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Observation of subtle dynamic transitions by a combination of neutron scattering, X-ray diffraction and DSC: A case study of the monoclinic L-cysteine

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ABSTRACT

The paper illustrates the benefit of combining several experimental techniques (incoherent elastic and inelastic neutron scattering, DSC, and X-ray diffraction) to study subtle dynamic transitions in a biologically important system, probing a broad time (frequency) range of the molecular motions in a wide temperature interval of 2–300 K. As a case study the crystalline form (a monoclinic polymorph) of L-cysteine ($^{+}NH_3$–CH(CH_2SH)–COO^{-}) — an essential amino acid — has been selected. Crystals of amino acids are widely used to mimic important structural and dynamic features of peptides. The conformational lability of cysteine and the dynamics of the thiol-side chains are known to account for various phase transitions in the crystalline state and for the conformational transitions important for the biological function in the peptides. The effect of temperature on the monoclinic polymorph of L-cysteine, metastable at ambient conditions, has been studied for the first time. A dynamical transition at about 150 K, marking a crossover of the molecular fluctuations between harmonic and non-harmonic dynamical regimes, was evidenced by evaluating the evolution of the mean-square displacement obtained from the elastic fixed window approach using the backscattering spectrometer IN10 located at the ILL. Although this transition does not manifest itself in the DSC, it was clearly observed by incoherent inelastic neutron scattering. By analyzing the dynamical susceptibility contribution ($\chi''(\omega)$) obtained using IN6 also at ILL we were able to evidence another relaxation process at a different time scale. The disordered soft L-cysteine structure has an excess of inelastic scattering at about 3 meV, analogous to the "boson peak" observed in glass-like materials and proteins. High-precision X-ray diffraction has revealed an anomaly in the changes of selected unit cell parameters and volume at about 240 K.

1. Introduction

1.1. Importance of the problem

Proteins are responsible for the metabolic processes. Due to the characteristic flexibility of the protein molecules, they can exist in multiple conformations, which may be very close in energy [1]. A description of a protein as a system that fluctuates over a large number of conformational sub-states is now generally accepted. To understand their function, the knowledge of the parameters characterizing the dynamics of individual molecular fragments in the biopolymers is very important.

Biological activities of proteins are intrinsically related to the structural organization and atomic motions [2]. Normal mode simulations for proteins have shown that the atomic fluctuations can be separated into local oscillations in the range of the sub-picosecond time scale (few hundreds of cm^{-1}) superimposed on motions with a more collective character, corresponding to frequencies in the range 3 to 30 cm^{-1} [3–5]. These collective motions are responsible for the variation in the magnitude of the fluctuation characterizing different parts of a protein, and likely to be of primary importance in biological function.

The unique physical characteristic of proteins, which is very important for their functions, is that the motions of different molecular fragments are activated at different temperatures and at different time scales [6–8]. For example, a review [9] based on the

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NMR line shape analysis of amino acids and proteins gave estimates of correlation times and activation energies for the dynamics of crystalline amino acids. Moreover, ring flipping motions in crystalline amino acids have been shown to occur on the microsecond time scale, whereas the methyl group motions happen on a fast-limit scale ($\tau_c < 10^{-8}$ s) [9]. Studies of the membrane proteins bacteriorhodopsin using carbon and deuterium NMR indicate that loops and termini are much more mobile than the membrane bound helical regions [10,11]. The buried residues in proteins exhibit symmetric jump motions analogous to the ring flips and the methyl rotations observed in crystalline amino acids.

Even subtle changes in a small molecular fragment can trigger instabilities resulting in a conformational transition, or even a complete denaturation of a protein [12]. In the crystalline amino acids intramolecular conformational changes also result in lattice instabilities and trigger phase transitions, changing the hydrogen bond network (see as examples the reviews [13–16], and Refs. therein). Since the parameters characterizing the motions of amino acids in crystals can be comparable with those describing their dynamics in biopolymers, possessing information on the structure and dynamics of single amino acids is of great interest for the biochemistry, biophysics, medicine and pharmacy. The structure and dynamics of crystalline amino acids can be followed in a wide temperature and pressure range by diffraction and various spectroscopic techniques, and the results can be interpreted in a simpler way giving the characteristic times of selected motions and the values of the related energy barriers.

1.2. The choice of the crystalline cysteine as a model system

Conformational changes in the crystalline amino acids can result in the rearrangements of the crystal structure that will manifest themselves in the DSC curves as well as by discontinuous changes in the cell volume and cell parameters, and by radical changes in the Raman spectra. Examples are also known, when the changes in the amino acids are not so dramatic, so that the resulting change in the crystal is considered as a subtle "dynamic transition" — it can remain "invisible" in a calorimetry study (the energy changes related to the changes in the orientations of the molecular fragments may be quite small), and hardly manifest itself in a routine X-ray diffraction study (the average positions of atoms are not changed in a discontinuous way). The subtle "dynamic transitions" can be, however, very well tracked by spectroscopic techniques, and, depending on the choice of the technique (NMR, IR, Raman, elastic or inelastic neutron scattering), the processes of different time scales will be registered [17,18]. Considering that amino acids are densely packed and that their compressibility is low when compared to liquids or solid polymers, conformational changes can only occur in a collaborative way. Therefore motions in fast dynamic regime (femtoseconds or picoseconds time scale) related to minor atomic rearrangements may act as precursors to slower motions. While incoherent inelastic neutron scattering can give important information in the picosecond regime (from 0.1 to few hundreds of picoseconds), dynamic processes by high-resolution spin-NMR can cover processes ranging from picoseconds to seconds [19,20].

