Anharmonic transitions in nearly dry L-cysteine I

T A Lima¹, E T Sato¹, E T Martins¹, P Homem-de-Mello¹, A F Lago¹, M D Coutinho-Neto¹, F F Ferreira¹, C Giles², M O C Pires¹ and H Martinho¹

¹ Centro de Ciências Naturais e Humanas, UFABC, Rua Santa Adélia 166, Santo André 09210-170, São Paulo, Brazil
² Instituto de Física ‘Gleb Wataghin’, UNICAMP, Campinas 13083-970, São Paulo, Brazil

E-mail: herculano.martinho@ufabc.edu.br

Received 8 December 2011, in final form 24 February 2012
Published 13 April 2012
Online at stacks.iop.org/JPhysCM/24/195104

Abstract

Two special dynamical transitions of universal character have recently been observed in macromolecules (lysozyme, myoglobin, bacteriorhodopsin, DNA and RNA) at \( T^* \sim 100–150 \) K and \( T_D \sim 180–220 \) K. The underlying mechanisms governing these transitions have been the subject of debate. In the present work, a survey is reported on the temperature dependence of structural, vibrational and thermodynamical properties of a nearly anhydrous amino acid (orthorhombic polymorph of the amino acid L-cysteine at a hydration level of 3.5%). The temperature dependence of x-ray powder diffraction patterns, Raman spectra and specific heat revealed these two transitions at \( T^* = 70 \) K and \( T_D = 230 \) K for this sample. The data were analyzed considering amino acid–amino acid, amino acid–water, water–water phonon–phonon interactions and molecular rotor activation. Our results indicated that the two referred temperatures define the triggering of very simple and particular events that govern all the interactions of the biomolecular: activation of CH\(_2\) rigid rotors (\( T < T^* \)), phonon–phonon interactions between specific amino acid and water dimer vibrational modes (\( T^* < T < T_D \)), and water rotational barriers surpassing (\( T > T_D \)).

(Some figures may appear in colour only in the online journal)

1. Introduction

The search for correlations between fluctuations, excitations and biochemical activity has recently motivated experimental and theoretical advances in the field of the physical properties of biomolecules. In biological systems, fluctuations and, consequently, excitations are intrinsic components of biomolecular activity. Their understanding is of utmost interest.

Recently two special dynamical transitions, apparently of universal character, were observed in macromolecules such as lysozyme, myoglobin, bacteriorhodopsin, DNA and RNA (see, e.g., [1, 2]). With cooling, the first transition occurred at \( T_D \sim 180–220 \) K and was observed in macromolecules with a hydration level \( h > 0.18 \). It is related to a transition from the anharmonic to harmonic regime where, for example, the hydrogen mean-squared displacement (msd) suddenly decreases and starts displaying linear temperature dependence [2]. The most remarkable character of this transition is its direct correlation to the biological activity. In fact, many studies indicated that it represents the lowest temperature at which the macromolecule can perform its physiological function [1–5]. It has been suggested [1, 4] that this temperature represents the activation of a number of relaxation processes coupled to atomic displacements, which are of vital relevance to the biochemical activity. For example, anharmonic movements are strongly coupled to the kinetic constants of the intermediate states in the photocycle and proton pumping in bacteriorhodopsin [6].

However, some important counterexamples are also presented in the literature. Lopez et al [7] showed that neither hydration water nor fast anharmonic dynamics are required for...
catalysis by the pig liver esterase enzyme. The msd measured in native and denatured hydrated lysozymes by Mamontov et al [8] revealed that the dynamical transition was not specific to biologically functional molecules. Schiro et al [9], comparing the dynamic behavior of myoglobin with that of an amino acid mixture having the same chemical composition but lacking the polypeptide chain, concluded that neither the protein dynamical transition nor the boson peak needed the protein polypeptide chain to be observed.

Based on quasi-elastic scattering (QENS) measurements on lysozyme, Chen et al [10] interpreted this transition as a fragile-to-strong dynamic crossover where the structured water makes a transition from a high-density to a low-density state. This scenario was based on studies that advocated a second critical point of water (liquid–liquid critical point) in confined supercooled water [11]. However, a recent high resolution QENS experiment performed by Doster et al [12] in fully deuterated C-phycocyanin protein showed no evidence of such fragile-to-strong character at \( T_D \). As pointed out by these authors, their experimental findings are consistent with a glassy transition of the hydration shell. However, the exact nature of the glassy state remains unclear. Moreover, the particular role of protein and water is still an issue. As an example, Frauenfelder et al [1] proposed that the protein dynamics is slaved to the bulk solvent fluctuations. On the other hand, the dynamics of tRNA compared to other studies on DNA and proteins by Khodadadi et al [13] contradicts the slave picture. Their results indicated the mutual influence of macromolecule and hydration water must be considered. Schiro et al [14] studied the dynamic behavior of different residues separately in order to assess the contribution of each type of side chain to the anharmonic dynamics of proteins. The contributions of the order of 10–20% from the motions of other groups are present. The dynamical transition occurring at \( \sim 230 \) K was attributed mainly to motions involving backbone fluctuations. Studies on hydrated protein samples [15] have shown that, while the pure backbone contribution does not depend on the hydration level at \( > 0.2 \) in the presence of side chains, the anharmonic fluctuations involved in the dynamical transition are enhanced by increasing the water content.

