Low frequency dynamics of lysozyme: A Raman scattering and low temperature specific heat study

C. Crupi a,*, G. D’Angelo a, U. Wanderlingh a, V. Conti Nibali a and C. Vasi b

a Department of Physics, University of Messina, Messina, Italy
b IPCF-CNR, University of Messina, Messina, Italy

Abstract. Raman scattering and low temperature specific heat ($C_p$) measurements have been performed to investigate the low frequency vibrational dynamics of hen egg white dry lysozyme. The occurrence of low energy extra excitations have been revealed both in the light scattering spectrum and in the $C_p/T^3$ vs. $T$ plot. A perfect agreement in the frequency and temperature position of the Boson Peak in this protein has been observed. At temperature below 3 K it has been found a contribution to the specific heat similar to that revealed in glassy systems. This, together with the other analogies discovered between thermal properties of amorphous and biological systems, suggests the possibility of making the most of the knowledge about the former in order to understand some glassy-like behaviours of proteins.

Keywords: Boson Peak, lysozyme, protein, specific heat, Raman scattering, low frequency dynamics

1. Introduction

It is generally accepted that proteins require internal flexibility for their activity. In the last decades, numerous studies on biological systems have lead to suppose that the specific conformation of a protein could influence its modes having frequency less than 200 cm$^{-1}$ [3–6,9,15] which are thought having a peculiar role for some biological functions, such as protein folding and catalytic functions of enzymes [12,18]. Thus, in order to understand the function properly, it is necessary to understand both the structure of the specific protein and the nature of its low frequency dynamics.

Many experimental and theoretical efforts have been done in order to understand the origin of these low frequency excitations but many questions remain still unsolved even though the interest in this research field is still increasing, together with the number of experimental evidences of the occurrence of low frequency vibrational motions in globular proteins. In particular these excitations have been observed by performing inelastic light [10] and neutron [2,3,6,8] scattering measurements. More precisely, these modes merge into a clearly observable asymmetric peak located in the frequency range below 300 cm$^{-1}$ in the Raman spectra and below 10 meV in the inelastic neutron scattering data. This feature of globular proteins represents a similarity to glassy systems, for which, this low frequency band, commonly named Boson Peak, is considered to be an excess of the vibrational density of states $g(\nu)$ with respect to the Debye value predicted for a crystalline material.

*Corresponding author: C. Crupi, Department of Physics, University of Messina, via Stagno D’Alcontres 31, 98166 Messina, Italy. E-mail: cristina.crupi@yahoo.it.
The idea that proteins can be described as disordered atomic structures performing specific biological functions is today largely diffused and continues to strengthen thanks to many evidences that they have also anomalous behaviours in their thermal properties similar to amorphous systems. For example, the low temperature specific heat in metmyoglobin crystals [14] and synthetic melanine [17] indicates the presence of a broad energy spectrum of localized, low-energy excitations similar to those found in other glasses.

Unfortunately, notwithstanding the numerous experimental techniques available for studying protein dynamics, the complexity of these biological macromolecules has prevented from combining all the acquired information into a cohesive picture which otherwise remains still piecemeal.

An innovative approach to reach a deeply knowledge about this subject would be to take advantage of the observed analogies between the dynamics of proteins and of glasses lavishing on biological systems what is known about amorphous materials. Thus, it would be useful to investigate biological systems by using the same techniques which have already given very important information about amorphous materials. In this regard, low temperature calorimetry could allow to study just those low frequency excitations supposed to be involved in the biological activity of proteins.

The main goal of this article is the study of the low energy vibrational dynamics of lysozyme by performing Raman scattering and low temperature specific heat measurements. This protein has been extensively studied by all conventional means and thus can be considered as the most convenient sample to be analyzed in order to compare the information collected by both techniques and to test the validity of low temperature specific heat measurements as a useful tool to investigate low frequency dynamics of biological systems.

2. Materials and methods

Lyophilized chicken hen egg white lysozyme powder has been used as purchased from Sigma without further purification.

Raman scattering experiments have been performed at room temperature by using an Ar⁺-ion laser (λ = 514.5 nm) with a power of 300 mW as the excitation source and a Yobin Yvon U-1000 double monochromator. The light scattering data have been acquired in the backscattering geometry with a resolution of the experimental set-up of 1 cm⁻¹. The sample has been prepared by sealing the powder between sapphire windows, which contributed a negligible intensity to the light-scattering spectra in the frequency range of interest (ν < 200 cm⁻¹).

Specific heat measurements have been carried out in the temperature range between 1.5 and 23 K by means of an automated thermal relaxation method [1]. The dry powders of lysozyme have been packed into a copper foil cell housing a total amount of about 15 mg of the sample.

3. Results and discussion

In Fig. 1 the light scattering spectrum of dry lysozyme is shown while in the inset of Fig. 1 it is plotted the normalized Raman intensity as I_{exp}/[(ν × [ν(ν) + 1)]) vs. ν which has been obtained normalizing the raw data to the Bose–Einstein population factor [ν(ν) + 1] and to the harmonic propagator 1/ν after having subtracted the background. This is the same procedure conventionally applied to study low frequency vibrational dynamics of glasses by light scattering measurements.
Fig. 1. The light scattering spectrum of dry lysozyme is plotted as solid line. In the inset the normalized Raman data in the frequency region below 250 cm$^{-1}$ are shown.

