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An experimental and theoretical study of the amino acid side chain Raman bands in proteins

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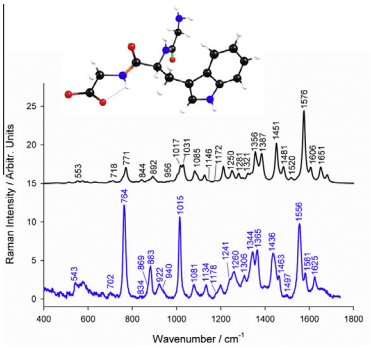
HIGHLIGHTS

- The Raman spectra of a series of tripeptides have been measured and calculated.
- A detailed band assignment has been made for all tripeptides.
- Raman frequencies of the tripeptides are blue shifted compared to free amino acids.
- Protein Raman spectra are interpreted using the position and band intensities of the tripeptides.
- The Raman bands of the tripeptides and those in the proteins show good agreement.

GRAPHICAL ABSTRACT



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ABSTRACT

The Raman spectra of a series of tripeptides with the basic formula GlyAAGly where the central amino acid (AA) was tryptophan, tyrosine, phenylalanine, glycine, methionine, histidine, lysine and leucine were measured in H_2O. The theoretical Raman spectra obtained using density functional theory (DFT) calculations at the B3LYP/6-311+G(2df,2pd) level of theory allows a precise attribution of the vibrational bands. The experimental results show that there is a blue shift in the frequencies of several bands of the amino acid side chains in tripeptides compared to free amino acids, especially in the case of AAs containing aromatic rings. On the other hand, a very good agreement was found between the Raman bands of AA residues in tripeptides and those measured on three model proteins: bovine serum albumin, β-lactoglobulin and lysozyme. The present analysis contributes to an unambiguous interpretation of the protein Raman spectra that is useful in monitoring the biological reactions involving AA side chains alteration.

Introduction

When amino acids (NH_2—C(H)R—COOH) combine via the peptide bond to form proteins it is the properties of their side chains (R) that will determine the specificity of the protein conformation and function. Moreover, the amino acids side chains are the preferred target of various reactants participating to the cell biochemistry [1]. Consequently, changes in their vibrational bands provide valuable information when the mechanisms of protein reactions are investigated. These changes can be monitored using the Raman and infra-red spectroscopy that are important noninvasive techniques for the structural characterisation of peptides and proteins [2–6]. However, the Raman spectra of proteins are often difficult to analyze due to their complex structure. Besides, it has been well documented that both the relative intensity and the frequency of protein Raman bands are sensitive to chemical changes and the microenvironment around the functional groups. For instance,

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the effect of the conformational changes of the disulphide bridges and that of the involvement of the tyrosine residues in hydrogen bonding are well known [7–9].

Vibrational spectra of the free amino acid in aqueous solution are often used in order to decipher the complexity of the protein spectra [10–13]. More recently, detailed assignments of amino acids vibrational bands based on *ab initio* calculations have been reported [11–14]. But there are very few studies which concern themselves with the Raman spectra of di or tripeptides or studies which aim to determine how Raman bands of amino acid side chains are affected by the presence of the peptide bond [15–18]. Obviously, this is a necessary step for an unambiguous assignment of Raman bands in proteins. In this paper we provide detailed spectra for a series of tripeptides with the basic formula of GlyAAGly for which the amino acid (AA) was either glycine, leucine, lysine, methionine, histidine, phenylalanine, tyrosine and tryptophan. In this series, the aromatic amino acids were selected in reason of their strong Raman bands providing a significant contribution to the Raman spectra of proteins. The remaining amino acids were selected in reason of their abundance in the structure of some model proteins here analysed. In addition to the experimental Raman spectra, the calculated Raman spectra obtained using DFT calculations are also presented and systematically used in the band attribution. The study is focused on the Raman bands of the AA side chains. Their bands were carefully separated from the tripeptide backbone contribution in order to be further used in the assignment of the protein Raman bands. This assignment was finally performed on three model proteins, Bovine serum albumin, lysozyme and β-lactoglobulin. Since all spectra were performed using the same experimental set-up, the Raman bands attribution in proteins was based not only on their frequencies (as is usually the case) but also on the corresponding band intensities observed in tripeptides.

