JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 9, No. 3, March 2007, p. 637 - 640

FT-Raman and NMR investigation of the protein extracted from barley aleurone cells

M. TOMOAIA-COTIŞEL, A. MOCANU, N. LEOPOLD[a], M. VASILESCU[a], V. CHIŞ[a], O. COZAR[a*]
Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Department of Physical Chemistry, Arany Janos Str., No. 11, RO-400028 Cluj-Napoca, Romania
[a]Babeş-Bolyai University, Faculty of Physics, Kogălniceanu Str., No. 1, RO-400084 Cluj-Napoca, Romania

Investigations by FT-Raman and solid ^{13}C NMR spectra of the major protein component of barley aleurone cells (PBAC) have been performed. The amide I band of the protein is present as a broad band at 1657 cm^{-1} and it suggests a dominant α-helix secondary structure for this protein. Also, this α-helix conformation is confirmed by the amide III band present at 1317 cm^{-1}. On the other hand, the low intensities of the bands below 1260 cm^{-1} in the FT-Raman spectrum of this protein, suggest some contribution from beta sheet and/or random coil conformations. ^{13}C NMR spectrum of protein in powder form has been recorded at room temperature and the chemical shifts of the carbon nuclei in the amino acids entering the protein sequence have been calculated by DFT method at B3LYP/cc-pVDZ level of theory. The proposed amino acid sequence is supported by theoretical chemical shifts calculations.

(Received November 15, 2006; accepted December 21, 2006)

Keywords: FT-Raman spectrum, ^{13}C NMR spectrum, Barley aleurone cells protein, Protein secondary structure, Geometry optimization

1. Introduction

The investigated protein belongs to the 7S globulins and it represents the major protein component of barley aleurone cells (PBAC). It has been purified and characterized by Yupsanis and coworkers [1] and its presence in the aleurone cells layer of barley was confirmed. In that study it was also proposed the N-terminal amino acid sequence for PBAC: ^{1}EQGDRSRPYVFGPRSF^{16}.

In this work we are aiming to get new insights into its structure and conformation and for these purposes we used Raman and NMR spectroscopy on solid state protein. Chemical shifts obtained by NMR spectroscopy are very useful quantities for the determination of molecular conformations [2-4]. Moreover, hydrogen bonds and ionic interactions are important factors for substrate recognition, in forming the secondary structure or in forming supramolecular assemblies in biological systems or in material science [5]. Previous studies [6-8] have shown that experimentally determined chemical shifts are very sensitive to the network effects like hydrogen bonds or short range electrostatic interactions. Thus, for a reliable assignment of the experimental spectra, it is often necessary to calculate the NMR spectra of the investigated compounds at a reasonable level of theory, theoretical results being very useful for structural analysis. Chemical shifts associated with ^{13}C nuclei in the aminoacids drastically depend on the functional group to which they belong, the corresponding values being spread in a large domain, from 10-200 ppm [6].

In order to calculate the chemical shifts corresponding to the C nuclei in the protein we used the Gaussian98 program package [9], adopting the following protocol: first we optimized the geometries of the aminoacids by using Density Functional Theory (DFT) approaches with the hybrid B3LYP [10] functional. We used the correlation consistent cc-pVDZ basis set which was shown previously [11] to perform very well for high accuracy calculations of chemical shifts associated with carbon nuclei.

2. Experimental

FT-Raman spectra were recorded in a backscattering geometry with a Bruker FRA 106/S Raman accessory equipped with a high sensitivity nitrogen cooled Ge detector. The 1064 nm Nd:YAg laser was used as excitation source, and the laser power was set to 350 mW. All spectra were recorded with a resolution of 4 cm^{-1} by co-adding 32 scans.

^{13}C RMN spectrum of the protein has been recorded using a Bruker AVANCE spectrometer operating at 100.63MHz at the National Center of Magnetic Resonance, Babeş-Bolyai University, Cluj-Napoca. The spectra have been obtained by using a single exciting pulse of 9μs, accumulating 400 FID decays and tetramethylsilane (TMS) was used as internal standard.