Dynamics of the crystalline cysteine is a very interesting example, to study and to test the power of different experimental techniques in detecting conformational changes and distortion of the hydrogen bonding. Cysteine can adopt many different conformations as a zwitter-ion in solutions [21–23], in the crystals of the individual compound [24–28], or of cysteine salts [29–33], as well as a molecule in inert matrix [34], or as a molecular fragment in the biopolymers [35]. The side chain, $-CH_2SH$, can form weak H-bonds, S–H…O, or S–H…S. Conformational versatility of cysteine is known to be of primary importance for the biological function of the biomolecules and biochemical reactions [36–38]. Cysteine SH-groups are the most chemically reactive sites in proteins at physiological conditions [36–38]. Besides, the structure of L-cysteine is very close to that of the selenocysteine [39,40], with an atom of selenium substituting sulfur, the biosynthesis of which is possible only *in vivo* under defined conditions. Therefore to get information on the dynamics of selenocysteine, cysteine can act as a cheaper and easier available model system to start with. While phase transitions have been clearly observed on cooling [28,41–46] and under pressure [47–49] in DL-cysteine and the orthorhombic L-cysteine, nothing was known about the effect of temperature on the monoclinic polymorph of L-cysteine prior to this work. L-cysteine turned out to be a challenging system. While no significant changes have been detected by DSC, X-ray diffraction analysis has revealed a change in the slope of the $V(T)$, $b(T)$, $c(T)$ curves at about 240 K, even though the curves were continuous between 100 and 300 K. Therefore it represents an excellent system showing subtle change in dynamic properties, and we decided to use it, to test the benefits of combining elastic and inelastic neutron scattering when studying dynamic transitions in the biomimetic and biological systems.

The aim of the present study was to follow the dynamic behavior of the monoclinic polymorph of L-cysteine at different time scales, from few nanoseconds to tenths of picoseconds, using for this purpose two different neutron spectrometers, IN10 and IN6, both located at the Institut Laue-Langevin (ILL, Grenoble).

2. Materials and methods

2.1. Materials

Powder samples of L-cysteine were obtained from Sigma-Aldridge. According to the X-ray diffraction analysis, the orthorhombic L-cysteine was the dominant phase in the purchased samples, with small amounts of the DL-cysteine and the monoclinic L-cysteine present as impurities. Recrystallization from solution gives the orthorhombic polymorph of L-cysteine [24,25]. The denser monoclinic polymorph can be obtained under very special experimental conditions only, like the crystallization from the supercritical CO_2 [50]. In this work the monoclinic L-polymorph has been obtained using an original biochemical technique [A. Kyriakopoulos, unpublished], which gives exclusively pure monoclinic L-cysteine ($a = 9.4814(14)$, $b = 5.2280(6)$, $c = 11.4829(19)$ and beta = 109.238(14) at 300 K) without any impurities either of other compounds, or of DL-cysteine and of the orthorhombic polymorph of L-cysteine. Biochemical techniques are widely used for chiral resolution and chemical purification, but, to the best of our knowledge, a biochemical technique has never before been described as a tool of obtaining a metastable crystalline polymorph.

Crystalline L-cysteine is not hygroscopic and does not form crystal hydrates. The powder samples used in this work did not contain any solvent inclusions at all (see [51] for the details of detecting solvent inclusions by DSC). When the sample was put in an oven with oxygen gas at a pressure of $<10^{-6}$ mm Hg and its mass was measured over 48 h, no changes in the sample mass could be detected.

2.2. Methods

2.2.1. Differential scanning calorimetry — DSC

A DSC-204-Netzsch calorimeter was used for calorimetry measurements at temperatures below ambient on reverse heating from 120, 135 and 155 K (in different series of experiments) to ambient at different heating rates. The samples of 9.21 mg were put into a standard aluminum crucible of 0.04 mL and studied in a dry argon flux (25 mL min^{-1}). Heating rate was 6 K min^{-1}.

