

APPLICATION OF COMBINED APPROACH FOR SPECTROSCOPIC AND STRUCTURAL ELUCIDATION OF SMALL PEPTIDES

Atanas G. Chapkanov

South-West University "Neofit Rilski", Department of Chemistry, 66 "I. Mihailov" str.,
2700 Blagoevgrad, Bulgaria

Abstract

The linear-polarized spectroscopy (IR- or Raman) of oriented colloids in nematic host coupled with advanced theoretical calculations are applied as a part of modern "combined approach" for spectral and structural elucidation of small dipeptides. L-phenylalanyl-L-tyrosine (H-Phe-Tyr-OH) and the hydrochloride of L-alanyl-L-phenylalanine stabilized as monohydrate (H-Ala-Phe-OH.HCl.2H₂O) are studied. The obtained information, especially for the characteristic IR-bands of amide C=O-NH fragments and out-of-plane vibrations of the aromatic residues provides a structural information about the configuration of amide O=C-NH group/s and corresponding amide planes in the peptide molecules, which are characterized with a large number of vibrations. In the aromatic peptides with more neighboring disposed amino acid, the typical π -repulsion leads to a limitations of the RHF or B3LYP methods. A good agreement between theory and experimental geometry parameters is obtained, when the calculations are performed at RI-MP2 level of theoretical approximation and high basis set.

Key words: small peptides; L-phenylalanyl-L-tyrosine (H-Phe-Tyr-OH), (H-Ala-Phe-OH.HCl.2H₂O); linear-polarized spectroscopy, theoretical calculations.

1. INTRODUCTION

The paper deals with a modern "combined approach" for spectral and structural elucidation of small peptides, using the possibilities of linear-polarized infrared (IR-LD) and Raman spectroscopy of oriented colloids in nematic host, combined with the advanced theoretical approximation tools for optimization of the electronic structure and vibrational properties (Ivanova, Arnaudov & Bontchev 2004, Ivanova, Tsalev & Arnaudov 2006; Ivanova et al. 2007; Kolev 2007). In review article (Kolev, Spiteller & Koleva 2010) is discussed the scopes and limitations of this modern complex approaches for investigation of small biologically active molecules, focusing on the aliphatic amino acids, their amides and small peptides. However in the case of peptides, containing aromatic amino acids, the analysis is difficult because of the presence a large number of characteristic IR- and Raman bands, belonging to aromatic fragments. On the other hand typical for these systems inter- and/or intra-molecular π -stacking (Kumpf & Dougherty 1993; Dougherty 1996,) limits the application of enough number theoretical tools in addition. Namely, these aromatic interactions have been described as excellent stabilizing agents of the secondary structure scaffold in peptides with neighboring aromatic amino acid in side-chains (Russell & Cochran 2000, Mahalakshmi, Raghothama & Balaram 2006). For these reasons some authors (Toroz & Van Mourik 2006) delimited spectroscopic properties and theoretical data interpretation for aliphatic and aromatic peptides. In this work novel and different accents are focused for the possibilities and limitations of the above mentioned complex tools for study of small peptides with aromatic amino acids in side chains. As a basis of this discussion is the analysis of large group amino acid derivatives and small peptides containing L-phenylalanyl-, L-tyrosyl-, L-tryptophyl- and/or L-histidyl-fragments (Kolev 2007; Ivanova & Miteva 2004; Ivanova 2006; Kolev et al. 2009; Koleva, Kolev & Spiteller 2007). Physical and chemical properties of these systems can be precisely calculated by means of *ab initio* and DFT methods at Hartee-Fock, MP2 and B3LYP level of theory, varying basis sets. The validity of the conclusions about the structure or vibrational properties of these systems have been supported, compared and/or additionally proved by the results from independent physical methods as ¹H and ¹³C-NMR, single crystal X-ray diffraction, HPLC tandem mass spectrometry and etc. However, a well ordered crystal must first be grown in order to determine the molecular structure by single crystal X-ray diffraction. Although the 3D structures of peptides have been determined over the past decades, peptides crystallization is a still a major obstacle to X-ray diffraction work as well and the presence of chiral centre/s makes measurements with difficulties. For this reason, the "combined approach" presented can be regarded as an alternative method, obtaining structural information in the solid-state. It is obviously, that only absolute crystallographic method gives geometric parameters as bond lengths and angles,

but the spectroscopic methods can provide information about the dihedral angles for *cis*- and *trans*-configured amide groups as well and mutual disposition of the aromatic fragments in peptides. Their validity is illustrated by comparison of the theoretical and spectroscopic results obtained with available crystallographic data. In this respect, the article could be represents a useful source of information not only in IR-spectroscopy area, but also in the field of structural analysis of these systems. Modern vibrational approaches have been applied for these systems by Chou (1983, 1984, 1988). The possibilities of the near-infrared diffuse reflection spectroscopy for the determination of the protein structure has been applied (Bai et al. 2006). The X-ray technique is often used for 3D structure determination of proteins/peptides, however not all proteins/peptides can be easily to crystallize, especially for membrane proteins. As a complement, NMR is indeed a very powerful mean for determination of these structures (Schnell & Chou 2006; Call et al. 2006; Schnell et al. 2005), however it is time-consuming and costly. Although, the using of various structural bioinformatics tools, particularly the homologous technique (Chou 2004, 2005), which can to help acquire protein 3D structures, unfortunately the number of templates for homologous modelling is quite limited. From this point of view it would be very useful to develop a “combined approach” by coupled of many different techniques and methodologies for introduction in this paper.