3. Computational details

The molecular geometry optimizations, vibrational frequencies and NMR chemical shifts calculations were performed with the Gaussian 98W program package [8] by using DFT approaches, with the hybrid Becke's three parameter and Lee, Yang and Parr (B3LYP) exchange-correlation functional [10].

The calculation of NMR spectra was performed using the GIAO (Gauge-Including Atomic Orbitals) method

[13], with the hybrid B3LYP exchange-correlation functional, in conjunction with cc-pVDZ basis set [12]. This correlation consistent basis set was shown to perform much better than the standard 6-31G(d) basis set for this purpose [10].

In order to express the chemical shifts in ppm, the geometry of the tetramethylsilane (TMS) molecule has been optimized and then its NMR spectrum was calculated by using the same method and basis set as for the investigated aminoacids.

4. Results and discussion

Raman spectrum

The FT-Raman spectrum of PBAC in powder form was recorded at room temperature (Fig. 1). The most important bands that can give information on the secondary structure of the protein are the amide I band due to the C=O stretching, amide II band due to R-NH deformations and amide III band due to the C-N stretching vibrations [14].

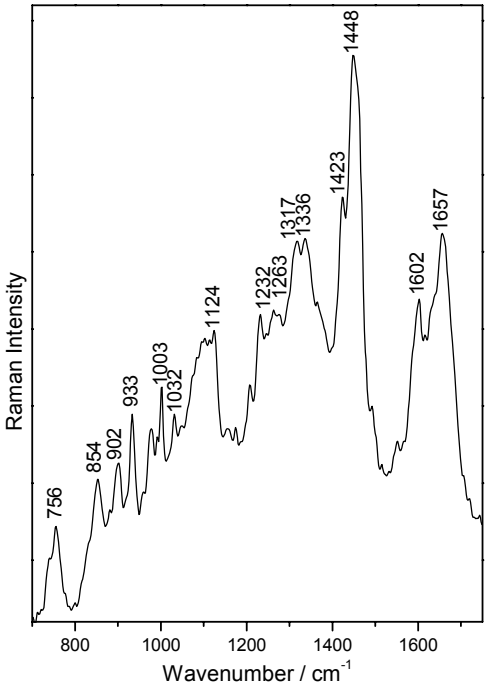


Fig. 1. Raman spectrum of PBAC.

Moreover, depending on the secondary structure, the amide I band appears in the 1658 - 1640cm^{-1}, 1680 - 1665cm^{-1} and 1666 - 1660cm^{-1} regions for α-helix, β-sheet and random coil structures, respectively. Characteristic to the same structures, amide III band appears in the following wavenumbers ranges: 1310-1260cm^{-1}, 1242-1235cm^{-1} and 1250-1240cm^{-1}.

Amide II band is usually weak in Raman spectra but rather strong in the infrared spectra.

In order to analyze the secondary structure of the protein we recorded its Raman in solid state and in water solution and the SERS spectrum on silver colloid. In the Raman spectrum of solid state protein, the amide I band is present as a broad band at 1657 cm^{-1}, suggesting a dominant α-helix secondary structure for the investigated protein. Moreover, this conformation is confirmed by the presence of the characteristic amide II band at 1317 cm^{-1}. In the wavenumber region below 1260 cm^{-1} the band intensities is very low and this fact can be associated with a β-sheet or random coil conformation.

In the solid-state Raman spectrum of the protein can be distinguished bands which are due to the vibrations associated with phenyl rings at 1003 and 1602 cm^{-1}. The CH_2 and CH_3 deformations contribute to the spectrum of the protein giving bands located at 1423 and 1448 cm^{-1}.

Unfortunately, due to the very low concentration of the protein in the water solution, the corresponding Raman spectrum shows only the bands associated with the water vibrations. In order to circumvent this problem we tried to use the high capabilities of the SER spectroscopy. SERS spectrum of this protein on Ag colloid shows only two bands at 1300 and 1440cm^{-1} which do not help in the analysis of the secondary structure of the PBAC protein.