The other important transition observed in macromolecules is a hydration independent sub-regime of harmonicity onset at \( T^* \sim 100–150 \) K [2]. The microscopic nature of this transition is still an object of debate. Discussed possibilities include quantum effects near a zero-point vibration [17] and methyl group rotation associated with anharmonicity [2, 18]. The elastic neutron scattering results by Schiro et al [14, 15] shows that methyl group rotations are unaffected by hydration and confirms that the dynamical transition is suppressed in dry samples. Wood et al [16] comparing the dynamics of a non-methyl-containing side-chain (lysine) protein membrane to a naturally abundant control protein sample concluded that the temperature inflection observed at \( T^* \) originated in the methyl dynamics.

Vibrational modes play a very important role in the conformational arrangement of different functional states and in the energetic balance of the chemical bonds [4, 5, 19]. Theoretical studies concerning anharmonic effects on phonons show that their frequency and linewidth analyzed by the inelastic light scattering (Raman effect) could vary due to the anharmonic potential shape of the simple oscillator and due to phonon–phonon interactions as well [20–22]. Knowledge of the underlying mechanism of the anharmonic effects, the role of water and of dynamical transitions could be of assistance in the design of bioprotectant materials, and also shed light on the life machinery process itself. Studies on short protein fragments such as amino acids could provide a very interesting approach to solve the problem. The low molecular weight amino acids are very suitable for computational modeling due to their comparatively small structures and can be used as functional prototypes of more complex structures such as proteins and lipids. In fact, subtle fluctuations in their physical properties could trigger the most significant changes detected in large macromolecules.

The present study is focused on the temperature dependence of structural, vibrational and thermodynamical properties of the orthorhombic polymorph of the amino acid L-cysteine, \( \text{C}_\text{H}_\text{S}_\text{O}_\text{N}_\text{O} \). The amino acid possesses a very simple chemical structure and high biological relevance. The thiol or sulfhydryl group in the residues of L-cysteine is the most chemically reactive site in proteins under physiological conditions [23]. Therefore, the knowledge of its dynamics is very important.

2. Materials and methods

2.1. Experimental details

2.1.1. Sample. The sample used was a commercial powder from Sigma-Aldrich (St Louis, MO, USA) with purity \( \geq 97\% \). The pellet made was stored in a dry ambient (small glass recipient with silica gel) to avoid moisture variations. The water content was determined by titration with the Karl Fisher method as being 3.4% in mass (nearly one water molecule per unit cell on average). The water presence was also checked by the presence of the water association band (WAB) and water combination band (WCB) at \( \sim 2080 \) cm\(^{-1}\) and \( \sim 5400 \) cm\(^{-1}\), respectively, by Fourier transform infrared (FTIR) spectroscopy [24, 25].

2.1.2. FTIR spectroscopy. FTIR measurements were performed on a Varian 660 FTIR spectrometer using a Miracle diamond ATR accessory (Pike Tech).

2.1.3. Raman spectroscopy. The Raman scattering excitation was performed employing a 514 nm line of an Ar-ion laser (Innova 70C, Coherent, Santa Clara, CA, USA). The typical stimulated Raman spectroscopy experiment is excited with a very low bandwidth laser (\( \sim 5 \) MHz at 514 nm or \( \sim 0.02 \) \( \mu \)eV). The impinging photons interact with the vibrations mediated by the electron–phonon coupling. After excitation, the system undergoes a transition to a virtual state which decays to a vibronic state with emission of an inelastic
2.1.4. X-ray diffraction: room temperature, Rietveld refinement. The crystallographic phase was checked performing the x-ray diffraction (XRD) experiments at room temperature on a Bruker D-8 Foccus Diffraction meter (Lynxeye 1D detector) with Ni-filtered Cu Kα1 radiation in the range from 8° to 40° (0.02° increment). After the measurements, the diffractograms obtained were refined by the Rietveld method [26].

2.1.5. X-Ray diffraction: temperature dependence. For the measurements of XRD pattern temperature dependence, the powdered sample was loaded to fill ~5 cm in a 0.7 mm diameter borosilicate glass capillary, which was kept spinning. An Oxford Cryosystem 700 was used to follow the behavior of thermal expansion of the unit cell of L-cysteine, with temperature ranging from 10 to 300 K, with a variation of 15 K. Cu Kα1 radiation, obtained by a VariMax Cu HR Confocal Max-Flux multilayer system, from a Rigaku UltraX-18HF rotating anode source was used. The Debye rings of L-cysteine were recorded by using a MAR345 imaging-plate detector. After the measurements, the unit cell parameters were obtained from the diffractograms for each temperature studied.

2.1.6. Specific heat. The calorimetric measurements were performed using the commercial automated heat-capacity measurement system option of the Physical Properties Measurements System—PPMS (Quantum Design, San Diego, CA, USA). They were performed using the relaxation method. A determined heat power is delivered to the sample over a specific time. As a result the sample temperature was raised some fraction of a degree. The relaxation to the environmental temperature is monitored, and this data is fitted to obtain the specific heat of the sample. The timescale is governed by the thermal conductivity of the sample itself. It employs a thermal relaxation calorimeter that operates in the temperature range of 1.8–395 K [27].

2.2. Computational

Density functional theory (DFT) [28, 29] was used in order to obtain the equilibrium geometries and harmonic frequencies for crystalline L-cysteine using two approaches: free molecule (fm) and periodic boundary condition (pbc) calculations using a fixed unit cell volume. The fm approach used the B3PW91 hybrid functional [30, 31] as implemented in the Gaussian 03 revision E.01 suite [32], while the pbc calculations used the BLYP functional [33, 34] augmented with dispersion corrections for the proper description of dispersion interactions [35, 36] as implemented in the CPMD code [37]. A 6-311G(d, p) Pople basis set was used in all atoms for the fm calculations. The pbc calculations used a 70 Ryd energy cutoff, a cubic cell of 24 Å in length and norm-conserving Troullier–Martins pseudopotentials.