2. EXPERIMENTAL PART

The investigated peptides are Bachem organics compounds. The IR-spectra were measured on a Bomen-Michelson 100 FTIR-spectrometer ($4000\text{--}400\text{ cm}^{-1}$, $\pm 2\text{ cm}^{-1}$ resolution, 150 scans) equipped with a Perkin Elmer wire-grid polarizer. The non-polarized solid state IR spectra were recorded using KBr disk technique. The oriented samples were obtained as suspension in a nematic 4'-cyano-4'-alkylbicyclohexyl mixture (ZLI-1695 in the Merck notation), mesomorphic at room temperature. The poor IR-spectrum makes possible to record the guest-compound bands in the whole $4000\text{--}400\text{ cm}^{-1}$ range. The presence of an isolated nitrile stretching IR-band at 2233 cm^{-1} additionally serves as an orientation indicator. The effective orientation of the solid state sample was achieved through the following procedure: about 5 mg of the compound studied was added to the appropriate liquid crystal substance until a slightly viscous mixture is obtained. The suspension thus prepared was pressed between two KBr-plates rubbed out in advance in one direction by means of fine sandpaper. The grinding of the prepared mull in the rubbing direction promotes an additional orientation of the sample (Koleva et al. 2005). The Raman spectra were recorded on Horiba Jobin Yvon 6000 Raman Spectrometer.

Quantum chemical calculations are performed with GAUSSIAN 98 and Dalton 2.0 program packages (Frisch et al. 1998; Dalton 2.0). The result files are visualized by means of the GausView03. The geometry parameters of the aromatic peptides (as zwitterionic) with one aromatic amino acid residue were calculated by *ab initio* and Density functional theory (DFT) calculations. The geometry of (1) was optimized at two levels of theory: second-order Moller-Pleset perturbation theory (MP2/RI-MP2) and DFT using the 6-31⁺⁺G** basis set. The DFT method employed is B3LYP, which combines Becke's three-parameter non-local exchange function with the correlation function of Lee, Yang and Parr. Molecular geometries of the studied species were fully optimized by the force gradient method using Bernys' algorithm. For every structure the stationary points found on the molecule potential energy hypersurfaces were characterized using standard analytical harmonic vibrational analysis. The absence of the imaginary frequencies, as well as of negative eigenvalues of the second-derivative matrix, confirmed that the stationary points correspond to minima of the potential energy hypersurfaces. The calculation of vibrational frequencies and infrared intensities were checked to establish which kind of performed calculations correspond to the best experimental data. The empirical scaling factors 0.9614 and 0.8929 are made to achieve better correspondence between the experimental and theoretical values. Calculated geometry of the *H-Phe-Tyr-OH* is carried out at RI-MP2/6-31⁺⁺G** level of approximation.

3. RESULTS AND DISCUSSION

3.1. Spectroscopic and structural elucidation of small aromatic peptides

The application and illustration of linear-polarized vibrational spectroscopy as a part of “combined approaches” to study aromatic peptides systems is the carried out L-phenylalanyl-L-tyrosine (*H-Phe-Tyr-OH*) and hydrochloride of L-alanyl-L-phenylalanine stabilized as monohydrate (*H-Ala-Phe-OH.HCl.2H₂O*) analysis. We choose these examples with a view to compare the scopes and limitations of the polarized vibrational tool, studding the IR- and Raman spectra (Figs. 1 and 2) as well as ones of the widely used theoretical approaches for prediction of the molecular geometry of small peptides. At first it is necessary to notes, that in these examples as well as other aromatic peptides, the characteristic IR-bands of NH_3^+ -group, typical for zwitterionic and