^{13}C NMR spectrum

^{13}C NMR spectrum of the PBAC protein recorded at room temperature is given in Fig. 2. For the carbon nuclei correspondence we based on the IUPAC numbering system in which the alpha carbon (C^{α}) is C2, and C^{β}, C^{ε}, C^{γ}, etc ate the next carbon atoms in the side chain.

The signal at 174.9 ppm is assigned to the ^{13}C nuclei in the carboxyl groups and carbon nuclei in the amidic groups of the aminoacids entering into the protein sequence. This value is in very good agreement with those corresponding to ^{13}C nuclei in glycine and L-alanine [5].

The complex structure of this signal is most probably due to the presence in this region of the signals from C^{γ} nuclei. Thus, in the NMR spectrum of L-asparagine, these carbon nuclei give a peak at 175.6 ppm [5]. However, since L-asparagine is not a constituent of the protein, the splitting of the signal at 174.9 ppm is assigned to the C^{γ} nuclei of glutamine.

The chemical shifts in the 100-135ppm range, (i.e. the signals at 101.4, 114.2, 126.9 and 134.5 ppm) are assigned to the carbon nuclei in the aromatic rings of phenylalanine and tyrosine.

According to other studies reported in the literature [4], the chemical shifts at 60.8 and 72.1 ppm are assigned to the C^{α} nuclei in the peptide chains and the weak signals at 53.2 and 46.2 ppm are due to the valine residues in the investigated protein.

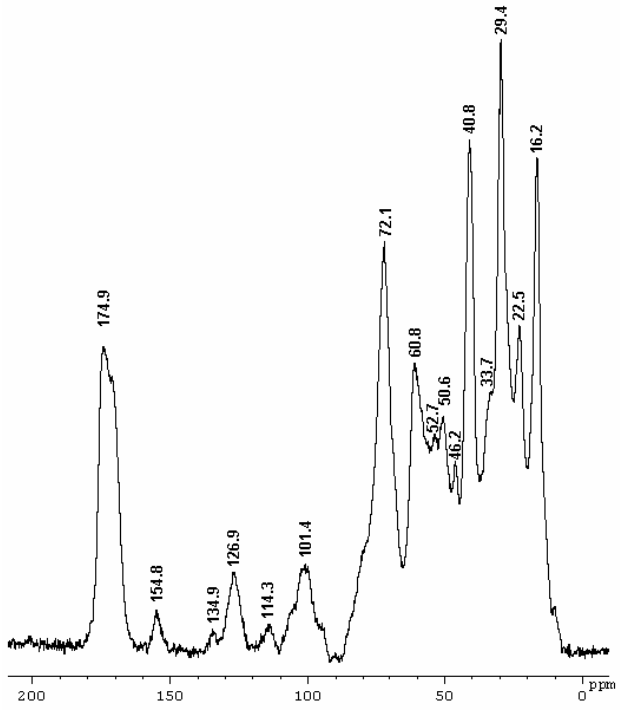
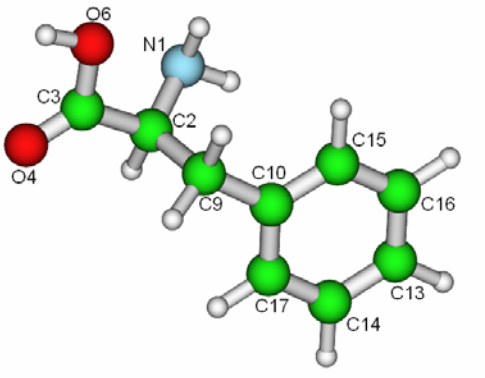


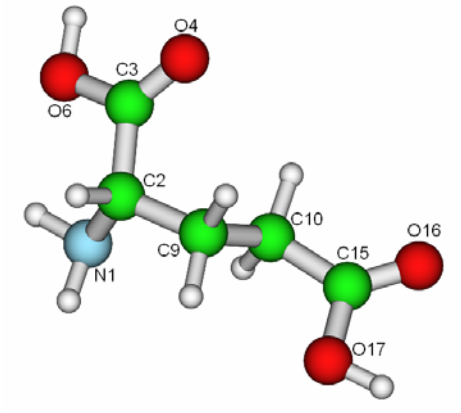
Fig. 2. ^{13}C NMR spectrum of PBAC.