3. Results and discussion

Figure 1(a) shows the room-temperature XRD pattern (open circles) of the studied sample. The Rietveld refinement (solid line) indicated that the predominant phase is orthorhombic (P2₁2₁2₁ space group) with unit cell parameters \( a = 8.069(11) \text{ Å}, b = 12.0988(12) \text{ Å} \) and \( c = 5.3887(13) \text{ Å} \). Our findings are consistent with others previously reported in the literature [38, 39]. As commented above, the water content was determined by the Karl Fisher titration method and its presence confirmed by FTIR spectroscopy data. The presence of water was also checked by analyzing the possible changes in the Rietveld refinement when incorporating small water content (one and two water molecules) in the unit cell. The difference between observed and calculated diffraction patterns is shown in figure 1(b) for each case. It could be seen that the refinement quality was very similar in all cases. Based on FTIR and Raman data, which will be discussed below, we have evidence for the presence of water dimers in the crystal structure. The refinement indicated that the water content is distributed as dimers in the unit cell with an occupancy of 0.25 with corresponding lattice parameters \( a = 8.132(2) \text{ Å}, b = 12.190(3) \text{ Å} \) and \( c = 5.443(1) \text{ Å} \). Large atomic motion amplitude was observed for the water molecules which could indicate a high disorder degree. It is important to observe that all expected diffraction peaks were present in the phase for each case, which

\[
\omega \propto \frac{1}{\tau} \propto \frac{1}{\text{thermal conductivity}}
\]

\[
\text{The timescale is governed by the thermal conductivity of the sample itself.}
\]

\[
\text{It employs a thermal relaxation calorimeter that operates in the temperature range of 1.8–395 K [27].}
\]

\[
\text{Density functional theory (DFT) [28, 29] was used in order to obtain the equilibrium geometries and harmonic frequencies for crystalline L-cysteine using two approaches: free molecule (fm) and periodic boundary condition (pbc) calculations using a fixed unit cell volume.}
\]

\[
\text{The fm approach used the B3PW91 hybrid functional [30, 31] as implemented in the Gaussian 03 revision E.01 suite [32], while the pbc calculations used the BLYP functional [33, 34] augmented with dispersion corrections for the proper description of dispersion interactions [35, 36] as implemented in the CPMD code [37].}
\]

\[
\text{A 6-311G(d, p) Pople basis set was used in all atoms for the fm calculations. The pbc calculations used a 70 Ryd energy cutoff, a cubic cell of 24 Å in length and norm-conserving Troullier–Martins pseudopotentials.}
\]

\[
\text{3. Results and discussion}
\]

\[
\text{Figure 1(a) shows the room-temperature XRD pattern (open circles) of the studied sample. The Rietveld refinement (solid line) indicated that the predominant phase is orthorhombic (P2₁2₁2₁ space group) with unit cell parameters \( a = 8.069(11) \text{ Å}, b = 12.0988(12) \text{ Å} \) and \( c = 5.3887(13) \text{ Å} \). Our findings are consistent with others previously reported in the literature [38, 39]. As commented above, the water content was determined by the Karl Fisher titration method and its presence confirmed by FTIR spectroscopy data. The presence of water was also checked by analyzing the possible changes in the Rietveld refinement when incorporating small water content (one and two water molecules) in the unit cell.}
\]

\[
\text{The difference between observed and calculated diffraction patterns is shown in figure 1(b) for each case. It could be seen that the refinement quality was very similar in all cases. Based on FTIR and Raman data, which will be discussed below, we have evidence for the presence of water dimers in the crystal structure. The refinement indicated that the water content is distributed as dimers in the unit cell with an occupancy of 0.25 with corresponding lattice parameters \( a = 8.132(2) \text{ Å}, b = 12.190(3) \text{ Å} \) and \( c = 5.443(1) \text{ Å} \). Large atomic motion amplitude was observed for the water molecules which could indicate a high disorder degree. It is important to observe that all expected diffraction peaks were present in the phase for each case, which}
\]
Figure 2. (a) Crystal structure of dry l-cysteine I and tentative suggestions of one-water and two-water structures observed along the ab planes. (b) FTIR spectra at high-wavenumber region showing the presence of WAB (subbands WAB₁ and WAB₂) and WCB water bands.

indicates the correctness of the space group and subsequent refinement. The observed differences were relatively small (∼1/1000 of the measured signal) and suitable for the kind of analysis we are performing, which is based on a previously determined crystal structure. Since the l-cysteine is not a hygroscopic amino acid the water molecules present in the structure are solvent inclusions in the crystal. However, it is important to notice, due to the low molecular weight of water, the x-ray powder diffraction technique is not the most sensible way to detect such a low moisture concentration. The corresponding tentative refined structures are shown in figure 2(a). The structure of dry l-cysteine is in perfect agreement with those reported in the literature. The structures with water molecules are only tentative suggestions. Figure 2(b) presents the high-wavenumber FTIR spectrum which confirms the presence of a low amount of water in the sample due to the presence of the WCB band. This band is an intramolecular water vibration ascribed to a combination of the water molecule bending and asymmetric stretching [24]. The WAB has an intermolecular character and is attributed to a combination of the bending mode of water with intermolecular vibrational modes involving hydrogen bonding forming groups [25]. In the present case the WAB appeared to be a superposition of two subbands labeled as WAB₁ and WAB₂. The deconvolution of the 2000–2160 cm⁻¹ spectral region to a sum of two Gaussian lineshapes confirms this finding. The variations of the WAB band shape contain information on structural rearrangements of the water dipoles network [24]. The two subbands indicate the presence of two different contributions to the intermolecular coupling of the band oscillator strength. One is the hydrogen bonding network of l-cysteine, which couples to the bending modes of water molecules, which originates the lowest frequency WAB₁ subband. The other contribution goes from the water–water interactions responsible for the WAB₂ subband. It is important to notice that the observation of the WAB₂ subband corroborates our Rietveld refinement findings about the existence of water clusters as dimers in the crystal structure.