protonated peptides such as NH_3^+ , NH_3^+ , NH_3^+ are low-intensive in corresponding Raman spectra. As could be seen, the bands between $3100\text{--}2500\text{ cm}^{-1}$ (NH_3^+ , NH_3^+), at 1681 cm^{-1} (NH_3^+) in *H-Phe-Tyr-OH* and at 1693 cm^{-1} , 1681 cm^{-1} (NH_3^+ , NH_3^+) in *H-Ala-Phe-OH.HCl.2H_2O* which are observed in the IR-spectra at Fig. 1.2 and Fig. 2.2 are low-intensive or/and disappeared in corresponding Raman spectra (Fig. 1.1 and 2.1). The same is valid for C=O (Amide I) and C=O (COOH) stretching vibrations (Figs. 1 and 2). On the other hand the characteristics out-of-plane (o.p.) modes for the IR- spectra aromatic residue of the amino-acids at 836 cm^{-1} (11-CH(Tyr)), 746 cm^{-1} (11-CH(Phe)) and 700 cm^{-1} (4-Ar (Phe)) in first example (Fig. 1.2) as well as at 735 cm^{-1} (11-CH(Phe)) and 700 cm^{-1} (4-Ar (Phe)) in the second peptide salt are low-intensive in corresponding Raman spectra (compare Figs 1.1 and 2.1 with 1.2 and 2.2). This is in accordance with the conclusions for other aromatic peptides (Ivanova 2005; Ivanova & Arnaudov 2006; Ivanova, 2006; Koleva et al. 2008; Kolev, Koleva, & Spiteller, 2008; Koleva, Kolev & Spiteller, 2007; Koleva et al. 2008; Kolev et al. 2006). From this point of view the local structural elucidation of these systems is better to be performed using polarized IR-spectroscopic tool. If the objects interested possess an in-plane (i.p.) vibration of A_1 type (Varsanyi 1969) for aromatic peptides, then the polarized Raman tool is more appropriated. As can be seen the vibration band of dipeptide *H-Phe-Tyr-OH* (**1**) at about 1000 cm^{-1} of the aromatic fragment (Varsanyi 1969) is one of the most intensive in the Raman spectra of tyrosyl or phenylalanyl -containing peptides (Figs. 1.1 and 2.1). All studied aromatic peptides are characterized with significant degree of macro orientation of suspended particles in nematic host (Ivanova, Arnaudov & Bontchev 2004; Ivanova, Tsalev & Arnaudov 2006; Ivanova et al. 2007; Kolev, Spiteller & Koleva 2010; Kolev, 2007).

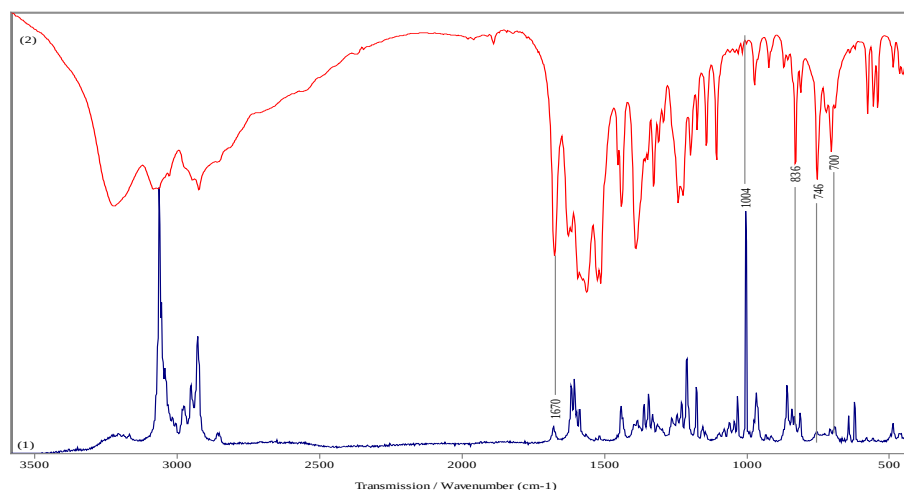


Fig. 1. Raman (1) and IR-(2) spectra of dipeptide *H-Phe-Tyr-OH* in solid-state

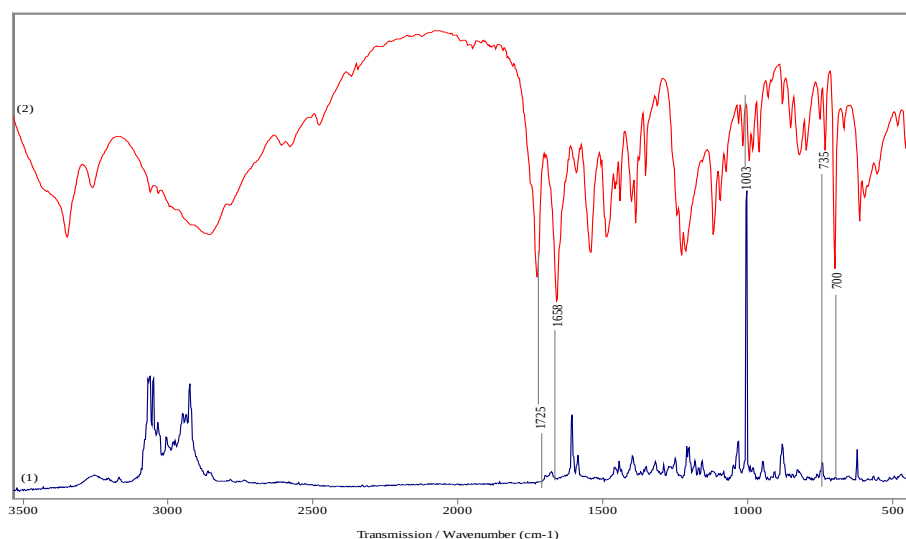


Fig. 2. Raman (1) and IR-(2) spectra of dipeptide *H-Ala-Phe-OH.HCl.H₂O* in solid-state