2.2.2. X-Rays powder diffraction

Bruker D8 Advance Diffractometer (flat plate geometry, CuK_α radiation, $\lambda = 1.5418$ Å, equipped with a Bruker Lynx Eye position sensitive detector, PSD, which allows fast time measurements without

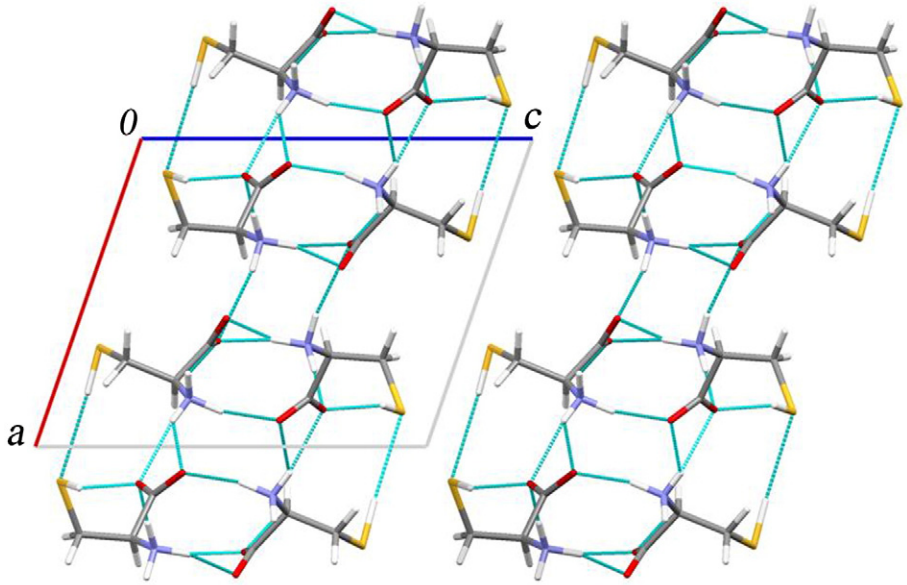

Fig. 1. A fragment of the crystal structure of the monoclinic L-cysteine ($^{+}NH_3$–CH(CH_2SH)–COO^{-}) at ambient conditions. Hydrogen bonds are shown by thin dotted lines.

lost of resolution) and STOE STADI-MP (Debye–Sherrer transmission mode, $CuK_{\alpha 1}$ radiation, $\lambda = 1.54060$ Å with a curved germanium (111) monochromator and a linear PSD detector) have been used for the phase analysis and for the variable-temperature X-ray diffraction studies at 300 K (Bruker), and of the complete powder patterns on step-wise cooling (STOE). An Oxford-700 cryostreams device (gas temperature stability of 0.1 K) has been used for the variable-temperature measurements.

With the STOE STADI-MP powder patterns were collected in moving PSD and fixing omega mode with 2θ range of 5°–70°, a step of 0.5° and a count time of 80 s. Temperature of the sample varied from 300 to 100 K with a step of 20 K. The cell parameters at each temperature were refined using the STOE Win XPOW program package. Full diffraction patterns were collected with a step size of 0.00125° and a count time of 0.5 s.

2.2.3. Incoherent inelastic neutron scattering experiments

The incoherent inelastic neutron scattering (IINS) experiments provide information on the energy transfer and neutron scattering distribution for a solid angle. Depending on the temperature and energy resolution (time) range, the measured scattering function ($S(Q, \omega)$), where Q is the magnitude of the scattering wave vector and ω is the energy transfer, will express different contributions allowing for distinguishing different types of motion. This function can be decomposed into three components: elastic, quasi-elastic (QE) and inelastic, as follows [52]:

$$S(Q,\omega) = S_{\text{elastic}}(Q,\omega = 0) + S_{\text{quasi}}(Q,\omega) + S_{\text{inelastic}}(Q,\omega) \quad (1)$$

The elastic component originates from neutrons without change in energy and the inelastic component is related to vibrational modes. QE scattering, which is a broadening of the elastic peak, describes the dynamical nature of the molecular motion, and in this particular study is dominated by the incoherent cross section of the hydrogen. Considering a purely incoherent signal, $S_{\text{elastic}}(Q, \omega = 0)$ provides information on the geometry of atomic motions and can be expressed as the spatial Fourier transform of the long-time-limit self correlation function [53], which evaluates the probability to find a particle around the position $\mathbf{r}$ at time t, knowing it was at $\mathbf{r} = 0$ at $t = 0$. Additionally in the L-cysteine sample the response of the H nuclei will dominate the signal because of its large cross section.

It is important to keep in mind that a dynamical transition will be experimentally observed if the timescale of the characteristic relaxation processes leading to the increased mean-square displacement, $\langle u^2 \rangle$, is faster than the energy resolution of the spectrometer. However the dynamic response will not be discernable from the response of a harmonic system if all relaxation processes (frequencies) are within the energy (time) resolution of the instrument, consequently $\langle u^2 \rangle$ will be proportional to T and extrapolate, at sufficiently low temperatures, to the finite zero point $T = 0$ vibration. A deviation from this behavior indicates a dynamical transition away from harmonic behavior [54].

In our experiments, fast motions, with characteristic times faster than 10 ps, could be probed using IN6 with an incident wavelength $\lambda = 4.14$ Å, and monochromatic focusing geometry gave an elastic resolution 170 μeV full width at half maximum (FWHM) with time focusing on the elastic line. On the other hand, slower motions were observed using IN10, with resolution ΔE of about 1 μeV (FWHM), which corresponds to a coherence time for neutron scattering on the order of 2 ns. Besides, the evolution of $S_{\text{inelastic}}(Q, \omega)$ as a function of temperature was monitored using IN6.