Figure 3 shows the Raman spectra at 30 and 300 K. An almost temperature-independent region between 550 and 620 cm⁻¹ could be observed. All bands above this spectral
window kept their shape, presenting a softening due to temperature variation. The exception was 815 and 830 cm\(^{-1}\) bands where an intensity transfer was observed between them. A completely diverse situation occurred below 550 cm\(^{-1}\) where some bands broadened at high temperatures. \textit{Ab initio} vibrational analysis calculations were performed to interpret the Raman spectra. The experimentally observed Raman frequencies and a summary of the calculated frequencies in the 34–2996 cm\(^{-1}\) range for fm and pbc calculations are shown in table 1. Those values in accordance with the experimental finding within 5% variations were considered for the assignment. It is important to stress that the fm frequencies that meet this criterion (second column of table 1) were only those modes related to carbon backbone torsion. The exception was the 1638 cm\(^{-1}\) vibration, which is related to N–H bending. Similar results were obtained by Pawlucojk \textit{et al} [39]. Bands in the pbc (third column) showed better agreement with experimental values with a larger set of concordant bands. Most crystal modes presented contributions of almost all atoms in the unit cell with their corresponding eigenvectors showing a more complex dynamics than the fm case. This fact is a manifestation of the role of the intra- and intermolecular interactions in the vibrational mode description, both responsible for the strong anharmonicity of this system.

Based on CPMD calculations our approach considered a detailed temperature dependence study for those modes where hydrogen bonds participate (marked with asterisks in figure 3 and table 1). These particular vibrations are candidates to mediate the water–molecule interactions. At this point, it is important to distinguish the two kinds of anharmonic contributions to the temperature dependence of the phonon frequency. The first one arises from anharmonic deviations of the single harmonic oscillator potential shape. The second one is related to phonon–phonon coupling [22]. The isothermal mode Grüneisen parameter \(\gamma_i = -\partial \ln \omega_i / \partial \ln V(T)\) is a parameter that quantifies the phonon anharmonicity of the first type. A good estimation for \(\gamma_i\) could be obtained scaling the phonon frequency variation, \(\Delta \omega(T) = \omega(T) - \omega(T_0)\) to the unit cell volume variation \(\Delta V(T) = V(T) - V(T_0)\), where \(T_0\) is the lowest experimentally measured temperature:

\[
\frac{\Delta \omega(T)}{\omega(T_0)} = -\gamma_i \frac{\Delta V(T)}{V(T_0)}
\]

After computing the \(\Delta \omega(T)/\omega(T_0)\) and \(-\Delta V(T)/V\) values for each temperature these two quantities were plotted in conjugated graphs (figures 4(a) and (b)). For a strictly harmonic phonon, it is expected that \(\omega(T) = \omega(T_0)\) is constant. Whether the temperature dependence arises from first type anharmonicity as cited above, it is expected that the \(\Delta \omega(T)/\omega(T_0)\) and \(-\Delta V(T)/V(T_0)\) followed the same temperature behavior. When other sources of anharmonicity are present characteristic deviations could be observed.

This scaling was performed on the phonons indicated by asterisks in table 1. We could distinguish three kinds of characteristic behaviors. Figures 4(a) and (b) display the representative behavior for each member of these groups. The right and left scales show the phonon frequency and the

![Figure 3. Temperature dependence of the Raman spectrum of L-cysteine I. Two spectra at 30 K (black line) and 300 K (red line) are shown. Those bands marked with an asterisk (*) had their detailed temperature behavior studied between 10 and 300 K.](image)

Table 1. Experimental frequencies (cm\(^{-1}\)) of L-cysteine I vibrational modes compared to those calculated by DFT considering the fm (selected frequencies) and the pbc calculations. The modes assigned with ‘*’ had their temperature dependence studied.

| Exp. (30 K) | fm | pbc |
|------------|----|-----|
| 77         | 78 |     |
| 101        | 96 | 105 |
| 118        | 116|     |
| 155        | 155|     |
| 177        | 166| 175 |
| 213        | 217|     |
| 284        | 286|     |
| 303        | 302|     |
| 377*       | 352|     |
| 455*       | 438|     |
| 538*       | 563| 654 |
| 645*       | 639| 654 |
| 691*       | 687| 691 |
| 755*       | 768| 766 |
| 771*       | 771|     |
| 815*       | 802|     |
| 824*       | 816|     |
| 874*       | 861| 875 |
| 945*       | 921|     |
| 1007*      | 1020| |
| 1071*      | 1062| |
| 1107       |    |     |
| 1142       | 1145| |
| 1201       | 1200| |
| 1268       | 1244| |
| 1296       |    |     |
| 1345       | 1355| |
| 1398       | 1389| |
| 1426       | 1431| |
| 1524       | 1521| |
| 1577       | 1574| |
| 1612       |    |     |
| 1647       | 1647| |
| 2552       |    |     |
| 2683       |    |     |
| 2817       |    |     |
| 2971       | 2965| |
| 2996       | 2997| |