The intense peaks at 40.7 and 29.4ppm correspond to the C^{α} and C^{β} carbons of the constituent aminoacids, respectively. Similar values have been reported for glycine (43.7ppm for C^{α}), L-alanine (51.1ppm for C^{α} and 20.6ppm for C^{β}), L-asparagine (51.1ppm for C^{α} and 34.8ppm for C^{β}) [4]. In the same range are also seen the signals at 25.4 and 16.2 ppm which are assigned to the ^{13}C nuclei in methyl groups of valine.

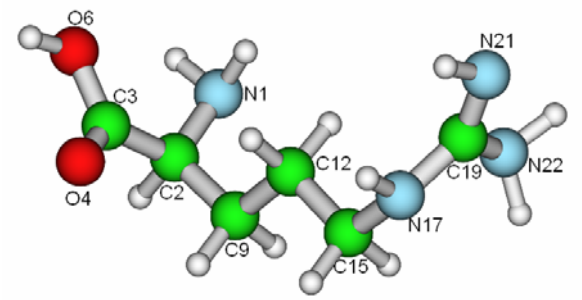
Geometry optimizations of the investigated aminoacids were performed without any constrain at B3LYP/cc-pVDZ level of theory and all the optimized structures represent true minima on the potential energy surface. The optimized geometries together with the atom numbering scheme are given in Fig. 3.



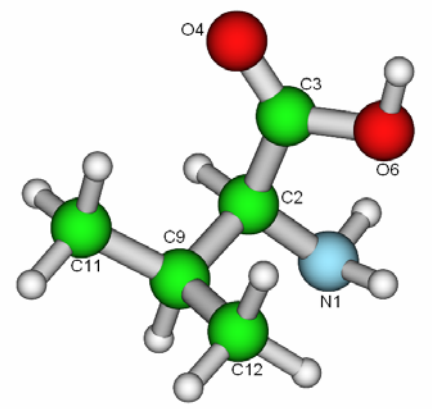
a



b



c



d

Fig. 3. B3LYP/cc-pVDZ optimized geometries of: a) phenylalanine (F) b) glutamine (Q) c) arginine (R) şi d) valine (V).

The same method and basis set have been used for the calculation of chemical shifts by using the GIAO (Gauge-Including Atomic Orbitals) method implemented in the Gaussian program [13] and theoretical results are collected in Table 1.

As seen in Table 1, the carbon nuclei in carbonyl groups of the aminoacids have associated chemical shifts in very good agreement with the corresponding experimental value and they are: 168.3ppm for phenylalanine C3 nucleus, 166.4 and

167.58ppm for glutamine C3 and C15, respectively, 171.0ppm for arginine C3 nucleus and 169.6ppm for valine C3.

The calculated values for C^{α} nuclei are: 62.5ppm, 55.8ppm, 57.1ppm and 63.4ppm for phenylalanine, glutamine, arginine and valine, respectively, again in very good agreement with the experimental values which are located in the 53-72ppm interval.

Table 1. B3LYP/cc-pVDZ calculated chemical shifts for the aminoacids investigated.