Experimental

Glycine, GlyGly and GlyGlyGly were obtained from Sigma Aldrich and were used as received. GlyLysGly, GlyLeuGly, GlyMetGly, GlyHisGly, GlyPheGly, GlyTyrGly and GlyTrpGly (Fig. 1) were purchased from Bachem and were again used as received. Crystallised bovine serum albumin (BSA) (⩾96% fatty acid free), lysozyme (chicken egg white, ⩾90%) and β-lactoglobulin (bovine milk, ⩾90%) were obtained from Sigma–Aldrich and were used without further purification. The spectra reported here for the tripeptides were measured in aqueous solutions at 40 mM or 20 mM. All solutions were adjusted to pH 7 using the dropwise addition of 2 M NaOH (the exceptions being for GlyHisGly and GlyTrpGly for which the pH was set at pH 11 due to solubility considerations). This method of pH adjustment was chosen in order to avoid the contamination of the measured spectra by the Raman bands of the pH buffer. The spectra reported for the proteins BSA, lysozyme and β-lactoglobulin were measured at pH 7 for a protein concentration of 1 mM (for BSA) and 2 mM (for β-lactoglobulin and lysozyme).

The Raman spectroscopy experimental set up has previously been described [19]. It includes a Spectra Physics Nd/YAG laser model LAB-170-10 which delivers pulses with a duration of 5 ns at a repetition rate of 10 Hz. The equivalent power density at the sample was 30 mW mm^{-2} for a beam diameter of 1 cm. From our experience, the advantage of using pulsed excitation in Raman spectroscopy is that the relative contribution of the sample fluorescence to the detected signal is reduced due to the saturation of the excitation (this saturation does not occur for the Raman scattered light). The spectra were recorded using the second harmonic emission wavelength (532 nm) of the laser. The scattered light was detected at 90° using a Princeton Instruments spectroscopy system which includes an Acton Spectra Pro 2500i monochromator with maximum resolution of 0.035 nm and a PIMAX-1024-RB CCD camera. In the present configuration, the spectral resolution of the system is 7 cm^{-1} as determined by the FWHM of the Raman spectrum of N_2 in air. Since the FWHM of isolated Raman bands of proteins and amino acids in aqueous solution is about 20 cm^{-1}, determining the position of the band maximum with high precision requires a special treatment. For the well resolved Raman bands, a fit with a Lorentzian function was applied that gave the position of the band maximum with an error that was generally inferior to 0.5 cm^{-1}. For the remaining bands, the position of the band maximum was visually evaluated with a reproducibility within ±2 cm^{-1}. Each spectrum was acquired using 10,000 laser shots and was corrected for the solvent contribution by subtracting the Raman spectrum of water.

The laser intensity was constantly monitored by measuring the intensity of the N_2 Raman band as obtained from the laser beam scattering on air. All spectra were then corrected with respect to the changes in laser intensity by dividing them by the intensity of the N_2 Raman band acquisitioned on 400 laser shots. This is equivalent to a "normalization" of the measured intensities with respect to the Raman band of N_2 in air. Consequently, the intensities of the different experimental Raman spectra reported in the present paper are measured in the same (arbitrary) unit and can be directly compared.

The theoretical Raman bands were calculated using the Gaussian 09 program [20]. Full geometry optimization for the all molecules were carried out by the DFT method using Becke's three parameter hybrid functional combined with the Lee–Yang–Parr correlation functional (B3LYP) with the 6-311+G(2df,2pd) basis set [21,22]. Vibrational frequencies were then computed at the same level of theory. All calculations were performed in aqueous solution using the polarizable continuum model (PCM) [23]. The normal mode analysis in terms of internal coordinates was provided by Gaussian 09 and visualized with the Chemcraft software using the calculated Raman intensities and a lorentzian broadening of 15 cm^{-1} for the FWHM [24]. It is known that in the *ab initio* programs the theoretical Raman intensities are calculated based on the third derivatives of the energy whose values are very difficult to predict with high precision. Therefore, the agreement between the calculated Raman intensities reported in the present paper and the experimental values is only qualitative. As the aim of these calculations is to help to decipher experimental spectra (rather than reproducing them accurately), only the most stable conformer of each tripeptide has been considered. It is here assumed that possible peak shifts due to the variations of the backbone structure are not significant enough to prevent a reliable attribution of the experimental bands. The cartesian coordinates of the structures used in these calculations are given as Supporting Information.

Results

Model systems

Glycine, GlyGly and GlyGlyGly

In order to correctly separate the contribution of the peptide backbone to the Raman spectra of the various tripeptides here analysed, we performed a preliminary study on the series glycine, GlyGly and GlyGlyGly (Fig. 1a–c, respectively). The corresponding experimental and simulated Raman spectra are presented in Fig. 2. As for all tripeptides here studied, the explored spectral range was 400–1800 cm^{-1} since this region provides the most useful information on the backbone as well as on the side chain vibrational modes. The Raman spectrum of glycine is relatively simple containing a few well defined peaks whose positions are in good agreement with those previously reported [12,17]. The detailed