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ORIGINAL PAPER

Analysis of vibrational modes from alpha-synuclein: a theoretical model using density functional theory and Raman spectroscopy

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Abstract

Parkinson's disease is a neurodegenerative pathology difficult to diagnose. Researches have confirmed the presences of death cells in the brain produced by the modification of a protein called alpha-synuclein synuclein in people with Parkinson disease. Currently, a great amount of research is conducted to identify its biomarkers for early diagnostics. Recently, a studio found differences between the alpha- synuclein of the skin from Parkinson's disease and normal patients. In this paper, we use Raman spectroscopy through a numerical model to simulate the vibrational modes of well-defined finite clusters of alpha-synuclein in normal and pathological state, using the Gaussian09 software. The results of the model in the range of $x - y\ cm^{-1}$ are in good agreement with the experimental Raman spectra acquired from human skin with alpha-synuclein in the normal and pathological state.

Keywords Raman spectroscopy · Parkinson's disease · Density Functional theory · Alpha-synuclein

1 Introduction

Neurodegenerative diseases (ND) imply progressive degeneration of neurons in some areas of the brain. Studies suggest that this degeneration is due to the conformational change of a protein called alpha-synuclein that cause toxicity, and finally induces degradation in functions generally associated with the nervous system [1, 2]. The older population (>60 years old) is affected by this kind of pathologies. According to the prevalence of these disorders, currently, these are considered a public health problem due to the rising life expectancy of the people. Parkinson's disease (PD) is one of the most prevalent NDs. It is diagnosed using clinical criteria, such as symptomatology, laboratory studies to detect smells, video fluorography, and neuroimaging studies. PD is characterized by the presence of tremor, rigidity, akinesia, bradykinesia, postural alteration, unilateral dystonia, ideomotor apraxia, dysphagia, personality changes, dementia, depression, and a decrease of olfaction. However, there is little knowledge of PD, and its biomarkers are still being determined. Consequently, PD is usually diagnosed once the symptoms are evident; that is, at its advanced stages [3–6]. Recent research has studied methods detecting of non-invasive biological markers for early and prompt diagnosis, and it has found that people with PD show aggregates of the alpha-synuclein protein in the brain. Such results suggest that these aggregates (overexpression) are responsible for cell death and have an important role in the neurodegeneration [7].

Alpha-synuclein is the largest fibril constituent from Lewy bodies in dopaminergic neurons in the substantia nigra, which are the main characteristic of the PD.

Recent research, based on biopsies of skin from people with PD has demonstrated differences in the alpha-synuclein morphology between skin from PD patients and healthy people (associated with the aggregates of alpha-synuclein in the brain) [8]. Thus, this study suggests the analysis of the conformational state of alpha-synuclein for diagnostic purposes. However, the use of skin biopsies is an invasive procedure that should be done by qualified personnel. As a consequence, this entails to the search of methods to quantify the difference between the healthy skin and PD, in an easy, quickly and noninvasive way.

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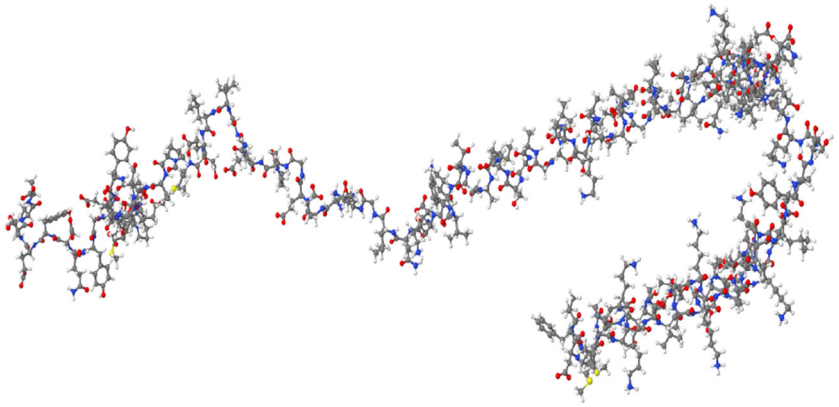


Fig. 1 Molecular geometry of protein alpha-synuclein in the normal state

In this sense, methods coming from Biophotonics have been incorporated as a tool by the analysis and treatment of some diseases [9]. One of these techniques is the Raman spectroscopy that identifies the vibrational modes of molecular bonds from a sample. As a result, Raman spectroscopy provides a Raman spectrum (RS) that contains information from a sample (a molecular fingerprint). Some advantages of Raman spectroscopy are that: it is a non-invasive technique, it is fast and safe, and samples can be in a solid, liquid or gaseous state. Such characteristics have allowed their inclusion in biomedical and biological applications. Thus, Raman spectroscopy has been used to characterize different types of proteins as well as their conformational changes. Recently, the conformational changes in the alpha-synuclein protein (normal state and associated with the PD) has been analyzed. The results have shown differences in the profile Raman related to the organic base amide I [7, 10, 11].