Nucleus	Phenylalanine (F)	Glutamine (Q)	Arginine (R)	Valine (V)
C2	62.6	55.8	57.1	63.4
C3	168.3	166.4	171	169.6
C9	48.2	32	32.8	39.7
C10	138.8	28.9	-	-
C11	-	-	-	22.2
C12	-	-	27.5	17.2
C13	125.5	-	-	-
C14	127.9	-	-	-
C15	128.8	167.6	48.9	-
C16	126.8	-	-	-
C17	128.2	-	-	-
C19	-	-	152.1	-

^{13}C nuclei in the methyl groups of valine have calculated chemical shifts of 17.2 and 22.2 ppm, in excellent match with experimental values of 16.2 and 22.5 ppm.

C^{β} carbon nuclei in the aminoacids have calculated chemical shifts of 48.2, 32.0, 32.8 and 39.7 ppm corresponding to phenylalanine, glutamine, arginine and valine and they are associated with the experimental values of 29.4 ppm and 40.7 ppm, respectively.

5. Conclusions

The amide I band located at 1657cm^{-1} suggest a dominant α-helix secondary structure for the investigated protein and this is sustained by the amide III band at 1317cm^{-1}. However, some contributions from beta-sheet and random coil structures are not excluded.

The calculated chemical shifts of the ^{13}C nuclei in the investigated aminoacids confirm their presence in the protein. Due to the very good agreement between the experiment and theory, the assignment of the experimental spectrum is sustained by the theoretical chemical shifts obtained by DFT methods, at B3LYP/cc-pVDZ level of theory.

Acknowledgments

We are grateful to Professor Yupsanis for the barley aleurone protein kindly offered for these studies. This research was financially supported by the Ministry of Education and Research (Scientific research project no.5/2005, within the Excellency Research Program).

References

[1] T. Yupsanis, S. R. Burgess, P. J. Jackson, P. R. Shewry, J. Exp. Botany **41**, 385 (1990).
[2] J. C. Facelli, J. H. Hu, M. S. Solum, R. J. Pugmuire, D. M. Grant, in Modeling NMR Chemical Shifts; (Facelli, J. C., de Dios, A. C., Eds.), ACS Symposium Series Vol. 732; American Chemical Society: Washington, DC, **1999**; p 162.
[3] M. Torrent, D. Mansour, E. P. Day, K. Morokuma, K. J. Phys. Chem. A **105**, 4546 (2001).
[4] Calculation of NMR and EPR Parameters, Chapter E (M. Kaupp, M. Bühl, V. G. Malkin, Eds.), Wiley-VCH Verlag, 2004.
[5] M. Strohmeier, D. Stueber, D. M. Grant, J. Phys. Chem. A, **107**, 7629 (2003).
[6] Y. Wei, A. C. de Dios, A. E. McDermott, J. Am. Chem. Soc. **121**, 10389 (1999).
[7] A. M. Orendt, J. C. Facelli, D. M. Grant, Chem. Phys. Lett. **302**, 409 (1999).
[8] J. C. Facelli, Chem. Phys. Lett. **322**, 91 (2000).
[9] M. J. Frisch, G. W. Trucks et al. Gaussian 98, Revision A.7, Gaussian, Inc., Pittsburgh PA, 1998.
[10] A. D. Becke, J. Chem. Phys. **98**, 5648 (1993); A. D. Becke, Phys. Rev. **38**, 3098 (1998); C. Lee, W. Yang, R. G. Parr, Phys. Rev. B **37**, 785 (1988).
[11] V. Chiş, A. Pîrnău, T. Jurcă, M. Vasilescu, S. Simon, O. Cozar, L. David, Chem. Phys. **316**, 153 (2005).
[12] D. E. Woon, T. H. Dunning Jr., J. Chem. Phys. **98**, 1358 (1993).
[13] R. Ditchfield, Mol. Phys. **27**, 789 (1974); K. Wolinski, J. F. Hilton, P. Pulay, J. Am. Chem. Soc. **112**, 8251 (1990).
[14] G. Socrates, Infrared and Raman characteristic group frequencies: tables and charts. 3 ed., 2000, John Wiley & Sons, Chichester, UK.

*Corresponding author: cozar@phys.ubbcluj.ro