# Ring-Opening Polymerization in Carnosine under Pressure

Chitra Murli,* A. K. Mishra, Susy Thomas, and Surinder M. Sharma

High Pressure and Synchrotron Radiation Physics Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

ABSTRACT: Our high pressure Raman scattering experiments on carnosine, a dipeptide of L-histidine and β-alanine, show pressure induced ring-opening polymerization involving the imidazole ring. While the onset of polymeric transformation is found to be at ~2.8 GPa, a substantial fraction of the monomeric solid becomes polymerized by 12 GPa. On release to ambient conditions, the observed Raman spectra do not contain any of the Raman modes of the ambient phase.

Crystal structure of Carnosine ((beta-alanyl-L-histidine)
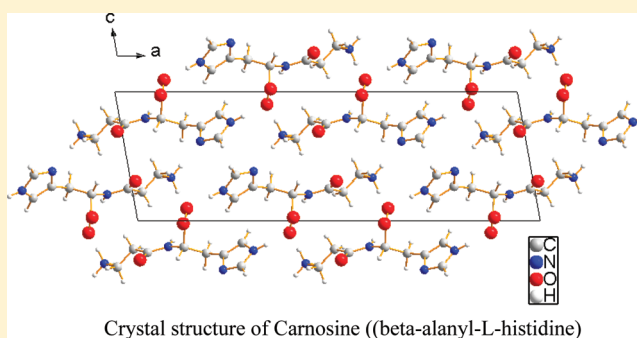
1. INTRODUCTION

Imidazole, a five-membered heterocyclic ring, plays an important role in biological activities and has several biomedical applications. Carnosine, a dipeptide of β-alanine (ala) and L-histidine (his), has an imidazole ring. In the pharmaceutical industry, carnosine[1,2] is used as an antiaging agent and hence called fountain of youth. It is also found in relatively high concentrations in several body tissues. The presence of an imidazole ring in carnosine makes it an interesting candidate for high pressure investigations, as these studies can throw light on possible ring-opening polymerization under pressure as reported for many cyclic monomers such as benzene[3−5] tribromobenzene,[6] pyridine,[7] etc. As imidazole is a five-membered heterocyclic ring, its behavior under pressure in this dipeptide can provide new reaction mechanisms, which can lead to novel material synthesis. The carnosine molecule (molecular formula $C_9H_{14}N_4O_3$) is made up of four planes; the β-alanyl residue, the peptide group, the carboxyl group and the imidazole ring as shown in Figure 1a. This compound crystallizes in the monoclinic space group C_2 (S. G. No. = 5)[8] with four formula units per unit cell with lattice parameters $a = 24.740$ Å, $b = 5.428$ Å, $c = 7.992$ Å, and $\beta = 100.12°$. Figure 1b shows the crystal structure of carnosine. In this structure, carnosine molecules are connected to each other by two types of hydrogen bonds viz., N−H···O bonds having H···O distances varying from 1.82 Å to 2.25 Å and N−H···N type having H···N distance as 1.96 Å. Under compression as these hydrogen bonds can be easily modified, one can expect significant changes in the crystal structure.[9] It would also be of interest to investigate the pressure range in which its structure and the corresponding properties, which are useful for pharmaceutical applications can remain unaltered. In addition, one can look for the formation of new high pressure phases whose properties can be exploited for material research applications. High pressure investigation of this compound could therefore be useful from the point of view of structural chemistry as well as biomedical research. Our Raman spectroscopic investigations

(a)