In this sense, the analysis of conformational changes of alpha-synuclein associated with PD pathology are analyzed in this work, using Raman spectroscopy through a numerical model. Accordingly, the Raman spectrum of different molecular modeling of the protein alpha-synuclein in a normal state, using the chemical software computational Gaussian09 was acquired. The results have shown the presence and differences in the profile amide I as was reported in previous works.

The work is organized as follows, in section 2 are provided information of the protein, the organic component amide I and all methods used for this work. On the other hand, section 3 are showed the results from the simulations of the protein and the comparison with the previous results, finally, in section 4 are presented the discussion and conclusions.

2 Methodology

2.1 Alpha-synuclein Protein and Amide I

The alpha-synuclein protein in normal state was downloaded (digital format) from the database of proteins (Protein Data Bank) [7]. Figure 1 shows the molecular geometry of the alpha-synuclein protein, which is composed of 140 amino acids, with 2017 atoms and 2028 bonds between them.

Alpha-synuclein is an unfolded natively protein, due to its nature has an exposure more consistent to the amide bonds. By adopting shapes alpha-helical and beta-sheet, Alpha-synuclein is characterized by adopting the shape of fibrils. According to the literature, Raman spectrum of amide I from unfolded protein is less complex than the structure of the folded protein, consequently, with the analysis of the Raman

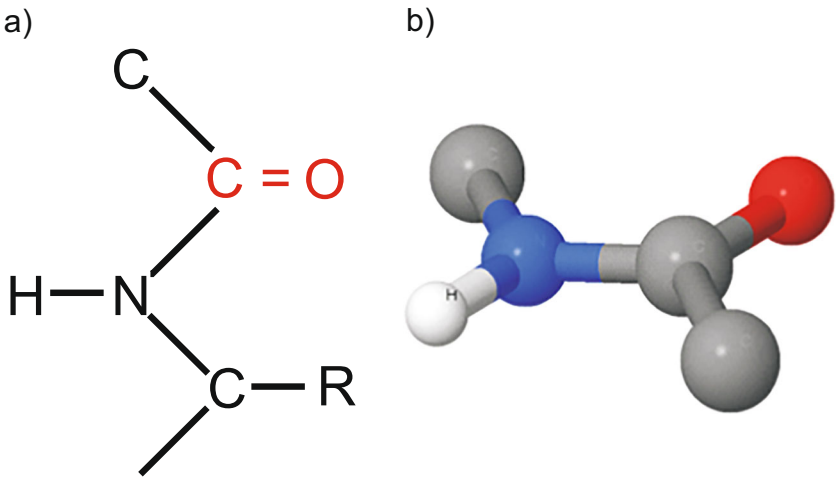


Fig. 2 Amide I

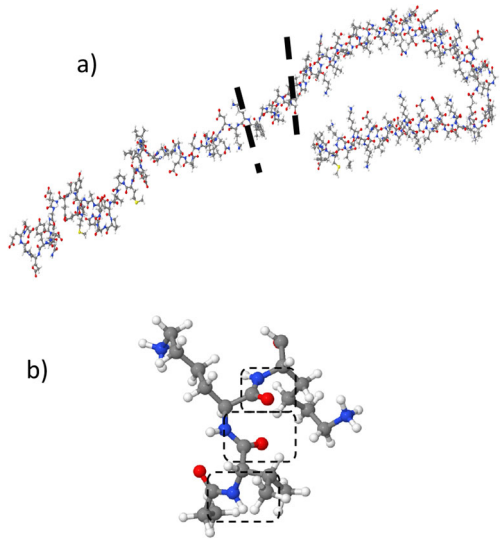


Fig. 3 **a** Alpha-synuclein and **b** first structure of 68 atoms

peak amide I (in the range of 1610 cm^{-1} to 1720 cm^{-1}) detailed information is acquired about of the protein structure and its conformational changes [11, 12]. Amide I is composed of one nitrogen (blue), one hydrogen (white), three carbons (grey), one oxygen (red) and the combination of this last with a carbon (carboxyl $C = O$) [13]. Figure 2 shows the geometry structure of the amide I component.

