found in egg, human milk and cow's milk. None of the 20 amino acids found in protein absorbs light in the visible range. Three amino acids, *viz.*, tyrosine, tryptophan and phenylalanine absorb light significantly in the ultraviolet region which is used in estimating the protein content. Tryptophan is utilised for synthesis of a plant growth hormone. The symptoms of tryptophan deficiency are identical to those of nicotinamide deficiency. This indicates that tryptophan plays a major role just like the nicotinamide substitute. The deficiency of tryptophan causes pellagra which was endemic in regions such as southern United States. This is caused due to the lack of tryptophan in corn proteins which was the major food in these areas.

The transaminator product of tryptophan yields indole-3-acetic acid or auxin. NAD$^+$ can be synthesised either from tryptophan or from the vitamin nicotinic acid[1,2].

Recently Craig *et al.*[3] studied the electrocapillary properties of the amino acid tryptophan in 0.165 M sodium chloride over the temperature range 20–40°C. Tsuboi *et al.*[4] studied the Raman tensors for the tryptophan side chain in proteins determined by polarised Raman microspectroscopy of oriented N-acetyl-L-tryptophan crystals. A knowledge of Raman tensors for the tryptophan side chain should facilitate the determination of indole ring orientation in biological complexes, amenable to investigation by the method of polarised Raman microspectroscopy. The static and dynamical aspects of tryptophan powders at various temperatures have been studied by fluorescence spectroscopy using synchrotron radiation by Taniguchi *et al.*[5] Meshalkin[6] used the method of two

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photon standard to determine two photon absorption cross sections of aromatic amino acids such as tryptophan, tyrosine, phenylalanine and proteins at the wavelengths of the second harmonic of an Nd^{3+}:YAG laser. Hasselbacher *et al.*[7] assessed the local environments of the four tryptophan residues of the extra cellular domain of human tissue factor from difference absorption and fluorescence spectra. Doering *et al.*[8] investigated the long life time component in the tryptophan fluorescence of some proteins. The change in permanent dipole moment for the transition from ^1L_a state to the ground state of tryptophan is the key photophysical parameter for the interpretation of tryptophan flurescence spectra in terms of static and dynamic dielectric properties of the surrounding medium. Pierce *et al.*[9] have studied this parameter by means of electric field effect (stark) spectroscopy and interpreted the steady state and time resolved spectroscopy of tryptophan. The use of a long life time component of tryptophan to detect slow orientational fluctuations of proteins was carried out by Doring *et al.*[10] Smiley *et al.*[11] investigated interfacial peptide-lipid interactions by optical second harmonic generation from tryptophan SHG studies of peptides and proteins absorbed to membrane mimetic lipid and phospholipid monolayers with impact ability of peptides and proteins to penetrate and interact with the membrane surface. The air/water interface of aqueous tryptophan and (t-butyloxycarbonyl)-tryptophan-tryptophan (boc-trp-trp) has been investigated with linearly and axially polarised incident radiation by Crawford *et al.*[12] using the technique of interfacial second harmonic generation.

Iketali *et al.*[13] studied the UV and X-ray double resonance absorption process in the case of 1 µm tryptophan. By adopting a three state model for UV absorption they calculated the excited molecular density. They have also calculated the X-ray transmittance. Hence, dependence on the UV laser photo flux using X-ray absorption cross section obtained by a calculation based on the atomic Hartre-Slater method. The FK 506 binding protein (FKBP 12) is important in the immunosuppressant action of FK 506 and rapamycin. Silva *et al.*[14] have investigated the tryptophan side chain dynamics in FKBP 12, with and without a bound immunosuppressant by measuring the tryptophan time-resolved fluorescence anisotropy decay r(t).

Human serum albumin is an important carrier of organic anions in humoral media of the human organism. It is capable of affinely binding compounds differing in their chemical nature such as fatty acids, bilirubin, tryptophan, bile acids, etc. Hence Ivanov *et al.*[15] have carried out infrared and Raman spectroscopic studies of the structure of human serum albumin under various ligand loads. Sulkes[16] has performed a confirmational analysis of jet-cooled tryptophan analysis and histamine using the MM3-94 force field. As the vibrational data for tryptophan is not available in the literature, the present investigation has been undertaken to provide spectroscopic information. The vibrational spectra of tryptophan have been studied using solid phase FT Raman and FT infrared spectra and by using SVFF to assign the analysis in-plane and out-of-plane vibrational frequencies.

EXPERIMENTAL

The pure commercial sample obtained from Ms. Fluka A.G., Switzerland in solid form at room temperature was used as such. The FTIR spectrum was recorded in the region 4000–400 cm^{-1} using Shimadzu FTIR 8101 spectrophotometer. FT Raman spectrum was recorded in the region 4000–100 cm^{-1} using Brucker IFS 66 FTIR with FRA 106 Raman module equipped with Nd:YAG laser source operating on 10.6 μm line with 200 MW power. The spectrum was recorded with a scanning speed of 30 cm^{-1} min^{-1} with a spectral width 2.0 cm^{-1}. The frequencies for all sharp bands were accurate to ±1 cm^{-1}.