(b)
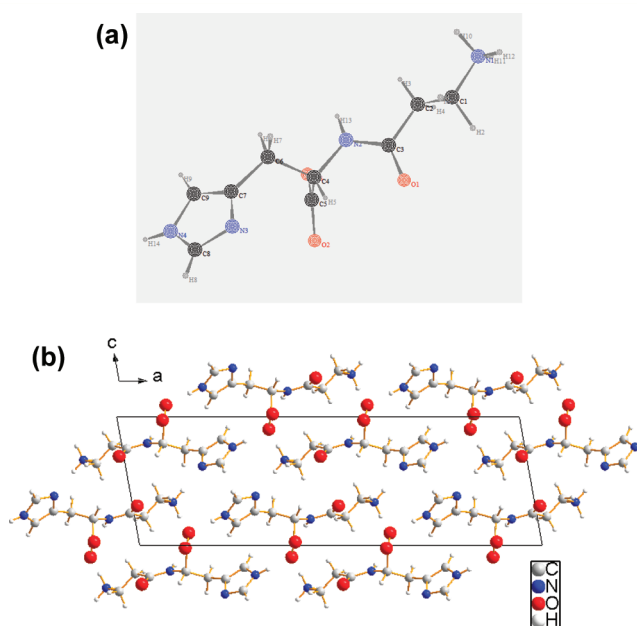
Figure 1. (a) Molecular structure of carnosine; (b) crystal structure of carnosine.

carried out up to 20 GPa reveal substantial spectral changes, which suggest pressure induced structural modifications leading to ring-opening polymerization in this compound.
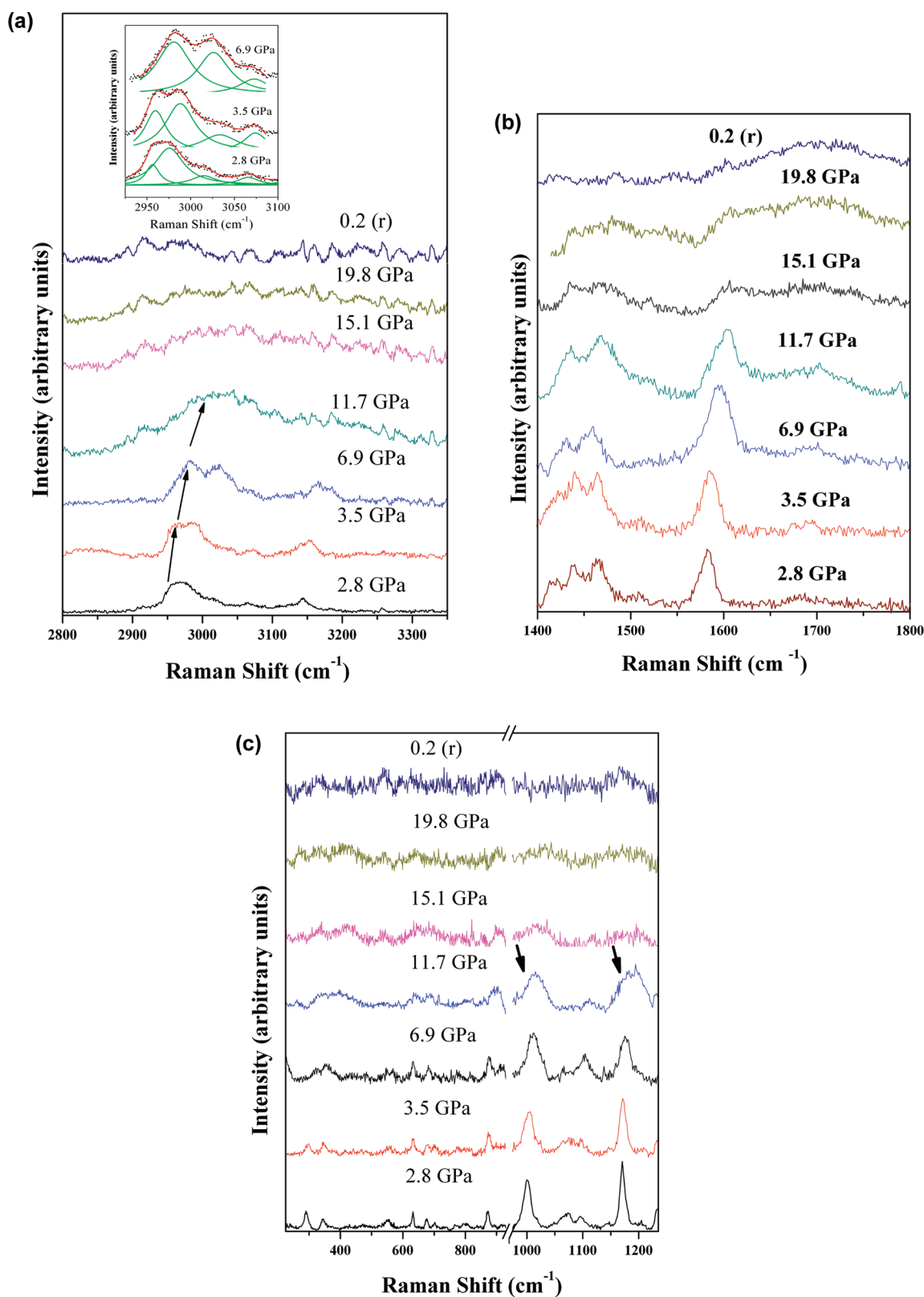
2. EXPERIMENTAL DETAILS