2.1.1 Simulation of Raman spectra

For the visualization of the geometric structures of the amide I and the protein (in three dimensions) was used Jmol. On the other hand, for the simulation of the Raman spectrum from the protein alpha-synuclein was used the software specialized Gaussian 09. The same software was used for the identification and simulation of the vibrational modes of molecular bonds, specifically of the profile amide I. As well as Gaussian View was used to display them. Gaussian is a computational software used in quantum chemistry, which performs calculations of the electronic structure from systems of many electrons using the density functional theory (DFT).

Fig. 4 Optimized molecular structure with Gaussian 09

DFT evaluate the bond energy of the electrons in a normal state, using as functional the spatial dependence of electronic density [14–16]:

$$E_{DFT} = E_{NN} + E_T + E_V + E_{coul} + E_{exch} + E_{corr}. \quad (1)$$

The methods based on DFT could be locals, no locals, and hybrids. The locals depend on the electronic density, the non-locals depend on the electronic density and its gradient, and finally, the hybrid methods incorporate the energy of exchange between elements from the system [15].

The optimization of the geometry of balance with the functional PBEPBE was obtained using Gaussian. The optimized model was generated with the vibrational frequencies and the Raman active. For characterization of the orbital molecules was used the basis set 6-31G.

These parameters were selected according to the information existing in the literature since it allows an adequate optimized geometry and vibrational frequencies accurately.

3 Results

Through the structure of Fig. 1 was made the simulation of the Raman spectrum, but given the high quantity of atoms, it was considered a small structure located in the central section of the protein; to subsequently increase the number of atoms in the simulation, ensuring that the structure amide I is present mainly in the structure selected. The first structure of approximately 68 atoms was processed with Gaussian to obtain its vibrational modes and its Raman spectrum. In Fig. 3 is shown the selected area. The dotted lines in Fig. 3a delimit the structure of 68 atoms, which was used during the first Raman spectrum. On the other hand, in Fig. 3b is presented the molecular structure analyzed, the square dotted indicates the sections where the amide I is located.

Subsequently, the optimized model with the specifications described above is shown in Fig. 4. We can observe the optimized structure where their atoms are in a balanced position with the potential energy minimum, is to say with minimal energy as occur regularly in nature. On the other hand, in Fig. 5 is shown the Raman spectrum obtained through the structure in Fig. 4. According to the information collected in the literature, as well as the results obtained in our experimental data, the spectral region in the range of 1610 cm^{-1} to 1720 cm^{-1} was analyzed. The results are shown in Fig. 6.

In Fig. 6 are shown three Raman peaks located at 1650 cm^{-1}(-with two subpeaks at 1648 cm^{-1} and 1652 cm^{-1}), 1666 $cm^{-1}$and 1688 cm^{-1} from profile amide I. The vibrational modes of each Raman peak are shown in Table 1, which summary the vibrational modes found in the analyzed structure, where the dotted