On IN10, data were collected during cooling and during heating over 16–24 h, while on IN6 data were continuously collected on cooling and at the selected temperatures for the IINS data on warming. No statistically significant differences were observed between the two sets of data. Thus in this time range one can suppose that at any temperature, the sample seems to be in equilibrium. Slab geometry with L-cysteine powder sample thickness of 0.4 mm (calculated normal-beam transmission of about 0.9) was used to achieve high total scattered intensities. An orientation angle of 135° with respect to the incident neutron beam direction was used for all samples, including vanadium. Raw data were corrected for cell scattering and detector response. For the evaluation of the vibrational information, one-dimensional spectra were obtained by summing the spectra collected at the different scattering angles. Finally, by dividing $S(\omega)$ by the Bose population factor, the dynamical susceptibility function $\chi''(\omega)$ was obtained [55].

3. Results and discussion

The monoclinic polymorph [26,27] crystallizes in the $P2_1$ space symmetry group and has two molecules — cysteine (A) and cysteine (B) — in the asymmetric unit (Fig. 1), with conformations differing from each other[1], and from that in the orthorhombic polymorph [24,25]. Similar to all the amino acid crystals, in the two polymorphs of

[1] One with a gauche + conformation ($\chi = 74.39(10)°$), and the other with an anti-conformation ($\chi = -170.15(7)°$).

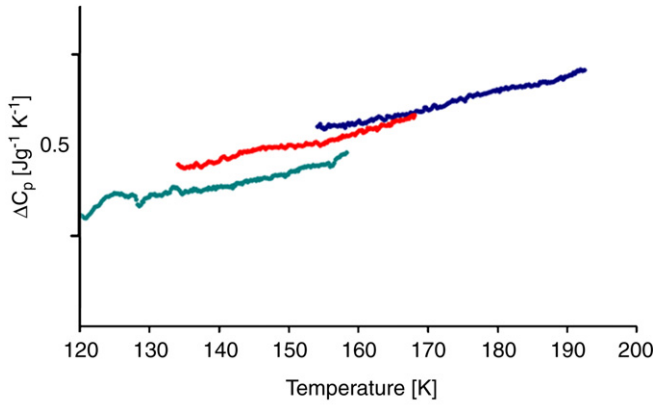

Fig. 2. DSC curves measured for the monoclinic L-cysteine on different heating rates. The slope and the visible absence of any first- or second-order phase transitions are quite reproducible. Each curve has been measured twice, with an empty crucible for a comparison.

L-cysteine, zwitter-ions of cysteine are linked via NH…O hydrogen bonds between the NH_3^+ and COO^- groups into infinite head to tail chains. The –SH-group forms additional weak hydrogen bonds either with oxygen, or with sulfur atoms: one of the two molecules in the asymmetric unit forms exclusively S–H…O hydrogen bonds, another — exclusively S–H…S hydrogen bonds [27] (Fig. 1).

In the orthorhombic polymorph of L-cysteine, an extended phase transition in a wide temperature range (around 70 K) has been observed by adiabatic calorimetry and DSC [42,43]. This transition has been studied in details by polarized Raman spectroscopy of the oriented single crystals [44], and these data, in addition to the low-temperature X-ray diffraction data [41] have shown the phase transition to be related to the ordering of the SH-groups on cooling: at ambient temperature, part of the S–H groups forms S–H…S, another part — S–H…O hydrogen bonds, and on cooling the S–H…S hydrogen bonds dominate. Variable-temperature IR-spectroscopy evidenced, that the motions of the SH– CH_2, CH–, NH, COO groups are activated at different temperatures [45].

A DSC curve for the monoclinic L-cysteine is plotted at Fig. 2. No obvious sign of a phase transition seems to be present. An X-ray diffraction analysis with a step-wise change in temperature has revealed no discontinuities in the changes of cell volume and cell parameters on cooling, which might indicate a structural phase transition, but an obvious change in the slope of the $V(P)$, $a(P)$, $b(P)$, $c(P)$ curves at about 240 K–250 K was observed (Fig. 3).

One can suppose the changes in the slopes of the cell parameters/volume *vs*. temperature are related to subtle changes in the interactions of the cysteine molecules with the crystalline environment, possibly of dynamic origin. Since the SH…S and SH…O interactions are weak, the changes in the system energy related to such changes may be too small, to be detected in a DSC experiment. However, they can be expected to manifest themselves in the neutron scattering experiments over a broad energy range at variable temperatures.