These results caused the successes fully application of the reducing-difference procedure for polarized spectra interpretation. The elimination of 3230 cm^{-1} peak, provokes a disappearance of 1670 cm^{-1} band, indicating their ν_{NH} and $\nu_{\text{C=O}}$ character and *trans*-configuration of the amide fragment as well (Fig. 3.2). The elimination of 1681 cm^{-1} peak (Fig. 3.3) leads to vanished of $8a$ and $19a$ modes at 1614 cm^{-1} and 1585 cm^{-1} respectively. The carried out elimination indicates a collinear disposition of $\delta^{\text{as}}_{\text{NH}_3^+}$ and A_1 - in-plane peaks of p-disubstituted benzene system. On the other hand, Fig. 3.3 shows a disappearance of $\nu^{\text{as}}_{\text{COO}^-}$ maximum at 1525 cm^{-1} . The obtained results correlated well with the theoretical predicted structure of zwitterionic *H-Phe-Tyr-OH* shown in Fig. 3. However, the illustrated last elimination leads to another fact, namely the simultaneously vanishing of out-of-plane B_1 -maxima for both benzene rings. As far as in the frame of one molecule concerned, the transition moments of A_1 - and B_1 -peaks is impossible to be eliminated simultaneously (their mutual orientation is perpendicular). The result supposed presence of more than one near to perpendicular oriented molecules in the unit cell. This phenomenon is typical for peptides which should be discussed as well in the next example of *H-Ala-Phe-OH.HCl.2H₂O* (Koleva et al. 2007).

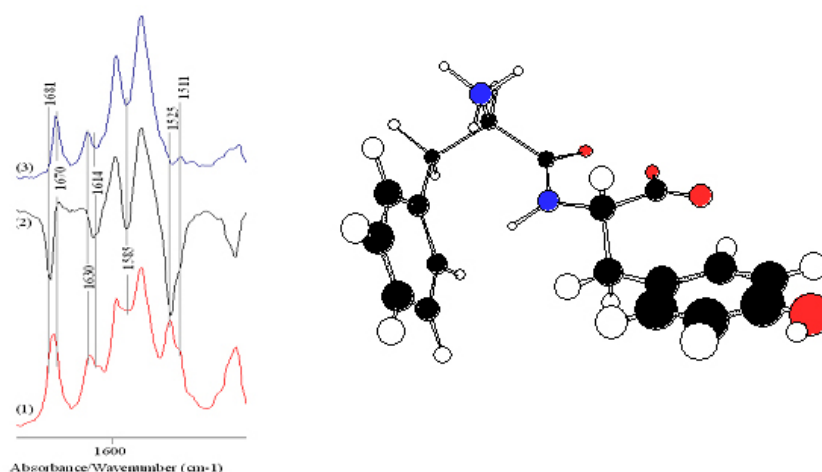
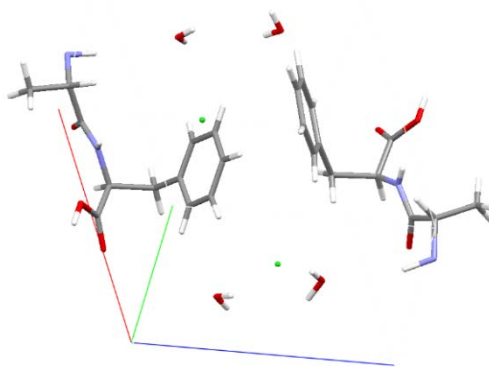


Fig. 3. Non-polarized IR-(1) and reduced IR-LD spectra after elimination of the bands at 1670 cm^{-1} (2) and 1681 cm^{-1} (3); Calculated geometry of the *H-Phe-Tyr-OH* at RI-MP2/6-31++G** level of approximation

According to crystallographic data, *H-Ala-Phe-OH.HCl.2H₂O* crystallized in $P2_1$ space group and the unit cell contains two mutual oriented molecules shown in Scheme 1. The disposition assumed a near to co-linear orientation of Amide I and $\nu_{C=O}$ (COOH group) transition moments in the frame of neighbouring molecules, which is confirmed by a simultaneously elimination of corresponding peaks at 1725 cm^{-1} and 1656 cm^{-1} . The parallel peak at 3266 cm^{-1} is also reduced due to the determined flat *trans*-amide group $C=O-NH$ disposition with torsion angle $175.9(9)^\circ$ (Chapkanov & Zareva, 2009). On the other hand, the amide group, carboxylic one and benzene ring planes are oriented at angles of $30.0(4)^\circ$, $42.1(6)^\circ$ and $55.3(3)^\circ$, respectively (Scheme 1). These data allows a determination of corresponding out-of-plane $\gamma_{C=O}$ (Amide VI), $\gamma_{C=O}$ (COOH group), $11-\gamma_{CH}$ and $4-\gamma_{Ar}$ modes (both B_1 symmetry class). Elimination of 738 cm^{-1} and 701 cm^{-1} peaks in equal dichroic ratio proves their assignment as $11-\gamma_{CH}$ and $4-\gamma_{Ar}$ modes of mono-substituted benzene ring. The pair peaks at 587 cm^{-1} and 555 cm^{-1} are observed during the last procedure. The elimination of the first one leads to out-of-plane peaks observation of benzene fragment as well as the maximum at 595 cm^{-1} . These data assigned 587 cm^{-1} and 555 cm^{-1} bands as $\gamma_{C=O}$ (Amide VI) and $\gamma_{C=O}$ (COOH group) out-of-plane modes.