In fact, it is known that the low frequency Raman scattering in glasses is related to the vibrational density of states $g(\nu)$ [7,13] by the equation:

$$I_R(\nu) = \frac{I_{exp} \nu}{[n(\nu, T) + 1]} = C(\nu)g(\nu),$$  \hspace{1cm} (1)

where $I_R$ is the so-called Raman intensity and $C(\nu)$ represents the light-vibration coupling coefficient, which turns out to have a complicated dependence on $\nu$.

Thus, according to Shuker and Gammon [13], the spectrum in the inset of Fig. 1 represents the product between the vibrational density of states as $g(\nu)/\nu^2$ and the coupling constant:

$$I_S(\nu) = \frac{I_{exp}}{\nu[n(\nu, T) + 1]} = \frac{C(\nu)g(\nu)}{\nu^2}. $$  \hspace{1cm} (2)

With the exception of some peaks due to the sapphire windows and lying in the region of frequency behind 400 cm$^{-1}$, the spectrum of dry lysozyme is characterized by a quasi-elastic tail at frequency below 15 cm$^{-1}$ followed by an inelastic contribution.

Due to the high temperature, the strong quasi-elastic scattering prevents from having a high definition for the profile of the inelastic part of the spectra since the low frequency tail of the bump is covered by this contribution. Anyway, according to previous light scattering study [16] of dry lysozyme, it is possible to observe the occurrence of the Boson Peak, centred at about $\nu_{BP} = 25$ cm$^{-1}$ and, furthermore, in the region behind 50 cm$^{-1}$, the presence of a shoulder (see the inset in Fig. 1) probably derived by the overlapping between a second band and the right tail of the first bump.
In Fig. 2 it is shown the temperature dependence of the reduced specific heat $C_p/T^3$ of dry lysozyme. The revealed trend is characterized by the presence of an upturn at $T < 3$ K and a well-defined asymmetric broad peak centred at $T_{\text{max}} \approx 6$ K which put in evidence a strong deviation from the expectation of the Debye model. In glasses, the former contribution is usually ascribed to the existence of tunneling states [11], the microscopic nature of which is still unknown. The detection of the same contribution in lysozyme leads to consider the possible existence of these extra excitations also in biological systems. The treatment of this subject, however, would require more accurate measurements at lower temperatures and it is anyway beyond the topic of this paper.

Moreover, it is worth to emphasize that low-temperature specific heat and low-energy vibrational density of states are closely connected by the following equation:

$$C_p \approx C_V = 3N\kappa_B \int_0^{\nu_0} g(\nu) \cdot \left( \frac{\hbar\nu}{\kappa_B T} \right)^2 \cdot \frac{\text{e}^{\hbar\nu/(\kappa_B T)}}{\left[ \text{e}^{\hbar\nu/(\kappa_B T)} - 1 \right]^2} \cdot d\nu,$$

where $N$ is the Avogadro number, $\kappa_B$ is the Boltzmann constant and $\nu_0$ is the Debye’s frequency. In the light of this statement, it appears clear how the reduced low temperature specific heat can be directly compared to the normalized Raman intensity since these two techniques allow to investigate the same low frequency dynamics. More precisely, this agreement is reflected into the correspondence between the positions of the bump in the specific heat and in the Raman scattering data. As a matter of fact, the temperature position $T_{\text{max}}$ of the bump refers directly to the Boson Peak frequency $\nu_{\text{BP}}$, being its equivalent value in temperature in the dominant phonon approximation ($\hbar\nu_{\text{BP}} = 4\kappa_B T_{\text{max}}$).

Furthermore, the peak in the $C_p/T^3$ shows an asymmetric shape mostly observable in the right tail of this bump which could arise from the overlapping of two contributions to the specific heat: the first due to the Boson Peak modes and the second due to the incoming modes at higher frequencies which merge into the shoulder observed in the Raman scattering spectrum. The extension of the investigated
temperature range over 30 K would give more information about this last contribution allowing to study in detail the modes contributing to the specific heat whose equivalent frequency values are higher than 50 cm$^{-1}$.

4. Conclusions

The low frequency dynamics of dry lysozyme have been investigated by performing room temperature Raman scattering and low temperature specific heat measurements. The comparison between the experimental results obtained by using these two techniques have shown a perfect agreement in settling the position of the Boson Peak in terms of frequency and of its equivalent temperature. A further correspondence is expected to exist also for the modes at frequency higher than 50 cm$^{-1}$ whose contribution in the Raman spectra is clearly visible and in the reduce specific heat trend is supposed to be masked by the asymmetric shape of the Boson Peak and only barely present due to the restricted temperature range investigated.

Moreover, low temperature specific heat measure has allowed to reveal the presence of an upturn at temperature below 3 K, a contribution that, in glassy systems, is usually ascribed to two level systems. Further investigation at lower temperatures could help in detailing this contribution and could give more information about the occurrence of these extra excitations also in biological systems.

In the light of these results it appears clear that low temperature calorimetric measurements can be surely considered as a very useful and promising tool to investigate low frequency dynamics of biological systems.

References

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