\[\text{J. Phys.: Condens. Matter 24 (2012) 195104 T A Lima} \]
Figure 4. \( \% \Delta \omega / \omega \) scaled to \( \% \Delta V / V \) as a function of temperature. The frequency and volume changes are shown in the left (black) and right (blue) scales, respectively, for G1 (open circles, a), G2 (open diamond, b) and G3 (open square, b) phonons. The frequencies corrected by the thermal expansion \( \% \Delta \omega / \omega_{\text{corr}} \) are shown in the right side (c) and d)). The quadratic and linear behaviors are clearly seen. The solid lines show the fitting to the corresponding polynomial functions. The insets represent the unit cell motion evolved in each phonon group.

negative of the unit cell volume variations, respectively. The first group (G1) comprises the 455, 874 and 945 cm\(^{-1}\) phonon modes. Figure 4(a) (open circles) shows the representative temperature dependence for the 455 cm\(^{-1}\) vibration. With heating, an overall softening for all members of this group following the lattice expansion was observed. However, the softening rate displayed a smooth increase between \( \sim 100 \) and 250 K compared to \( \Delta V / V \). The second group (G2) comprised the 538, 755 and 771 cm\(^{-1}\) phonons. The observed behavior was the opposite of the one observed following lattice expansion, as can be seen in figure 4(b) (open diamond) for the 771 cm\(^{-1}\) mode. The third group (G3) has a single member (1007 cm\(^{-1}\) phonon) and presented a very peculiar behavior (open square symbol in figure 4(b)). Up to 70 K, its frequency was almost constant. Thereafter, a sudden hardening was observed between 70 and 150 K followed by a softening. In order to carry out a detailed analysis of this contribution, we subtracted the scaled behavior from the phonon temperature dependence. The data is shown in figures 4(c) and (d). Those modes of G1 group presented a characteristic quadratic behavior (figure 4(c)). The data for G2 and G3 group phonons displayed a clear linear dependence. Above \( \sim 200 \) K a tendency to change was observed for G3, which presented a smaller slope than the G2 one. It is important to notice that recent reports in the literature claimed the occurrence of signatures of subtle phase transitions at \( \sim 70 \) K in L-cysteine by specific heat, Raman spectroscopy, x-ray diffraction and neutron scattering [40–42]. This kind of behavior can be understood by considering phonon–phonon interactions. Balkanski et al [21] developed a detailed theory of the higher-order phonon anharmonic decay in order to explain the temperature dependence of the frequency and linewidth of the optical phonons probed by Raman scattering. Using the high-temperature limit of the theory, the decay of one phonon with frequency \( \omega \) in two and three phonons will provide specific temperature contributions given by

\[
\Delta \omega (T)/\omega (T_0) \propto T
\]

for a three-phonon decay process and

\[
\Delta \omega (T)/\omega (T_0) \propto T^2
\]

for a four-phonon decay process, which adequately were fitted to the data of figures 4(c) and (d). In order to explain the observed temperature behavior of the three groups aforementioned one needs to find phonons that fit the sum rule and would participate in the decaying process. In the present context, the water vibrational bands are natural candidates. In fact, our vibrational analysis calculations of the water dimer showed that water dimer presented a set of low frequency intermolecular vibrations at 93 cm\(^{-1}\) (donor twisting/acceptor rocking), 161 cm\(^{-1}\) (donor rocking/acceptor wagging), 195 cm\(^{-1}\) (acceptor rocking) and 251 cm\(^{-1}\) (O–O stretching) which could participate in the decay process. For G1 phonons,
we can establish the following correlation:

\[
\begin{align*}
455 & \rightarrow 93 + 161 + 196 \\
874 & \rightarrow 455 + 251 + 161 \\
945 & \rightarrow 455 + 2 \times 251
\end{align*}
\]

which is correct within 2% precision. Each phonon decays into a combination of water and/or G1-member phonon. Likewise, the correlation

\[
\begin{align*}
538 & \rightarrow 455 + 93 \\
755 & \rightarrow 538 + 195 \\
771 & \rightarrow 538 + 251
\end{align*}
\]

which is correct within 3% precision could be established for G2 phonons.

Finally, for G3 (1007 cm\(^{-1}\)), we have

\[
1007 \rightarrow 755 + 251
\]

correct within 2% precision.