Powdered samples of L-carnosine (β-alanyl-L-histidine) from Aldrich were loaded along with a ruby chip of size 5 to 10 μm in a 140 μm hole of a tungsten gasket preindented to a

Figure 2. Raman spectra of carnosine in the pressure range 0.1 MPa to 2.8 GPa; (a) in the spectral region 2800−3350 cm^{-1}. Insert shows the convoluted peaks at 1.3 GPa, 1.8 and 2.8 GPa. (b) In the spectral regions 1400−1800 cm^{-1} and (c) 200−1250 cm^{-1}; ν indicates stretching ; τ represents torsion; δ indicates deformation; rg indicates ring; ρ indicates rock.

thickness of 70 μm in a diamond anvil cell. Raman scattering studies were carried out using an indigenous micro-Raman system assembled around a single state HR 460 Jobin Yvon spectrograph with a liquid nitrogen cooled Spectrum One CCD detector. A diode-pumped solid-state laser with the wavelength 532 nm was used as an excitation source. High pressure investigations have been done under nonhydrostatic conditions to avoid any interaction of pressure transmitting medium with the sample. Pressure inside the diamond anvil cell was measured using ruby R-lines.[10] Neon lines were used to monitor and correct for any possible drift in the spectrometer.

3. EXPERIMENTAL RESULTS

Raman spectra of carnosine have been recorded at different pressures in the pressure range 0.1 MPa to 20 GPa. We have

presented all the spectral changes, which have been noted in the spectral region 200−3400 cm^{-1} excluding 1300−1400 cm^{-1} where the intense Raman peak of diamond appears. The observed spectral changes are summarized along with their structural implications in section 4. The observed Raman modes are assigned on the basis of earlier work on carnosine, histidine, and alanine,[11−14] and these assignments are mentioned in the text and also shown in the figures containing ambient spectra. For Raman modes below 2000 cm^{-1}, mode assignment has been taken from ref 12, and for various modes above 2800 cm^{-1}, assignment of the modes is followed from the work on β-alanine[14] and a complex of histidine.[11] Figure 2a−c shows the Raman spectra of carnosine recorded in close intervals in the pressure range 0.1 MPa to 2.8 GPa. The Raman spectra in the pressure range 2.8−20 GPa are shown in Figure

Figure 3. Raman spectra of carnosine in the pressure range 2.8 GPa to 20 GPa; (a) in the spectral region 2800−3350 cm^{-1}. Insert shows the convoluted peaks at pressures 2.8, 3.5, and 6.9 GPa; In the spectral region (b) 1400−1800 cm^{-1} and (c) 200−1250 cm^{-1}.