Scheme 1. Crystal structure of *H-Ala-Phe-OH.HCl.2H₂O* (Cotrait & Barrans 1974)

Table 1. Calculated dihedral angles of protonated *H-Ala-Phe-OH* and experimental crystallographic data of *H-Ala-Phe-OH.HCl.2H₂O*

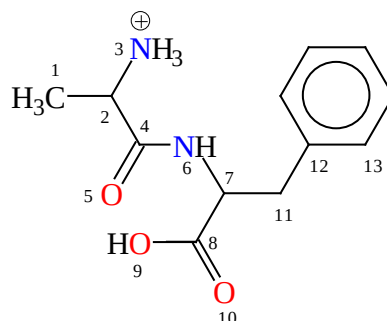
Name, definition	Dihedral angles [degree]*			
	UHF/6-31G**	RI-MP2/6-31++G**	(B3LYP/6-31++G**	X-ray data ***
D(1,2,4,5)	95.8	95.9	95.1	95.9
D(2,4,6,7)	175.0	174.2	177.1	174.3
D(3,2,4,5)	25.5	26.6	24.2	26.5
D(5,4,6,7)	0.0	0.2	0.0	0.3
D(6,7,8,9)	178.0	177.1	178.9	177.2
D(6,7,11,12)	57.2	60.0	57.1	59.3
D(7,11,12,13)	92.8	93.1	91.1	93.2
D(4,5,6,H)	176.0	175.8	177.2	175.9
C _{Ar} -C _{Ar} -C _{Ar} -C _{Ar} torsion angles are within: 0.2° to 0.1° (<i>H-Ala-Phe-OH</i>); 0.5° to 0.3° (<i>H-Ala-Phe-OH.HCl</i>), 0.2° to 1.1°				

*The atom numbering is presented in Scheme 2

**The calculations are performed in the presence of one H_2O molecule

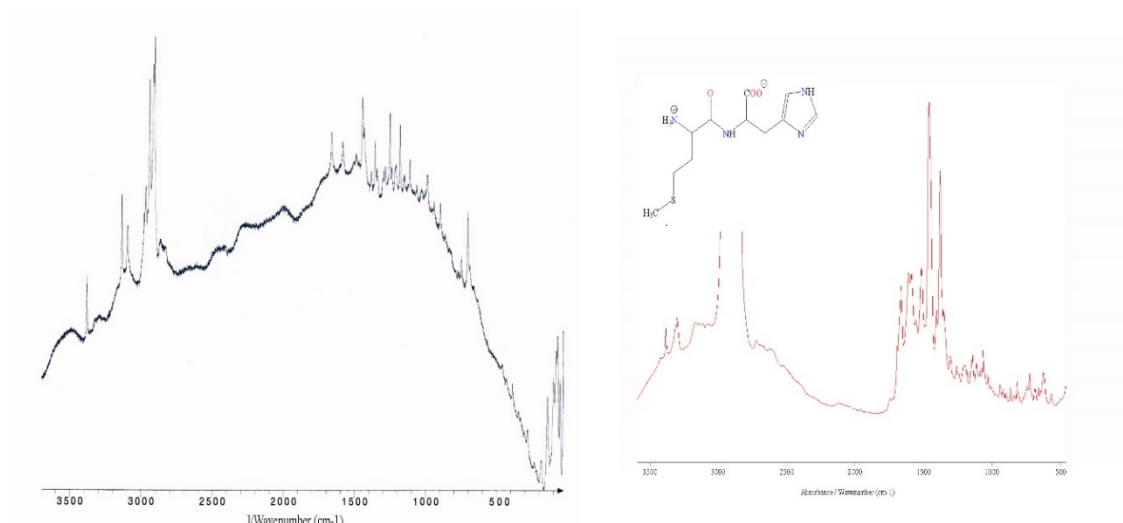
*** According Cotrait & Barrans (1974)

The polarized IR-spectroscopy of oriented colloids could be widely used for aromatic peptides analysis, due to that more of these objects are characterized with the broad fluorescence band in the corresponding Raman spectra. Especially, this is valid for the L-tryptophyl- and/or some cases of L- histidyl-containing peptides. As can be seen in Fig. 4 the H-His-Met-OH dipeptide can be elucidated both by polarized IR- and Raman methods, while in the case of H-Met-His-OH, the first vibrational tool is appeared to be unique for interpretation of the vibrations in solid-state (Ivanova & Arnaudov 2006).