Normal coordinate analysis

The chemical structure of tryptophan is shown in Fig. 1. This molecule contains indole with alanine side chain. The spectra of tryptophan can be compared to benzene rings, pyrole rings and alanine. The benzene ring vibrations in the presence of substituents were investigated by several workers[17-19].
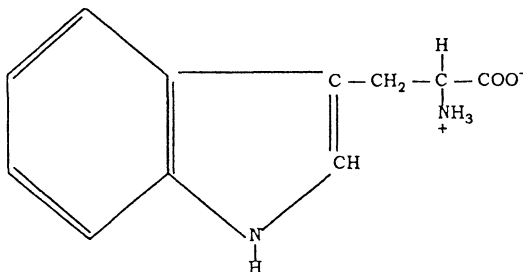

Fig. 1.   Structure of Tryptophan

The total number of atoms in tryptophan are 27 and hence the number of vibrational frequencies come to 75. They are distributed as 51 in-plane vibrations and 24 out-of-plane vibrations. The observed frequencies, the probable assignments for the observed bands are presented in Table-1. One H in alanine is replaced by indole which consists of benzene and pyrole rings.

A partial normal coordinate analysis is carried out for the molecule under study using simple valence force field due to the non-availability of all the vibrational frequencies from the spectra. These calculations were carried out using Wilson's F-G matrix method with the modified computer program used in the earlier work[20]. The SVFF has been shown to be very effective in the normal coordinate analysis and also the valence force constants can be transferred between the related molecules which is found to be very useful in the normal coordinate analysis of polyatomic molecules. The force constant used in the case of indole was transferred[21] and slight alterations were made in a few constants to obtain a close fit between the selected observed and calculated frequencies of tryptophan.

TABLE-1
OBSERVED AND CALCULATED FREQUENCIES OF TRYPTOPHAN

FTIR (cm^{-1})	Intensity	FTR (cm^{-1})	Intensity	Calculated frequency (cm^{-1})	Assignments	PED (%)
		155	vs		1610–1454	
		163	s		1455–1295	
		188	w		C-C-N out-of-plane bending	
		233	vw		C-C-C out-of-plan bending	
		264	vw		C-O-OH in-plane bending	
		300	vw		C-N-H$_2$ out-of-plane bending	
		350	vw		C-C-C out-of-plane bending	
		395	vw		C-C-C out-of-plane skeletal deformation	
420	s				C-C-C out-of-plane bending	
428	s	428	vw		C-N-H$_2$ in-plane bending	
450	m				C-C-C out-of-plane bending	
		466	vw		C-C-C out-of-plane bending	
503	m	505	vw		C-C-C inplane bending	
527	m	532	vw	526	C-C-C inplane skeletal deformation	68
		546	w		C-C-C out-of-plane bending	
584	w	583	vw		C-C-C in-plane skeletal deformation	
		596	m		N-H out-of-plane bending	
625	vw	627	vw		N-H$_2$ wagging	
660	vw	666	vw		C-C-C in-plane skeletal deformation	
699	vw	700	vw		C-C-C out-of-plane skeletal deformation	
743	m	743	s		C-C-C in-plane skeletal deformation	
		756	vs	751	Ring breathing	75
772	vw	773	m		C-C-C out-of-plane bending	
845	vw	837	w		C-H out-of-plane bending	
866	vw	866	s		C-C-C out-of-plane bending	
		882	m		C-C-C in-plane skeletal deformation	
922	vw	920	vw		C-H out-of-plane bending	
963	vw	966	vw		C-H out-of-palne bending	
		984	w		C-N-H$_2$ stretching	
1012	w	1008	vs	1001	Trigonal bending	81
1061	vw	1066	w		NH$_2$ rocking	
1095	w	1100	vw	1095	C-H in-plane bending	70
1128	vw	1119	w		C-H in-plane bending	68
		1150	vw		C-H in-plane bending	
1160	w	1162	w		C-H in-plane bending	
1202	vw	1207	w		C-C-O-OH stretching	