3a−c. Pressure induced variation of Raman shifts are given in Figure 4a−c. For convenience, in the following sections, increase in the frequency of the modes would be termed as stiffening, while a reduction of the same is termed as softening.

3.1. Spectral Changes in the Region 2800−3400 cm^{-1}. As one can see from Figure 2a, CH$_2$ stretching modes of alanine and histidine are observed as strong peaks at 2935 cm^{-1} (ala), 2945 cm^{-1} (ala), 2955 cm^{-1} (his), and 2974
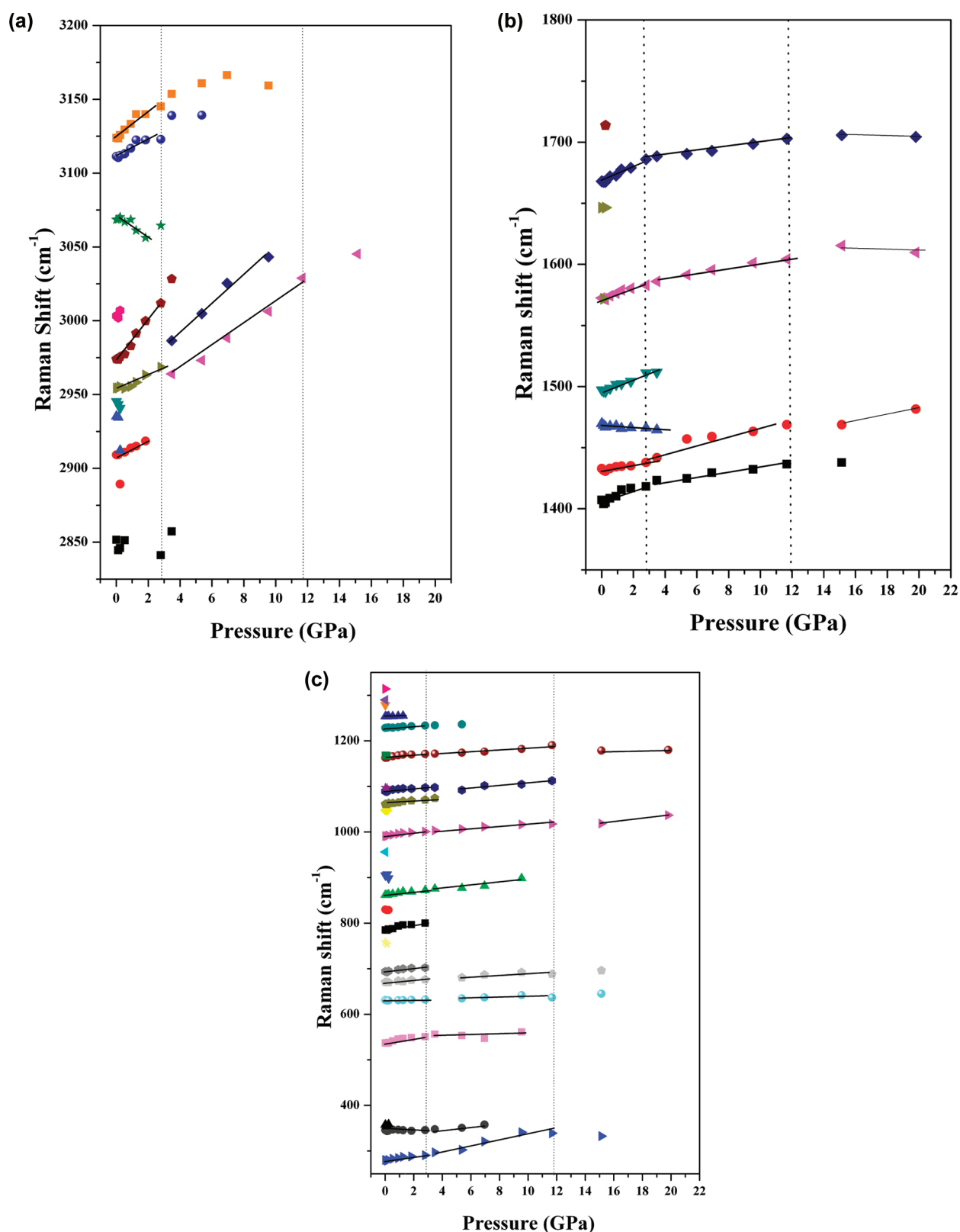
Figure 4. Variation of Raman shifts with pressure in the spectral region (a) 2800−3350 cm^{-1}; (b) 1400−1800 cm^{-1}; and (c) 200−1250 cm^{-1}. Lines are drawn as a guide to the eyes.