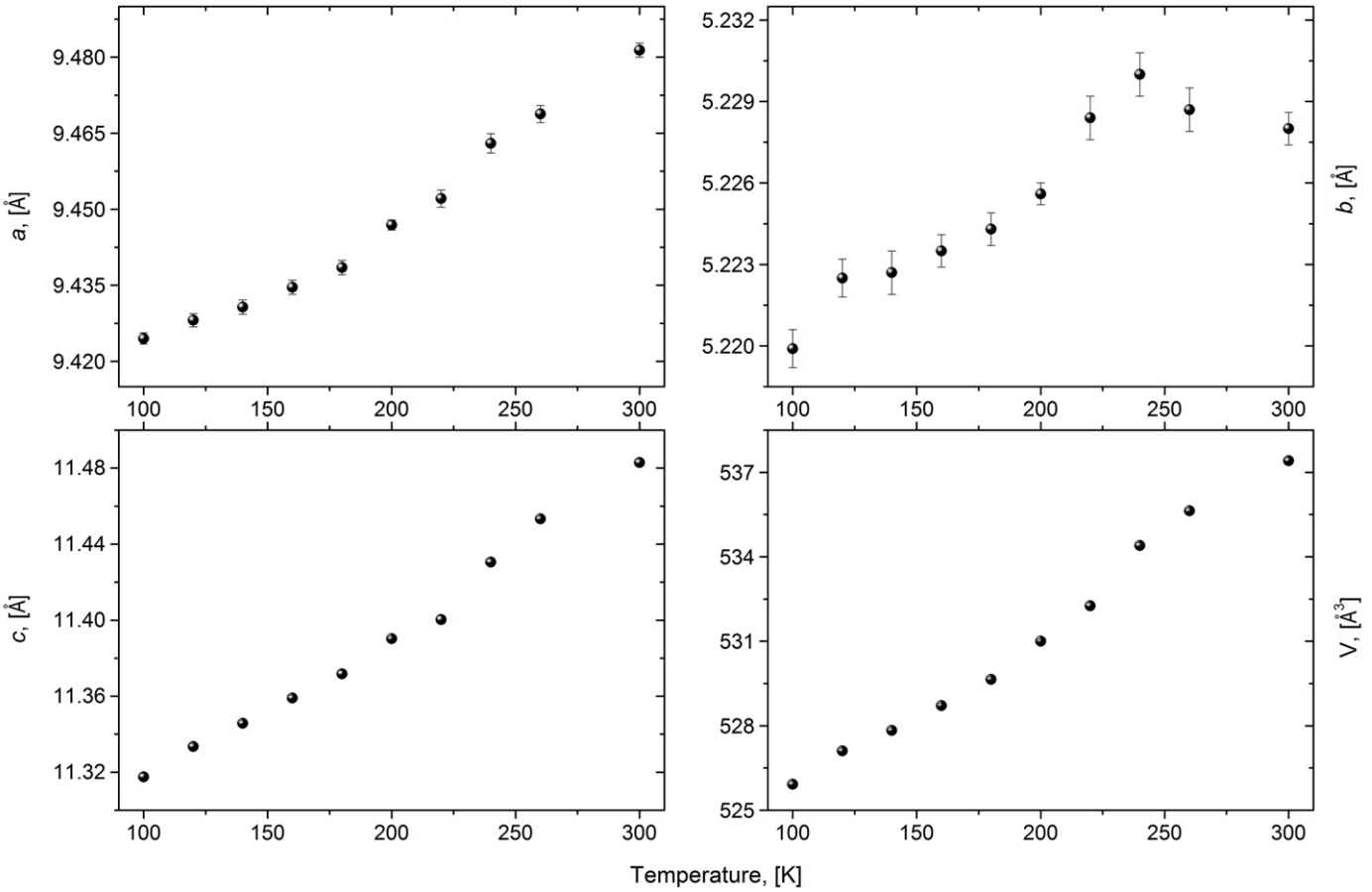

Fig. 3. The changes of the unit cell volume $V(T)$ and parameters $a(T)$, $b(T)$, $c(T)$ of the monoclinic L-cysteine on cooling.