The eigenvectors of the vibrational modes for each group also presented characteristic patterns. The G1 group was characterized by a stretching motion of the unit cell along the three crystallographic axes (see the inset of figure 4(c)). The atomic motions in the G2 and G3 groups resulted in the stretching of the cell along two perpendicular axes (see the inset of figure 4(d)). For G2, the movement was along \(b\) and \(c\) directions. In the G3 mode, the movement was confined in the \(ab\) plane. This symmetry may be relevant to the understanding of the interactions between L-cysteine and water. In fact, our results concerning the vibrational analysis water dimer indicated that the dipole derivative unit vector lay along the donor oxygen and acceptor hydrogen directions (main component along the O–O axis) for 93 and 161 cm\(^{-1}\) modes, being restricted to the perpendicular directions for the other modes. Changes in the relative orientation of the hydrogen atoms of the water dimers might have a strong influence on the coupling to the L-cysteine modes. The rearrangement of the hydrogen bonding pattern due to thermally activated tunneling pathways among configurations of water dimers with exchange of acceptor and donor hydrogens [43] is an option that should be considered. Our results could be consistently explained once considering the presence of one water dimer in the L-cysteine unit cell (see figure 2(a)). Since the G2 phonons do not change their behavior above 70 K (figure 4(d)) we argue that the dimers have their O–O axis along the \(a\) direction. This hypothesis has support in the Rietveld refinement data of figure 1(b). At 200 K, there is enough thermal energy to overcome the three water dimer tunneling pathways (bifurcation, interchange and acceptor switching, see Keutsch et al in [43]). This might explain the slope change observed in the G3 phonon frequency behavior (figure 4(d)).

The specific heat \(C_p\) results are shown in figure 5. Figure 5(a) shows the data between 50 and 300 K. Very subtle transitions are seen at 220 and 80 K as broad and tiny bumps near these temperatures. The uncertainty of the measurements is \(\Delta C \sim 3 \text{ J mol}^{-1} \text{ K}^{-1}\) (diameter of the open circle in figure 5(a)) and the jumps at transitions are \(\sim (3-4)\Delta C\). Calorimetric measurements on orthorhombic L-cysteine in the literature reported only a subtle transition at \(T^* \sim 70\) K [40]. Incoherent inelastic neutron scattering on monoclinic L-cysteine [42] revealed an anharmonic transition at \(T_D = 150\) K. Our Raman spectroscopy results indicated that the temperature of this transition is \(\sim 70\) K. The available data in the literature revealed that it could vary depending on the technique of measurement, energy resolution and timescale probed. In fact, for neutron scattering, it was observed that the onset depends on the spectrometer resolution. For example, it has been reported \(T^* = 100\) K with 1 meV resolution (timescale of 20 ps) [2] for lysozyme and \(T^* = 150\) K with 8 meV resolution (timescale of 0.1 ns) for some protein hydrated powders [14] which is expected for thermally activated processes. Neutron spectroscopy results at two different experimental conditions (nanosecond and picosecond timescales) on pig liver esterase of Lopez et al [7] showed global anharmonic dynamics on nanoseconds timescale are suppressed in the anhydrous enzyme. The timescale of the Raman process is of the order of a few femtoseconds and the lowest temperature transition of \(\sim 70\) K for the first transition was measured. In the calorimetric measurements performed using the relaxation method the

---

**Figure 5.** Specific heat data for L-cysteine. (a) Specific heat at a constant pressure \((C_p)\), where it is possible to distinguish two tiny transitions at \(T^* \sim 80\) K and \(T_D \sim 230\) K. The solid line is a simulation of the phononic and rigid rotor contributions to \(C_p\) which accounts for the background behavior and \(T^*\) transition, respectively, as explained in the text. The inset displays the \(C/T\) versus \(T^2\) for the low-temperature data. (b) Specific heat involved in the dynamical transition \((C_D)\) calculated by subtracting the above-cited simulated curve. The data was fitted to a Schottky anomaly with three levels of energy at 250, 600 and 900 K (solid line).
timescale is governed by the thermal conductivity of the sample itself, and for this reason depends on the typical lattice vibration velocity (sound velocity). Crude estimates give a timescale of microseconds. Thus, it is reasonable to associate the two transitions observed for L-cysteine to the T_D and T^* ones, which are commonly seen in proteins and other macromolecules.

According to the literature, the low-temperature transition at T^* is related to methyl group rotations in proteins [1, 2, 15–18]. Since the specific heat of anisotropic rigid rotors is well known [44], this hypothesis could be easily checked. Thus, considering additional linear (electronic) and cubic (phononic) contributions, the specific heat is written as

$$C_p = \gamma T + \beta T^3 + C_{\text{rot}}(T, I_{xy}, I_z)$$

where $C_{\text{rot}}(T, I_{xy}, I_z)$ is given by expressions (3) and (4) of [44], with $I_{xy}$ and $I_z$ the inertia momenta in the xy plane and z axis, respectively. The simulated curve is shown as a solid line in figure 5(a). The set of parameters that furnished the best simulated curve for 2 K < T < 150 K were found to be $\gamma < 0.03$ J mol$^{-1}$ K$^{-2}$, $\beta = 7.5 \times 10^{-4}$ J mol$^{-1}$ K$^{-4}$ and $I_{xy}/I_z = 0.7$. The $\gamma$ value could be considered negligible within the experimental error bar. The ratio $I_{xy}/I_z$ found is consistent with a prolate symmetric top rotor (CH$_3$ rotation). The inset of figure 5(a) displays $C/T$ versus $T^2$ for the low-temperature data showing the good agreement between simulated and experimental data.

The observation of the dynamical transition at T_D at very low water content (3.5%) as described herein is a remarkable fact. This water concentration is equivalent to one water molecule per unit cell on average. Lopez et al [7] studied the activity and dynamics of the near-anhydrous enzyme pig liver esterase. They observed some enzymatic activity at very near zero hydrations (three water molecules per molecule of enzyme) concluding that significant solvation was not an absolute requirement for biological activity in this case. However, within the experimental accuracy of the performed neutron scattering experiments, they did not observe the dynamical transition at T_D for their near-anhydrous sample. It is important to mention that this apparent discrepancy relies on the sensitivity of both methods. Elastic neutron experiments probe the displacements of non-exchangeable hydrogen atoms (usually amide hydrogen atoms) averaged over all molecules [45]. Thus, the sensitivity of neutron scattering to events mediated by water will be small at lower hydration. The Raman scattering experiment probes particular vibrational states, which involve a specific set of atoms in the unit cell. In this point of view, it is a more adequate microscopic probe for low hydration studies in biomolecules because it can observe particular vibrations close to the water site. However, in the higher hydration limit, the situation will be opposite since almost all vibrational modes will be coupled by hydrogen bonding paired by the neighboring water smearing out the ability to probe specific sites. In this limit, the neutron experiment will be more useful to probe the complex dynamics at T_D.