cm^{-1}(his).[11,14] CH stretching mode of histidine observed at 3004 cm^{-1}(his) is a relatively weaker peak. The frequencies of all these modes are found to increase with pressure and are observed as two broad bands above 0.2 GPa. These two bands

continue to stiffen with pressure. However, at 2.8 GPa, we observe a new band at 2958 cm^{-1}, i.e., near the shoulder of the strong CH$_2$ band as indicated by an arrow in Figure 2a. Figure 3a shows that these bands continue to stiffen, whereas the

width and intensity of the new band keeps increasing with pressure up to 11.7 GPa. However, at 15.1 GPa, the whole spectral region is covered by a very broad weak band. As shown in Figure 2a, among the ring C−H stretching (his) modes[11] observed at 3111 cm^{-1} and 3124 cm^{-1}, the intensity of the latter reduces drastically at pressures as low as 0.2 GPa, whereas the intensity of the CH stretching mode (3111 cm^{-1}) shows a slight decrease up to 2.8 GPa. Both the modes stiffen with a slope change across 2.8 GPa as can be seen from Figure 4a. Above 2.8 GPa, the relative intensity of the CH stretching mode (3124 cm^{-1}) continues to reduce up to 11.7 GPa. At further higher pressures, as shown in Figure 3a, ring CH stretching modes (3111 cm^{-1} and 3124 cm^{-1}) are observed as a weak and broad shoulder to the new band, which emerged at 2.8 GPa. The broadening of the ring stretch modes above this pressure indicates that the ring structure is disordered. At pressures above 15 GPa, these modes merge with the polymer band, and a very broad band is observed in this spectral region (2800−3400 cm^{-1}).

As seen in Figure 2a, the relative intensity of all the NH stretching modes is quite weak even at ambient conditions. A weak band observed at 2852 cm^{-1} has been assigned as the NH stretching mode of alanine in the earlier Raman study of β-alanine.[14] The frequencies of NH stretch modes are found to be at lower values for amino acids in which N−H is involved in a stronger N−H⋯O hydrogen bond.[15] Raman mode observed at 3069 cm^{-1} is the ring NH stretch and is found to show softening up to 2.8 GPa. NH stretching mode (peptide) (3250 cm^{-1}), which is observed as a weak and broad band at ambient conditions, is not observable beyond 0.2 GPa.

3.2. Spectral Changes in the Region 1400−2000 cm^{-1}. As shown in Figure 2b and 4b, the ring C=C stretch mode is observed at 1573 cm^{-1} at ambient conditions, and this mode shows stiffening with a small slope change across 2.8 GPa. As shown in Figure 3b, the relative intensity of this mode with respect to the C=O stretch mode observed at 1668 cm^{-1} shows considerable decrease at pressures above 11.7 GPa. The C=O stretch mode does not show much change in its intensity at higher pressures, though its peak position increases with a small slope change across 2.8 GPa (Figure 4b). The mode at 1625 cm^{-1} (NH_3 deformation) is weak at ambient conditions and is not observed at higher pressures. The mode at 1470 cm^{-1} (ring CN stretch/NH bend) shows softening up to 6.9 GPa and merges with CH_2 deformation mode (1430 cm^{-1}) as shown in Figure 2b and 3b. The modes observed at 1430 cm^{-1} (CH_2 deformation) and at 1407 cm^{-1} (COO stretch) stiffen with pressure.