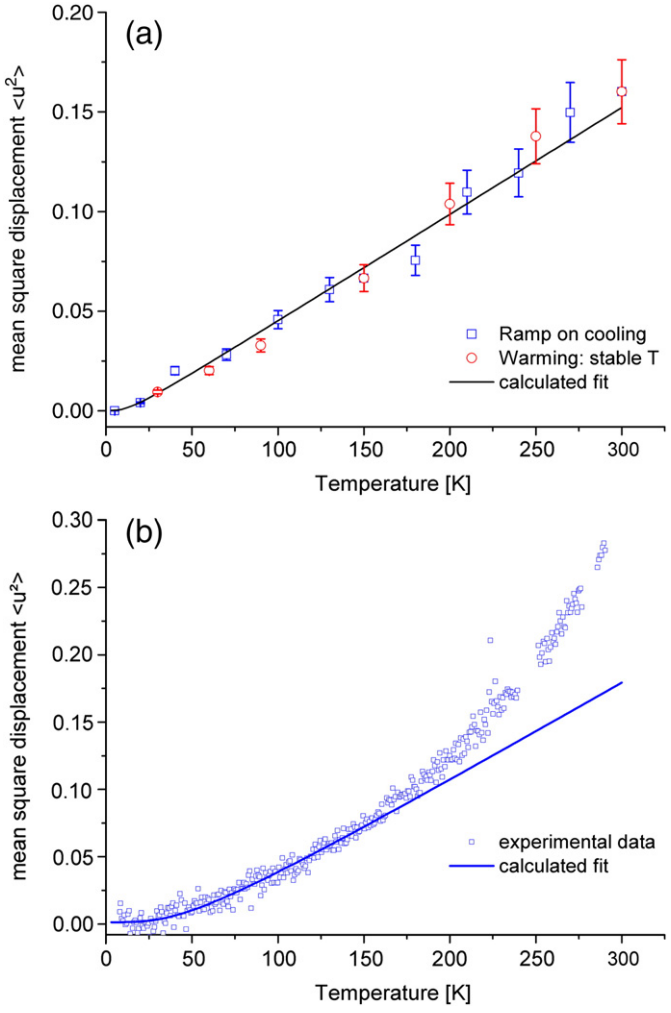

Fig. 4. $\langle u^2\rangle$ dependence calculated by evaluating the Q dependence of $S_{\text{elastic}}(Q, \omega=0)\,(T)$ / $S_{\text{elastic}}(Q, \omega=0)(T\sim 0\ \text{K})$ for the monoclinic L-cysteine. The dashed lines are the mean-squared displacements calculated from elastic neutron scattering measurements, (a) resolution 127 μeV and (b) resolution 1.1 μeV, as described in the text. During the temperature ramp problems were detected on one instrument located downstream of IN10, and the beam had to be turned off. Therefore points are missing around 250 K and again around 270 K.

The low-frequency vibrational contribution to the specific heat of the monoclinic L-cysteine could be determined from $S_{\text{elastic}}(Q, \omega=0)$. The evolution of $\langle u^2\rangle$ *vs.* was assessed by evaluating $[S_{\text{elastic}}(Q, \omega=0)\,(T)/S_{\text{elastic}}(Q, \omega=0)(T\sim 0\ \text{K})]$, as shown in Fig. 4. Then, assuming that the Debye model is applicable, the data given in Fig. 4 were fitted in terms of the following expression [56].

$$\langle u^2\rangle = \frac{3\hbar^2 T}{mk_B\theta_D^2}\left[\Phi\left(\theta_D/T\right) + \frac{1}{4}\left(\frac{\theta_D}{T}\right)\right], \tag{2}$$

where $\hbar$ is the reduced Planck constant, T is the temperature, m represents the mass of the scatterers, k_B is the Boltzmann constant, θ_D is the Debye temperature. $\Phi(x)$ is the Debye integral. At high temperatures, $T \gg \theta_D$, the Debye–Waller factor will be linear with temperature,

$$\langle u^2\rangle = \frac{3\hbar^2 T}{mk_B\theta_D^2}. \tag{3}$$

For the low temperatures where $T \ll \theta_D$, the Debye–Waller factor becomes temperature independent, and for low temperatures it reaches the zero point oscillation:

$$\langle u^2\rangle = \frac{3\hbar^2}{4mk_B\theta_D^2} \tag{4}$$

Within the statistical accuracy of our data and the measured time scale, in the monoclinic L-cysteine the mean-square displacements obey the harmonic model in the picosecond time scale (IN6 data, Fig. 4a) in the whole temperature range analyzed. This indicates that the relaxation frequencies of the dynamics determining the mean-square displacement are too slow to be detected by the finite energy resolution instrument. However, non-harmonic contributions above 150 K are evident in the nanosecond time scale (IN10 data, Fig. 4b), which would characterize the relaxation process connected to the onset of a new state, hereafter state II. A Debye temperature, θ_D, of about 200 K was obtained for L-cysteine. Such a value agrees well with the values reported for the orthorhombic L-cysteine and DL-cysteine based on calorimetry [42,46], as well as with the values reported for other amino acids (L-alanine [57], polymorphs of glycine [58,59], L-, DL-serine [60], glycilglycine [61], diglycilglycine [62], polyglycine, poly (L-alanine), poly(L-valine) [63], as well as with the values reported for proteins based on nuclear gamma resonance [64].

To explore the possibility of the existence of low-frequency motions of relaxational origin or fast conformational fluctuations, the QE response of the IN10 spectra was analyzed. From Fig. 5, it can be seen that in the monoclinic polymorph the spectra are identical within the instrumental resolution for 100 K$<T<$2 K. Actually, neither a QE contribution, nor very low inelastic vibrations are seen. In addition, no changes in the elastic intensity indicate that the zero point oscillation was reached, as would be expected thermodynamically. This observation characterizes the dynamics of the monoclinic cysteine in a new state, where the relaxation motion is no longer observable, hereafter state III.

In order to check for changes in the vibrational part of the spectra, related to fast motions, the dynamical susceptibility $\chi''(\omega)$ for the monoclinic L-cysteine obtained from the IN6 data was analyzed. In Fig. 6 the most important modes are identified at 300 K, based on the assignments proposed previously in [28,44,45,65–67].