FTIR (cm^{-1})	Inten- sity	FTR (cm^{-1})	Inten- sity	Calculated frequency (cm^{-1})	Assignments	PED (%)
1231	vw	1231	vw		C-H inplane bending	
		1252	w		C-H inplane bending	
		1295	m		C-N stretching	
1315	m	1312	m		C-N stretching	
1309	w			1315	C-C stretching	94
1347	m	1341	m	1340	C-C stretching	88
		1358	m	1351	C-C stretching	96
1413	s			1420	C-C stretching	92
		1427	vs	1428	C-C stretching	91
1454	m	1455	m	1462	C-C stretching	86
1492	w	1488	vw	1495	C-C stretching	85
		1555	vs	1549	C-C stretching	84
		1573	m	1572	C-C stretching	80
1583	vs			1576	C-C stretching	92
1610	vs	1616	w	1614	C-C stretching	94
1663	s				N-H inplane bending	
1916	vw				3403–1488	
1880	vw				3056–1162	
2104	vw				1095 + 1012	
2467	w				2 × 1231	
2545	w				2 × 1095 + 350	
2743	w			2741	C-H stretching	100
2853	m	2847	m	2849	C-H stretching	86
		2905	m	2900	C-H stretching	97
		2937	m	2931	C-H stretching	95
2962	m	2968	w	2961	C-H stretching	88
3051	s	3043	m	3038	C-H stretching	85
		3056	vs	3042	C-H stretching	85
		3119	w		N-H stretching in amino group	
3402	vs	3403	m	3410	N-H stretching	96
3402	vs	3403	m		N-H stretching in amino group	

Predicted in the present work from NCA.

RESULTS AND DISCUSSION

Carbon Vibrations: In benzene, the bands between 1650 and 1300 cm^{-1} are assigned to C-C stretching modes. In the present case, the bands observed at 1309, 1347, 1358, 1413, 1427, 1454, 1492, 1555, 1573, 1583 and 1610 cm^{-1} are assigned to C-C stretching for tryptophan. The C-C ring breathing mode and

C-C-C trigonal bending are assigned to the bands at 756 and 1008 cm^{-1} for tryptophan. The above assignments agree well with Murray and Calloway[22], Golse and Thio[23].

The in-plane carbon bending vibrations are obtained from the non-degenerate b_{1u} (1010 cm^{-1}) and degenerate e_{2g} (606 cm^{-1}) modes of benzene. The e_{2g} degenerate frequency in this case has been observed at 532 and 584 cm^{-1}. The carbon out-of-plane bending vibrations are defined by degenerate e_{2g} (404 cm^{-1}) modes of benzene. This split into two non-totally symmetric components and the bands are observed at 420 and 395 cm^{-1} for tryptophan.

C-H vibrations: The aromatic structure shows the presence of C-H stretching vibrations in the region around 3000 cm^{-1}. Hence the bands at 2743, 2853, 2905, 2937, 2962, 3051 and 3056 cm^{-1} of tryptophan have been assigned to C-H stretching modes. Studies on the spectra of benzene show that there are two degenerate e_{2g} (1178 cm^{-1}), e_{1u} (1032 cm^{-1}) and a_{2g} (1340 cm^{-1}) vibrations involving C-H in-plane bending vibrations. The frequencies 1095, 1128, 1150, 1160, 1202, 1231 and 1295 cm^{-1} are assigned to C-H in-plane bending vibrations for tryptophan molecule. These assignments are in agreement with the values in the literature[24, 25].

The C-H out-of-plane deformations result from b_{2g} (985 cm^{-1}), e_{2u} (671 cm^{-1}), e_{1g} (850 cm^{-1}) and e_{2u} (671 cm^{-1}) modes of benzene and they are expected to occur in the region of 1000–600 cm^{-1}. The changes in the frequencies of these deformations from their values in benzene are almost determined exclusively by the relative position of the substituents and are almost independent of their nature[26, 27]. Hence the bands at 845, 922 and 963 cm^{-1} have been assigned to C-H out-of-plane bending vibrations for tryptophan molecule.

N-H stretching: Tsuboi (27) reported N-H stretching frequency at 3481 cm^{-1} in aniline. In line with his observation, N-H stretching is assigned to the band at 3402 cm^{-1} for tryptophan molecule. N-H out-of-plane bending deformation is assigned to 596 cm^{-1} in the present work for tryptophan molecule.

NH$_2$ group vibration: It is noted that in primary amines the N-H stretching vibrations occur in the region 3500–3300 cm^{-1}. Due to this, the bands at 3119 and 3403 cm^{-1} are assigned to N-H symmetric stretching vibrations for tryptophan molecule. The NH$_2$ scissoring mode normally lies between 1590 and 1650 cm^{-1} in primary aromatic amines. In the present case, the frequency at 1663 cm^{-1} is assigned to NH$_2$ scissoring mode in tryptophan. According to Varsani[28] at (the NH$_2$ rocking takes place around 1050 cm^{-1}. In the present work NH$_2$ rocking for tryptophan molecule occurs at 1061 cm^{-1}.

The rest of the frequencies are accounted for as resulting from allowed combinations and overtones of the selected fundamentals which give additional suport to their choice.

Conclusion

A partial vibrational assignment for the tryptophan amino acid has been attempted successfully here. Due to the instrument limitations, all the frequencies of this molecule are not observed in the spectra. Accordingly, normal coordinate analysis was carried out for a few vibrations only.

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