3.3. Spectral Changes in the Region 200−1300 cm^{-1}. As seen from Figure 2c, the skeletal torsional mode at 225 cm^{-1} shows drastic reduction in intensity, splits at 0.9 GPa, and completely disappears at 2.8 GPa. The mode observed at 281 cm^{-1} stiffens with pressure, and the rate of stiffening increases beyond 2.8 GPa. The relative intensity of this mode reduces with pressure, and it is not observable above 11.7 GPa. The large pressure induced stiffening of this mode suggests that this could be assigned to a C−C torsional mode as similar high pressure behavior of this mode is reported for materials such as acrylic acid,[16] acrylamide,[17] etc. The Raman mode at 347 cm^{-1} has a weak shoulder peak at 336 cm^{-1} and a stronger shoulder peak at 357 cm^{-1}. The peak at 357 cm^{-1} reduces in intensity at higher pressures. The mode at 347 cm^{-1} shows a tendency to soften at higher pressures up to 2.8 GPa. The mode observed at 536 cm^{-1} is CO_2 rocking mode (ala), which shows an initial

increase in the intensity at 0.2 GPa, and at further high pressures, its intensity decreases. This mode shows broadening above 2.8 GPa and is found to split as two weak peaks above 6.9 GPa and is not observable beyond 11.7 GPa. The mode at 631 cm^{-1} shows a small pressure induced stiffening and shows considerable reduction in its intensity at 11.7 GPa. The modes at 670 cm^{-1} and 693 cm^{-1} stiffen with pressure and the latter one is not observable beyond 6.9 GPa. The modes at 758 and 785 cm^{-1} become too weak to be observed at higher pressures. The mode at 868 cm^{-1} (C−C stretch) is observed as a medium intense peak and adjacent to it a weak band is observed at 905 cm^{-1}. The former mode (868 cm^{-1}) shows stiffening and broadening with pressure. Its intensity reduces considerably and it becomes as weak as the adjacent peak (905 cm^{-1}), and both of them merge in to a single weak broad band. The peak at 991 cm^{-1}, which is a ring deformational mode, appears with increased intensity at 0.2 GPa, and at higher pressures, its intensity reduces. The full width at half-maximum of the ring deformation mode increases continuously up to 11.7 GPa and steeply increases above this pressure. The modes at 1048, 1061, 1089, and 1097 cm^{-1} stiffen with pressure and merge into a single weak broad band at pressures above 6.9 GPa. The mode at 1168 cm^{-1} (ring CN stretch) is a quite intense peak, which stiffens with pressure. The full width at half-maximum of the ring CN stretch does not increase much up to 11.7 GPa but increases very steeply across this pressure. The mode at 1230 cm^{-1} shows relatively a smaller stiffening rate up to 2.8 GPa, and above this pressure, its relative intensity changes considerably and is no more observable above 6.9 GPa. To summarize, many of the Raman modes show significant changes across 2.8 and 11.7 GPa.

4. DISCUSSION

The emergence of a new CH band around 2958 cm^{-1} is one of the key spectroscopic features that suggests that there is an onset of polymerization in this compound above 2.8 GPa. This is also accompanied by several spectral features such as softening of the ring CN stretch/NH bend at 1470 cm^{-1}, the reduction in the relative intensity of ring CH stretch modes (3111 cm^{-1} and 3124 cm^{-1}), and several others. Above 11.7 GPa, the relative intensity of the C=C stretch decreases considerably, and the new characteristic polymer CH band shows substantial broadening above this pressure. All the modes corresponding to the ring vibrations show significant broadening across this pressure. Above 15 GPa, the spectrum has a very broad band in the CH stretching and C=O stretching region. On release to ambient conditions, the retrieved phase does not contain any of the Raman modes of the initial phase. Hence, the observed Raman spectra indicate irreversibility of the phase transformations in this compound.