Our findings are inconsistent with the fragile-to-strong picture of Chen et al [10] since it is impossible to treat the aqueous content as a very low-density aqueous phase. In a similar way, the idea of a glassy transition of the hydration shell [11, 12] is not supported.

Thus, another explanation concerning the origin of the dynamical transition should be considered. By subtracting the above-mentioned simulated curve from the raw data, as can be seen in figure 5(b), we obtained an estimate of the heat capacity evolved in the transition T_D. The broad transition resembles the shape of a Schottky anomaly due to the thermal population of specific energy levels. One possible interpretation is to consider the anomaly as originated from thermal population of tunneling splitting energy levels in configurations of water dimers to be consistent with the analysis of Raman data in figure 4(d). We tested simulations with two, three, four and five energy levels and the best choice was obtained using the three-level scheme with energies $E_1 = 250$ K (174 cm$^{-1}$), $E_2 = 600$ K (420 cm$^{-1}$) and $E_3 = 900$ K (629 cm$^{-1}$). The agreement between the experimental data and simulation was reasonable considering the data dispersion. Keutsch et al [43] calculations in the gaseous phase indicated that the three distinct water dimer low barrier tunneling pathways—acceptor switching, interchange tunneling and bifurcation tunneling—have energies of ~157, 207 and 394 cm$^{-1}$, respectively. It is expected that the dimer energy scheme will dramatically change once it is placed in a hydrogen-bonded network. The extra constraints imposed by the environmental hydrogen bonds will increase the tunneling splitting. Therefore, the energies obtained in the simulation compared to the gaseous phase dimer are expected to be greater. Our results indicate that the dynamical transition T_D is not directly correlated to specific structural motions of amino acids. The configuration changes of water dimers have a direct impact on the mst of the hydrogen atoms of the biomolecule due to its strong coupling. We argue that the abrupt change in the water dimer hydrogen positions stretches the hydrogen bonding which increases the mst beyond the harmonic limit and distorts the harmonic potential being necessary to include anharmonic terms to describe it. Our findings are in agreement with the work of He et al [46] concerning terahertz time domain measurements on hen egg lysozyme and polylysin. They concluded that the T_D occurrence needs neither tertiary nor secondary structure and the lack of structural dependence for the transition suggests it arises strictly from either the solvent or the side-chain diffusive motion and not from protein structural collective motions. The water fluctuations could also be coupled to backbone fluctuations, in agreement with the work of Schiro et al [14] Similar conclusions were obtained by Schirö et al in [9].

In conclusion, our work revealed that the widely reported transitions at T_D and T^* observed in several proteins and other macromolecules are also a characteristic property of amino acids such as L-cysteine I. One striking feature described herein is that the dynamic transition at T_D was detected in very low water content (3.5%) for L-cysteine I. Due to the relatively simple crystallographic structure and low water content, it was possible to establish a clear and consistent interpretation for these two transitions, which will contribute to shed light on the character of these universal phase transitions.
transformations. According to our results and analysis, these two temperatures delimit the activation of very simple and specific events that govern all the biochemical interactions of the biomolecule. In the low-temperature limit, \( T < T^* \), all available experimental data are interpreted within the harmonic approximation. With heating, the main process that takes place is the activation of CH\(_2\) rigid rotors (CH\(_2\)SH dynamics). Above \( T^* \) all rotors are activated and they drive the intra- and intermolecular couplings in the biomolecule, which are responsible for the anharmonic regime seen in the \( T^* < T < T_D \) interval. In this regime, the water-mediated phonon–phonon interactions play the main role. When all the water rotational barriers are surpassed at \( T_D \), new water molecule configurations are accessible and the connected hydrogen bonding network leaves the system in a strongly anharmonic regime which could be one important ingredient to drive biological activity. In our proposed picture, the observed transitions are almost independent of the specific macromolecule structure, being restricted to the presence of rotors and a minimum amount of water molecules, which act as ‘slaving’ via phonon–phonon interaction. It is important to notice that probably anharmonicity alone is not sufficient to explain the onset of the biological activity, as pointed out by Mamontov et al. [8] in their study comparing native and denatured hydrated lysozyme.

Acknowledgments

HSM would like to thank the Brazilian agencies CNPq (301018/2006-5) and FAPESP (2011/06618-0) for their financial support. The authors acknowledge the technical, scientific and administrative staff of the Multi-user Central Facilities at UFABC (CEM-UFABC) for providing conditions to perform the experiments described in this work.