Scheme 2. Atom numbering scheme of the dipeptide *H-Ala-Phe-OH*

The using of the “combined approach”, including the theoretical approximation tools as well it must be taking into account the following statement. The calculations of the zwitterionic aromatic peptides with only one aromatic amino acid residue by the *ab initio* and DFT calculations (Table 1) gives a reasonable results for the geometry parameters (Dian et al. 2002; Valdes et al. 2006). In the case of second theoretical tool, the calculations of the peptide/H₂O system must be done.



(1)

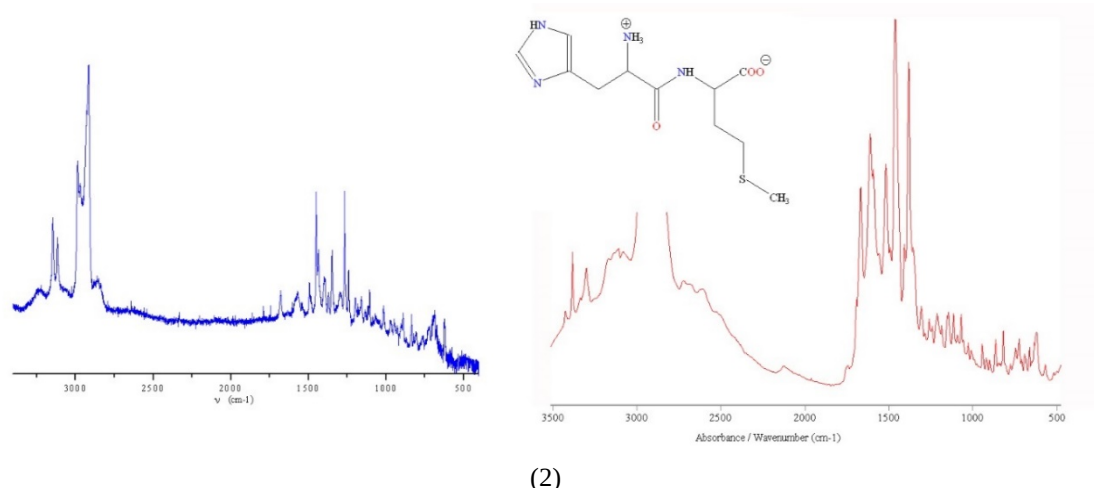


Fig. 4. Raman and IR-spectra of H-Met-His-OH (1) and H-His-Met-OH (2) in solid-state

However, if the object interested is a system with more than one aromatic amino acid side chain, then the RI-MP2 calculations gives a more precise structure of the molecules. In these systems the π -electron repulsion of the neighbouring aromatic fragment in the peptides limits the usage of the other level of theory (see Table 1) for adequate optimization of the geometry parameters of these systems. Using the large number of systematic investigations, part of which are cited here, the presented tool possess unquestionable advantages, but and limitations as well.

4. CONCLUSION

On the base of the obtained results we could summarize the possibilities and limitations of the used tools for analysis of small peptides containing aromatic residue in a following way: (i) The main advantages of the presented “combined approach”, based on linear-polarized vibrational spectroscopy is the ability to provide local structural information in solid state of the aromatic peptides, independent of their amorphous or polycrystalline character. For these systems the method appears to be unique providing any structural information and the possibility to defines precisely the configurations of amide $\text{O}=\text{C}-\text{NH}$ group/s and corresponding mutual disposition of the amide planes in the frame of the peptide molecules. The analysis of the aromatic fragments, are characterized with a large number of vibrations in whole $4000 - 400 \text{ cm}^{-1}$ region. In this respect the polarized IR-spectroscopy leads to obtained of more and precise information, due to these peptides are characterized with more intensive bands in the corresponding IR-spectra, specially the characteristic IR-bands of amide $\text{C}=\text{O}-\text{NH}$ fragments and out-of-plane vibrations of the aromatic residues. In contrast to, if the objects interested possess in-plane vibrations of the aromatic fragments, then the Raman spectroscopy is more suitable for analysis, because these bands are characterized with higher intensity. (ii) Similar to the analysis of aliphatic small peptide, the method is based on the possibility for precise and complete elucidation of the vibrational characteristics of the suspended substances and small peptides in particular. Linear polarized IR-(or Raman) spectroscopies are the only methods for experimental proof and assignment of the bands to corresponding molecular vibrations. (iii) In the cases of L-tryptophyl- and/or L-histidyl-containing peptides, where often the Raman spectra are characterized with broad fluorescence bands, the polarized IR-spectroscopy is appeared to be unique for vibrations interpretation of these systems in solid-state. (iv) In contrast to the aliphatic peptides, where the *ab initio* and DFT calculations give a reasonable optimized geometry parameters and vibrational properties, in the case of aromatic peptides with more than one neighboring disposed amino acid with aromatic fragments, the typical π -electron repulsion leads to a limitations of the used RHF or B3LYP level of theory. Good correlation between theory and experimental geometry parameters is obtained in the case, when the calculations are performed at RI-MP2 level of theoretical approximation and high basis set.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Prof. B. Ivanova for her continuously support of this research.