4.1. Ring Opening Polymerization under Pressure. Pressure induced polymerization in several linear[16−21] and cyclic[3−7,22,23] monomers have been reported in the literature. In the case of benzene, Pruzan et al.[3] first reported the onset of intermolecular coupling between adjacent molecules based on the emergence of the CH infrared band, at pressures above 23 GPa. At high pressures, coupling of the aromatic molecules can happen through π bonds, and as a result, an acyclic system is produced. In benzene, it is reported that the aromatic ring is completely altered, and the transformation is found to involve ring-opening polymerization. However, on release from 30 GPa, a mixture of benzene and high pressure species were recovered, which indicates that the transformation is only

4675

dx.doi.org/10.1021/jp300248b | J. Phys. Chem. B 2012, 116, 4671−4676

partial. However, complete transformation of the monomer has been reported with compression—decompression cycle at further higher pressures of 50 GPa reported by Ciabini et al.[23] The interaction of the nearest neighbor molecules is reported to form a network of interconnected benzene units with loss in the aromatic character, which indicates transformation to an acyclic system. However, bond breaking is reported to occur mainly during decompression cycle. In the case of pyridine, which is isoelectronic to benzene, Raman and infrared absorption studies indicate the loss of ring structure at pressures above 22 GPa leading to the formation of an amorphous polymer-like substance.[7] In tribromobenzene, chemical transformations are reported to occur at pressures above 25 GPa, and the Raman spectrum was found to contain a broad and diffuse band around 1600 cm^{-1} indicating disordered polymers.[6] For this compound, a metathesis reaction leading to a macrocycle formation is reported to be the possible reaction pathway. Under high pressure, the addition reaction is supposed to occur by overlapping and opening of the adjacent C=C double bonds of the macrocycle, and the interchain addition leads to a cross-linked compound.[23] In our study, the onset of polymerization in carnosine takes place at pressures ~2.8 GPa as indicated by the emergence and strengthening of the characteristic CH stretching band across this pressure. The drastic reduction in the relative intensity of the C=C stretching mode with respect to C=O modes at pressures above 11.7 GPa suggests that the C=C double bonds are broken and a significant fraction of the monomer is transformed to a polymer phase above 12 GPa. The broadening of the modes which correspond to ring vibrations also indicates deformation in the ring structure. At pressures above 15 GPa, the Raman spectra indicate a polymeric phase with no signature of any of the monomer peaks of the initial phase. In comparison to benzene and pyridine, which show polymerization during decompression, carnosine displays polymerization during compression. On release to ambient conditions, Raman modes of the ambient phase are not observed, which indicates irreversibility of the transformations. The mechanism of ring-opening polymerization in imidazole ring of carnosine under pressure may involve possible reorganization of the bonds within the ring. The structure of the high pressure phase and the retrieved phase need to be investigated in detail to understand the nature of the polymer network formed in this compound. The onset of polymer phase at a pressure of 2.8 GPa indicates that the chemical properties of carnosine will be significantly altered above this pressure range. However, the properties of the polymeric form of this compound need to be evaluated from the point of view of biomedical applications.

5. CONCLUSIONS

Our Raman spectroscopic investigations on carnosine up to 20 GPa indicate the onset of ring-opening polymerization involving the imidazole ring at pressures ~2.8 GPa, and a substantial fraction of the monomer is converted to a polymer network at pressures above 12 GPa. On release to ambient conditions, a disordered phase is retrieved with no signature of the ambient phase.

■ AUTHOR INFORMATION

Corresponding Author
*Tel: 91-22-25591326. Fax: 91-22-25595296. E-mail: cmurli@barc.gov.in.