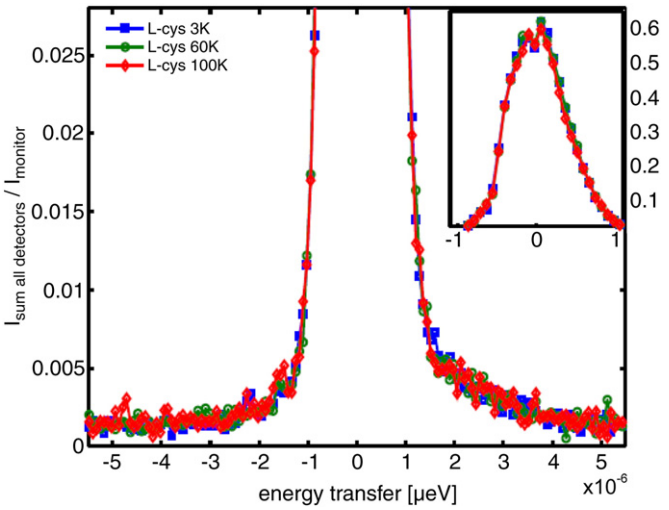

Fig. 5. Energy scans $I(Q, \omega)$ of the monoclinic L-cysteine measured on IN10. All spectra are normalized to the maximum intensity determined at the respective temperatures that are indicated in the figures. Please note, that no QE broadening was observed and that there is no difference between the $I(Q, \omega)$ at different temperatures.

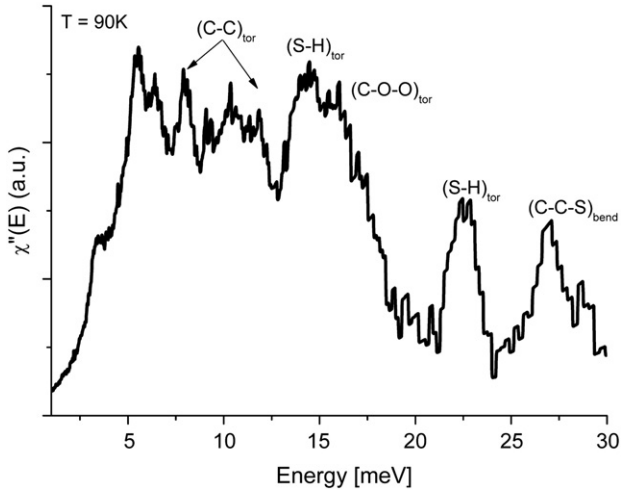

Fig. 6. Dynamical susceptibility $\chi''(E)$ of the H-atoms in the monoclinic L-cysteine at 90 K showing the modes discussed in the text.

Excitations due to harmonic vibrations should have a susceptibility that fits into the same master curve. In the range between 300 and 250 K (state I) the spectra remain almost unchanged, while a re-distribution of the intensity of the bands is noticeable below 200 K — state II (Fig. 7). Besides, in the frequency region of the lattice modes (below 30 meV) the intensities of specific modes — $(S–H)_{tors}$ at 22 meV and $(C–C–S)_{bend}$ at about 26 meV assigned to the conformation L-cysteine(A), and $(SH)_{tors}$ at 15 meV assigned to the conformation L-cysteine(B), show pronounced changes between room temperature and 150 K — states I and II, and then again between 150 and 60 K (Fig. 7a — state III). Moreover, the low-frequency lattice vibrations, as well as the $(C–C)_{tors}$ and the $(COO)_{tors}$ modes, sharpen dramatically below 100 K, and the re-distribution of the intensity under $\chi''(\omega)$ is obvious (Fig. 7b). These changes in the spectra can be interpreted as resulting from rapid jumps between equivalent torsion positions of the carbon and oxygen atoms in L-cysteine, as was observed for the NH_3 group in glycine [68], L-alanine [69] and in acetanilide [70]. Based on this observation, the conformational changes in the monoclinic L-cysteine are not continuous but rather go through a series of intermediate states; in this respect the behavior on cooling is similar to a series of the structural changes observed in the monoclinic L-cysteine with increasing pressure, only two of which have resulted in structural phase transitions [49]. Each transition to a new conformational state changes the dynamics of a cysteine molecule and manifests itself in the variation of the density of states. One can consider, for instance, that as the rotation motions are freed, their coupling with the phonon modes results in a very complex interaction and manifests itself in a dramatic amplitude modification of the vibrational modes. The evolution of the width of the $(S–H)_{tors}$ and $(C–C–S)_{bend}$ bands changes noticeably between 150 and 200 K, the broadening is clearly related to the disorder of the local environment as "seen" by the S atoms, implying that the system may be in some state disordered with respect to the orientation of the thiol-groups. Of even more interest is the observation of an inelastic excitation around 3 meV (Fig. 7c). This bump in the phonon spectrum, generally observed in glassy-like materials and proteins [71,72], is referred to as the 'boson peak', and can be considered as an indication of the behavior of disordered systems. The origin of this peak can be attributed to the localized vibrations that coexist and interact with acoustic waves.