REFERENCES

- Bai, Y Wang, D Yu, ZX Jia, Y Zhu, FY Wei, DQ Zhong, WZ Chou, KC 2006, Ecdysterone determination of niuxi by the near-infrared diffuse reflection spectroscopy (NIRDRS)', *Spectroscopy*, vol. 21, pp. 40-43.
- Call, ME Schnell, JR Xu, C Lutz, RA Chou, JJ Wucherpennig, KW 2006, 'The structure of the zetazeta transmembrane dimer reveals features essential for its assembly with the T cell receptor', *Cell*, vol. 127, pp. 355-368.
- Chapkanov, A Zareva, S 2009, 'Structural and IR-spectroscopic elucidation of dipeptide *L*-threonyl-*L*-methionine in solid-state', *Protein & Peptide Lett.*, **2009**, 16(11), 1277-1280.
- Chou, KC 1983, 'Low-frequency vibrations of helical structures in protein molecules', *Biochem. J.*, vol. 209, pp. 573-580.
- Chou, KC 1984, 'The biological functions of low-frequency phonons: 3. Helical structures and microenvironment', *Biophys. J.* vol. 45, pp. 881-890.
- Chou, KC 1988, 'Low-frequency collective motion in biomacromolecules and its biological functions', *Biophys. Chem.*, vol. 30, pp. 3-48.
- Chou, KC 2004, 'Structural bioinformatics and its impact to biomedical science', *Current Medicinal Chem.*, vol. 11, pp. 2105-2134.
- Chou, KC 2005, 'Coupling interaction between thromboxane A2 receptor and alpha-13 subunit of guanine nucleotide-binding protein', *Journal of Proteome Research*, vol. 4, pp. 1681-1686.
- Cotrait M & Barrans, Y1974, 'Structures cristallines de dipeptides aromatiques. I. Dérivés de la phenylalanine', *Acta Crystallogr.*, vol. 30B, pp. 1018-1021.
- Dalton 2.0 Program Package, <http://www.kjemi.uio.no/software/dalton/dalton.html>
- Dian, C Longarte, A Mercier, S Evans, D Wales, D Zwier, T 2002, 'The infrared and ultraviolet spectra of single conformations of methyl-capped dipeptides: N-acetyl tryptophan amide and N-acetyl tryptophan methyl amide', *J. Chem. Phys.*, vol. 117, pp. 10688-10702.
- Dougherty, DA 1996, 'Cation- π interactions in chemistry and biology', „A New view of benzene, Phe, Tyr and Trp. *Science*, vol. 271, pp. 163-168.
- Frisch, MJ Trucks, GW Schlegel, HB Scuseria, GE Robb, MA Cheeseman, JR Zakrzewski, VG Montgomery JA Stratmann, RE Burant, JC Dapprich, S Millam, JM Daniels, AD Kudin, KN Strain, MC Farkas, Ö Tomasi, J Barone, V Cossi, M Cammi, R Mennucci, B Pomelli, C Adamo, C Clifford, S Ochterski, J Petersson, GA Ayala, PY Cui, Q Morokuma, K Salvador, P Dannenberg, JJ Malick, D K Rabuck, AD Raghavachari, K Foresman, JB Cioslowski, J Ortiz, JV Baboul, AG Stefanov, BB Liu, G Liashenko, A Piskorz, P Komáromi, I Gomperts, R Martin, RL Fox, DJ Keith, T Al-Laham, MA Peng, CY Nanayakkara, A Challacombe, M Gill, PMW Johnson, B Chen, W Wong, MW Andres, JL Gonzalez, C Head-Gordon, M Replogle, ES Pople, JA1998, Gaussian 98, Gaussian, Inc., Pittsburgh, PA.
- GausView03, <http://www.softwaredcientifico.com/paginas/gaussian.htm>
- Ivanova, B. Arnaudov, M. Bontchev, P 2004, 'Linear-dichroic infrared spectral analysis of Cu (I)-homocysteine complex', *Spectrochim. Acta, Part A*, vol. 60, no. 4, pp. 855-861.
- Ivanova, B. Tsalev, D. Arnaudov, M. 2006, 'Validation of reducing-difference procedure for the interpretation of non-polarized infrared spectra of n-component solid mixtures', *Talanta*, vol. 69, pp. 822-836.
- Ivanova, B. Simeonov, V. Arnaudov, M. Tsalev, D 2007, 'Linear-dichroic infrared spectroscopy – validation and experimental design of the orientation technique as suspension in nematic liquid crystal. *Spectrochim. Acta, Part A*, vol. 67, no. 1, pp. 66-75.
- Ivanova, B & Miteva, M 2004, 'Au (III) interaction with methionine and histidine containing peptides', *J. Coord. Chem.*, vol. 57, pp. 217-221.
- Ivanova, B 2005, 'Solid state linear-dichroic infrared spectral analysis of dipeptide *L*-Phe-*L*-Phe and its mononuclear Au (III)-complex', *J. Coord. Chem.*, vol. 58, no. 7, pp. 587-593.