References

[1] Frauenfelder H, Chen G, Berendzen J, Fenimore P, Jansson H, McMahon B H, Stroe I R, Swenson J and Young R D 2009 Proc. Natl Acad. Sci. USA 106 5129
[2] Roh J H, Novikov V N, Gregory R B, Curtis J E, Chowdhuri Z and Sokolov A P 2005 Phys. Rev. Lett. 95 38101
[3] Doster W, Cusack S and Petry W 1989 Nature 337 754
[4] Frauenfelder H, Parak F and Young R D 1988 Annu. Rev. Biophys. 17 451
[5] Parak F and Knapp E W 1984 Proc. Natl Acad. Sci. USA 81 7088
[6] Pieper J, Buchsteiner A, Dencher N A, Lechner R E and Hauß T 2009 Photochem. Photobiol. 85 590
[7] Lopez M, Kurkal-Siebert V, Dunn R V, Tehei M, Finney J L, Smith J C and Daniel R M 2010 Biophys. J. 99 L62
[8] Mamontov E, O’Neil H and Zhang Q 2010 J. Biol. Phys. 36 291
[9] Schirò G, Caronna C, Natali F, Koza M M and Cupane A 2011 J. Phys. Chem. Lett. 2 2275
[10] Chen S H, Liu L, Fratini E, Bagliani P, Faraone A and Mamontov E 2006 Proc. Natl Acad. Sci. USA 103 9012
[11] Xu L, Kumar P, Buldyrev S V, Chen S H, Poole P H and Sciortino F 2005 Proc. Natl Acad. Sci. USA 102 16558
[12] Doster W, Busch S, Gaspar A M, Appavou M S, Wuttke J and Scheer H 2010 Phys. Rev. Lett. 104 098101
[13] Khodadadi S, Roh J H, Kisluk A, Mamontov E, Tyagi M, Woodson S A, Brier R M and Sokolov A P 2010 Biophys. J. 98 1321
[14] Schiro G, Caronna C, Natali F and Cupane A 2010 J. Am. Chem. Soc. 132 1371
[15] Schiro G, Caronna C, Natali F and Cupane A 2010 Phys. Chem. Chem. Phys. 12 10215
[16] Wood K, Tobias D J, Kessler B, Gabel F, Oesterhelt D, Mulder F A A, Zaccai G and Weik M 2010 J. Am. Chem. Soc. 132 (4990)
[17] Cordone L, Ferrand M, Vitrano E and Zaccai G 1999 Biophys. J. 76 1043
[18] Frick B and Fetters L J 1994 Macromolecules 27 974
[19] Balog E, Becker T, Oettl M, Lechner R, Daniel R, Finney J and Smith J C 2004 Phys. Rev. Lett. 93 28103
[20] Loudon R 2001 Adv. Phys. 50 813
[21] Balkanski M, Wallis R F and Haro E 1983 Phys. Rev. B 28 1928
[22] Holzapfel W B 2005 High. Press. Res. 25 187
[23] Friedman M 1973 The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins (New York: Pergamon)
[24] Giuffrida S, Cottone G and Cordone L 2006 Biophys. J. 91 968
[25] Giuffrida S, Cottone G, Vitrano E and Cordone L 2011 J. Non-Cryst. Solids 357 677
[26] Rietveld H 1969 J. Appl. Crystallogr. 2 65
[27] Lashley J C, Hundleby M F, Migliori A, Sarrao J L, Pagliuso P G and Darling T W 2003 Cryogenics 43 369
[28] Hohenberg P P and Kohn W 1964 Phys. Rev. B 136 864
[29] Kohn W and Sham L J 1965 Phys. Rev. 140 1133
[30] Perdew J P, Burke K and Ernzerhof M 1996 Phys. Rev. Lett. 77 3865
[31] Becke A 1993 J. Chem. Phys. 98 5648
[32] Frisch M J, Trucks G W and Schlegel H B 2003 Gaussian 03 Revision E 01
[33] Becke A D 1988 Phys. Rev. A 38 3098
[34] Lee C T, Yang W T and Parr R G 1988 Phys. Rev. B 37 785
[35] von Lilienfeld O A, Tavernelli I, Rothlisberger U and Sebastiani D 2005 Phys. Rev. B 71 195119
[36] Chun Lin I, Coutinho-Neto M D, Felsenheimer C, von Lilienfeld O A, Tavernelli I and Rothlisberger U 2007 Phys. Rev. B 75 205131
[37] CPMD www.cpmd.org Copyright IBM Corp 1990–2008 Copyright MPI für Festkörperforschung Stuttgart 1997–2001
[38] Moggach S A, Clark S J and Parsons S 2005 Acta. Crystallogr. B 62 296
[39] Pawluk A, Padureanu I and Aranghel D 2005 Spectrochim. Acta. A 61 2474
[40] Paukov I E, Kovalevskaya Y A and Boldyreva E V 2008 J. Therm. Anal. Calorim. 93 423
[41] Kolosov B A, Minkov V S, Boldyreva E V and Drebuschak T N 2008 J. Phys. Chem. B 112 12827
[42] Bordallo H N, Boldyreva E V, Fischer J, Koza M M, Seydel T, Minkov V S, Drebuschak V A and Kyrliakopoulos A 2010 Biophys. Chem. 148 34
[43] Keutsch F N and Saykally R J 2001 Proc. Natl Acad. Sci. USA 98 10533
[44] Caride A O and Tsallis C 1984 J. Stat. Phys. 35 187
[45] Gabel F, Bicout D, Lehner F, Tehei M, Weik M and Zaccai G 2002 Q. Rev. Biophys. 35 327
[46] Englander S W and Kallenbach N R 1983 Q. Rev. Biophys. 16 521
[47] He Y, Ku Pei I, Ku Knab J R, Chen J Y and Markelz A G 2008 Phys. Rev. Lett. 101 178103