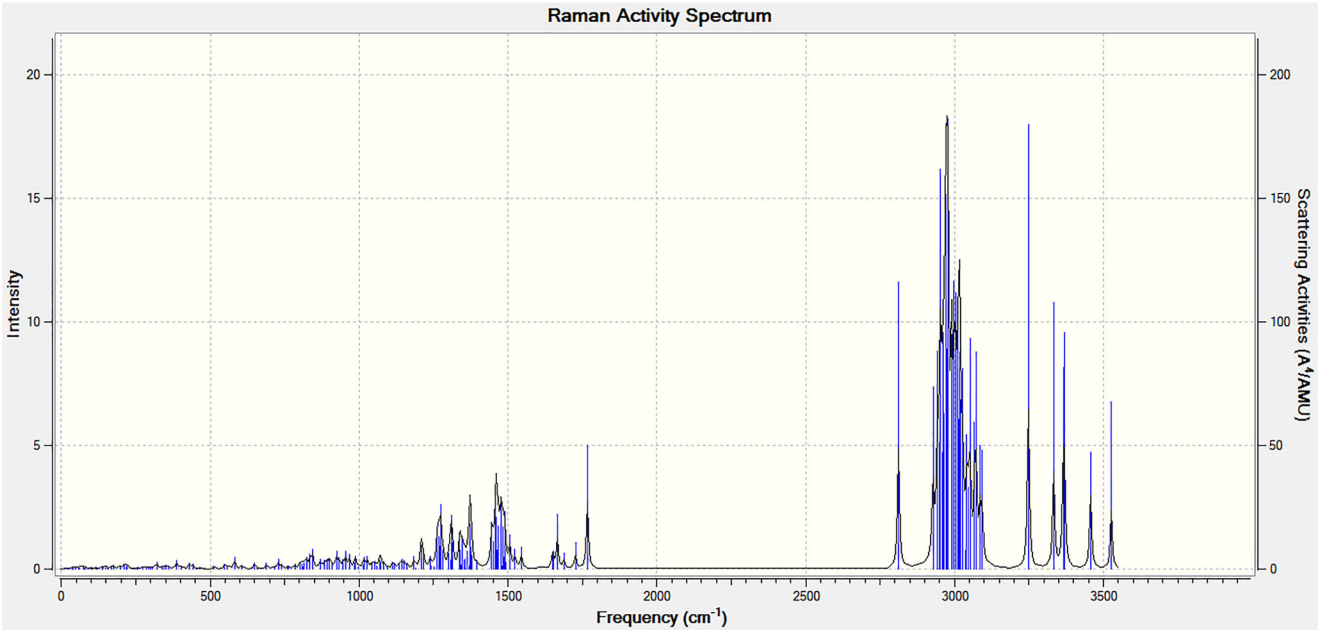


Fig. 5 Preliminary Raman spectrum of protein alpha-synuclein

squared shows the atoms that vibrate for each frequency. As is shown in the pictures and the Raman spectrum of Fig. 6, the vibrational modes identified are related directly to the amide I in the range spectral of 1610 $cm^{-1}$to 1720 cm^{-1}.

On the other hand, in Fig. 7 is shown the Raman spectrum obtained from a skin biopsy of a person with Parkinson's disease. The results, in Figs. 6 and 7, suggest the presences of the Raman peak of amide I in the skin of patients with Parkinson's disease as was observed in the Raman spectrum of the alpha-synuclein.

4 Discussion

Through a numerical model, a Raman spectrum was simulated according to experimental data based on the information in the literature. This suggests results with the same characteristics that the experimental data. This means, that the method could helps to make studies in Silico and reduce costs to studies the properties of the alpha-synuclein in persons with Parkinson disease.

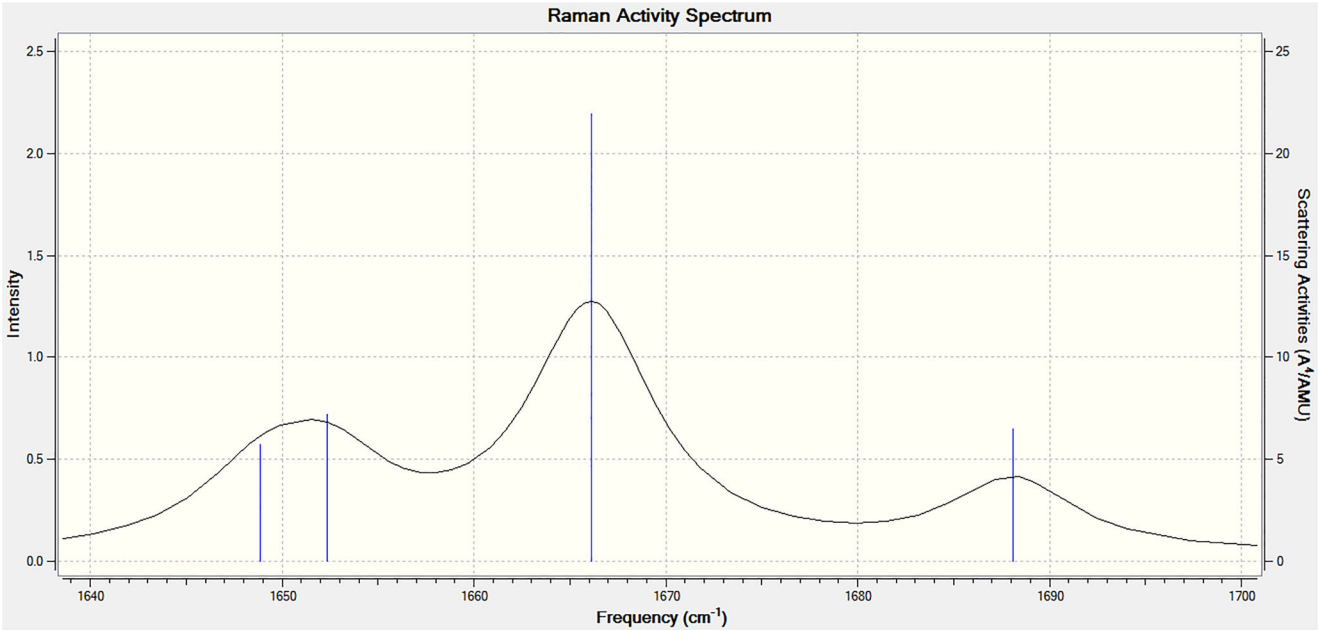
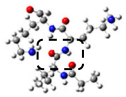
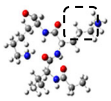