- Ivanova, B & Arnaudov, M 2006, 'Theoretical and solid state linear-dichroic infrared spectral analysis of aromatic dipeptides and their protonated forms', *Protein & Peptide Lett.*, vol. 13, pp. 889-896.
- Ivanova, B 2006, 'IR-LD spectroscopic characterization of L-Tryptophan containing dipeptides', *Spectrochim. Acta, Part A*, vol. 64, pp. 931-938.
- Koleva, B Kolev, T Zareva, S Spiteller, M 2008, 'Synthesis, spectroscopic, structural and theoretical characterization of hydrogensquarate and mononuclear Au (III)-complex of dipeptide phenylalanyltyrosine', *J. Mol. Struct.*, vol. 885, no. 1-3, pp. 104-110.
- Koleva, B Kolev, T Spiteller, M 2007, 'Mononuclear Au (III)-complexes with tryptophan-containing dipeptides: synthesis, spectroscopic and structural elucidation', *Inorg. Chim. Acta*, vol. 360, no. 7, pp. 2224-2230.
- Koleva, B Kolev, T Simeonov, V Spassov, T Spiteller, M 2005, 'Linear polarized IR-spectroscopy of partial oriented solids as a colloid suspension in nematic liquid crystal - new tool for structural elucidation of the chemical compounds', *J. Inclus. Phenom.*, vol. 61, pp. 319-333.
- Koleva, B Kolev, T Lamshöft, M Spiteller, M 2008, 'Synthesis, spectroscopic and structural elucidation of Au (III), Pd (II) and Pt(II) complexes with tripeptide glycyl-L-phenylalanyl-glycine', *Trans. Met. Chem.* vol. 33, no. 7, pp. 911-919.
- Kolev, T Koleva, B Seidel, R Mayer-Figge, H Spiteller, M Sheldrick, WS 2009, 'L-leucineamide hydrogensquarate – spectroscopic and structural elucidation', *Amino Acids*, vol. 37, no. 4, pp. 693-701.
- Kolev, T Koleva, B Spiteller, M 2008, 'Copper (II) complexes with hydroxyl containing dipeptides glycyl-L-serine and L-seryl-L-tyrosine', *J. Coord. Chem.*, vol. 61, no. 12, pp. 1897-1905.
- Koleva, B Kolev, T Zareva, S Spiteller, M 2007, 'The dipeptide alanylphenylalanine (H-Ala-Phe-OH) – protonation and coordination ability with Au (III)', *J. Mol. Struct.*, vol. 831, no. 1-3, pp. 165-173.
- Kolev, T Koleva, B Zareva, S Spiteller, M 2006, 'Au (III)-complexes of the alanyl-containing peptides glycylalanine and glycylalanylalanine–Synthesis, spectroscopic and structural characterization', *Inorg. Chim. Acta*, vol. 359, no. 13, pp. 4367-4376.
- Kolev, T. Spiteller, M. Koleva, B 2010, 'Spectroscopic and structural elucidation of amino acid derivatives and small peptides – experimental and theoretical tools', Minireview Article, *Amino Acids*, vol. 38, no. 1, pp. 45-50.
- Kolev, T 2007, 'Quantum chemical, spectroscopic and structural study of hydrochlorides, hydrogensquarates and ester amides of squaric acid of amino acid amides', „*New approaches in quantum chemistry*”, Nova Science Publishers, Inc., Hauppauge, NY 11788, USA, (Web: www.novapublishers.com).
- Kumpf, RA & Dougherty, DA 1993, 'A mechanism for ion selectivity in potassium channels: computational studies of cation- π interactions', *Science*, vol. 261, pp. 1708-1710.
- Mahalakshmi, R Raghothama, S Balaram, R 2006, 'NMR-Analysis of aromatic interactions in designed peptide β -hairpins', *J. Am. Chem. Soc.*, vol. 128, pp. 1125-1138.
- Russell, SJ & Cochran, AG 2000, 'Designing stable β -hairpins: Energetic contributions from cross-strand residues', *J. Am. Chem. Soc.*, vol. 122, pp. 12600-12601.
- Schnell, JR Chou, JJ 2006, 'Structure and mechanism of the M2 proton channel of influenza A virus', *Nature*, vol. 451, pp. 591-595.
- Schnell, JR Zhou, GP Zweckstetter, M Rigby, AC Chou, JJ 2005, 'Rapid and accurate structure determination of coiled-coil domains using NMR dipolar couplings: application to cGMP-dependent protein kinase I α ', *Protein Science*, vol. 14, pp. 2421-2428.
- Toroz, D & Van Mourik, T 2006, 'The structure of the gas-phase tyrosine–glycine dipeptide', *Molec. Phys.*, vol. 104, pp. 559-564.
- Valdes, H Reha, D Hobza, P 2006, 'Structure of isolated tryptophyl-glycine dipeptide and tryptophyl-glycyl-glycine, tripeptide: Ab Initio SCC-DFTB-D Molecular dynamics simulations and high-level, Correlated *ab Initio* Quantum chemical calculations', *J. Phys. Chem., B*, vol. 110, pp. 6385-6396.
- Varsanyi, G 1969, 'Vibrational spectra of benzene derivatives', Akademiai Kiado, Budapest.