4. Conclusion

The present study can serve as an example, that a combination of several techniques, which are sensitive to the processes with different

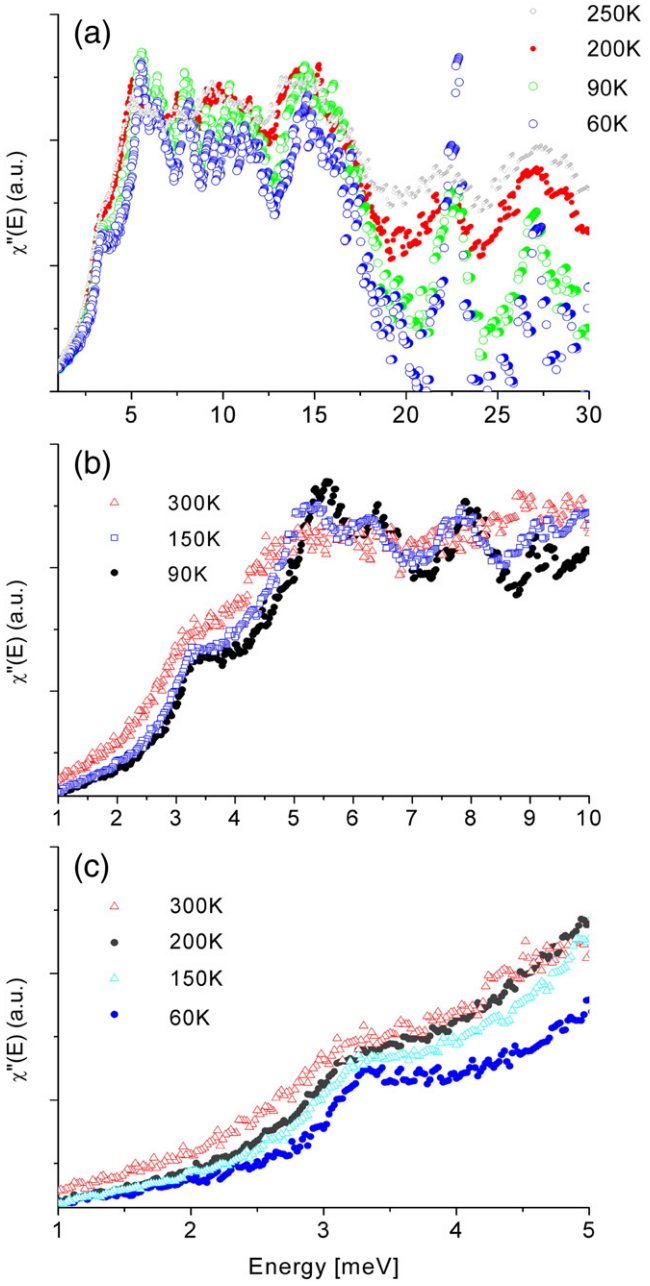

Fig. 7. Dynamical susceptibility $\chi''(E)$ of the H-atoms in the monoclinic L-cysteine at selected temperatures and energy range: (a) $\chi''(E)$ measured using IN6 in the energy range 1–30 meV between 250 and 60 K. Note the changes in the $(S–H)_{tors}$ at 22 meV, the $\tau(C–C–S)_{bend}$ at about 26 meV and the $(SH)_{tors}$ at 15 meV. Note also that changes occur step-wise; (b) The low-energy range for harmonic solids follow Bose dependence with temperature, therefore the spectra should overlap. This is the case for the monoclinic L-cysteine in distinct temperature ranges; (c) Temperature dependence of the "boson peak" (a localized vibration) may be related to the disorder in the crystal structure of the monoclinic L-cysteine.

time resolution, can be efficient in detecting even subtle dynamic transitions and in understanding their nature. The phase transitions in the crystals of cysteine teach us something new about the conformational dynamics of this amino acid. Generally, conformational changes involving cysteine residues in the biomolecules can be described theoretically in terms of transitions between local minima, which represent the stable conformers [73]. These transitions are mediated by transition states that are directly related to conformational motions, where dihedral angles changes induce variations of the torsion modes. As shown here, incoherent, inelastic neutron scattering spectroscopy (INS) is particularly well suited for the observation

of torsional motions: the $(S–H)_{tors}$ vibrations respond quite drastically to temperature. Of large interest is the evidence for the changes in the local minimum of the energy landscape above 150 K in the nanosecond time scale in neutron scattering experiments, and for the anomalies observed in the X-ray diffraction data at somewhat higher temperatures[2]. On warming, the molecular groups start re-orientation as their motions become "un-frozen". As the cooperative rearrangements of the crystal structure of the monoclinic L-cysteine take place continuously, the frustration of the locally favoured structures can explain the excess in the vibrational density of states, which looks like the boson peak. As discussed by Shintani and Tanaka [74], the lack of long-range structural order may be the cause of (quasi-) localized vibrational modes. To conclude, the approach described here is not limited to the crystalline amino acids as the changes in the bands related to SH-groups can equally provide important insights into the dynamics of these groups in biomolecules and on the identities of metal binding –SH in biomolecules and thin films at the surfaces.

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