Fig. 6 Raman spectrum previously simulated of amide I from protein alpha-synuclein

Table 1 Vibrational Mods

	1648cm^{-1}, associated with the Raman peak Amide I (Stretching).
	1652 cm^{-1}, not associated with the Raman peak Amide I (Bending).
	1666 cm^{-1}, associated with the Raman peak Amide I (Stretching).
	1678 cm^{-1}, associated with the Raman peak Amide I (Stretching).

The results suggest a correspondence between our experimental results, the results registered in the literature, and the theoretical results (simulate). This ensures that the alpha-synuclein is sensitive to be identified using Raman spectroscopy. Consequently, it is suggested that the Raman spectroscopy could be used to identify conformational changes of the protein alpha-synuclein in vivo, associated with the protein in shape of fibrils (over-expression present during the Parkinson's disease), analyzing the Raman peak of amide I.

5 Conclusion

The numerical model predicted in a fine manner the results of experimental acquisitions, this is interesting since it helps to minimize cost and save time to study the alpha-synuclein protein by avoiding extra experiments. On the other hand, through Raman spectroscopy simulation was possible to identify the Raman peak of amide I of the alpha-synuclein using Gaussian 09. Thus, the model theoretical confirmed that our findings of the protein are directly associated to specific

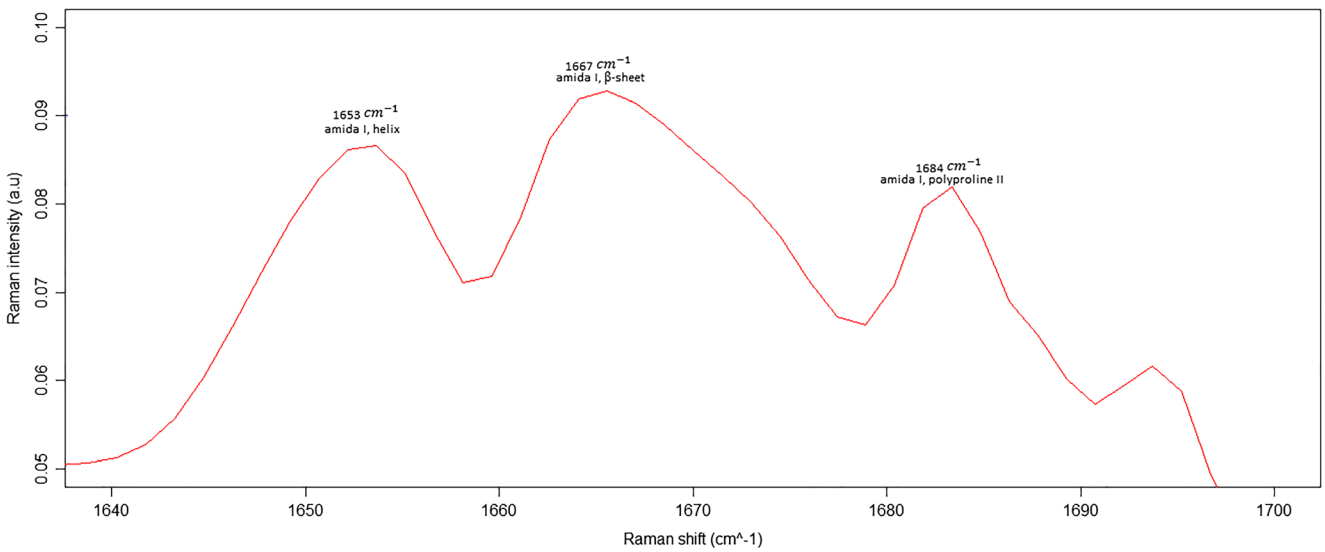


Fig. 7 Raman spectrum of the skin biopsy from a patient with Parkinson's disease

vibrational modes of the amide I to different frequencies and velocities as is registered in the literature.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent There is no informed consent.

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