Notes

■ REFERENCES

(1) Quinn, P. J.; Boldyrev, A. A.; Formazuyk, V. E. *Mol. Aspects Med.* **1992**, *13*, 379−444.

(2) Bonfanti, L.; Peretto, P.; Marchis, S. De.; Fasolo, A. *Prog. Neurobiol.* **1999**, *59*, 333−353.

(3) Pruzan, P.; Chervin, J. C.; Thiery, M. M.; Itie, J. P.; Besson, J. M.; Forgerit, J. P.; Revault, M. *J. Chem. Phys.* **1990**, *92*, 6910−6915.

(4) Ciabini, L.; Santoro, M.; Bini, R.; Schettino, V. *Phys. Rev. Lett.* **2002**, *88*, 085505(1)−085505(4).

(5) Chelazzi, D.; Ceppatelli, M.; Santoro, M.; Bini, R.; Schettino, V. *Nat. Mater.* **2004**, *3*, 470−475.

(6) Cansell, F.; Fabre, D.; Petitet, J. P.; Itie, J. P.; Fontaine, A. *J. Phys. Chem.* **1995**, *99*, 13109−13114.

(7) Zhuravlev, K. K.; Traikov, K.; Dong, Z.; Xie, S.; Song, Y.; Liu, Z. *Phys. Rev. B* **2010**, *82*, 064116(1)−064116(8).

(8) Itoh, H.; Yamane, T.; Ashida, T.; Kakudo, M. *Acta Crystallogr., Sect. B: Struct. Sci.* **1977**, *33*, 2959−2961.

(9) Mishra, A. K.; Murli, C.; Garg, N.; Chitra, R.; Sharma, S. M. *J. Phys. Chem. B* **2010**, *114*, 17084−17091.

(10) Piermarini, G. J.; Block, S.; Barnett, J. P.; Forman, R. A. *J. Appl. Phys.* **1975**, *46*, 2774−2780.

(11) Petrosyan, A. M. *Vib. Spectrosc.* **2007**, *43*, 284−289.

(12) Thomas, S.; Biswas, M.; Malkar, V. V.; Mukherjee, T.; Kapoor, S. *Chem. Phys. Lett.* **2010**, *491*, 59−64.

(13) Torreggiani, A.; Tamba, M.; Fini, G. *Biopolymers* **2000**, *57*, 149−159.

(14) Krishnan, R. S.; Katiyar, R. S. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 2098−2101.

(15) Krishnan, R. S.; Sankaran, V. N.; Krishnan, K. *J. Indian Inst. Sci.* **1973**, *55*, 66−116.

(16) Murli, C.; Song, Y. *J. Phys. Chem. B* **2010**, *114*, 9744−9750.

(17) Sharma, B. B.; Murli, C.; Sharma, S. M. *AIRAPT-23 International Conference on High Pressure Science and Technology*, Mumbai, India, Sept 25−30, 2011; Abstract P114, p 332.

(18) Ceppatelli, M.; Santoro, M.; Bini, R.; Schettino, V. *J. Chem. Phys.* **2000**, *113*, 5991−6000.

(19) Citroni, M.; Ceppatelli, M.; Bini, R.; Schettino, V. *Science* **2002**, *295*, 2058−2060.

(20) Chelazzi, D.; Ceppatelli, M.; Santoro, M.; Bini, R.; Schettino, V. *J. Phys. Chem. B* **2005**, *109*, 21658−21663.

(21) Aoki, K.; Usuba, S.; Yoshida, M.; Kakudate, Y.; Tanaka, K.; Fujiwara, S. *J. Chem. Phys.* **1988**, *89*, 529−534.

(22) Tabata, Y.; Shu, S.; Oshima, K. *Mol. Cryst.* **1969**, *9*, 435−452.

(23) Ciabini, L.; Santoro, M.; Bini, R.; Schettino, V. *J. Chem. Phys.* **2002**, *116*, 2928−2955.