A possible glass transition for poly(L-methionine) and poly(L-serine)

A. Xenopoulos*, K. Roles and B. Wunderlich
Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6197, USA and Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA
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The possibility of a low-temperature glass transition is proposed for two poly(amino acid)s: poly(L-methionine) and poly(L-serine). The suggestion is based on deviations of the experimental heat capacity from values calculated from the vibrational spectrum. The discrepancy consists in an increase of the heat capacity over a wide temperature range (260–400 K) to values more typical of liquids. Qualitative experiments are performed that show the rubbery nature of the samples above room temperature and brittleness below. The broadness of the proposed glass transition may be caused by the presence of some low level of crystallinity. It is speculated that the motion begins in the side groups around room temperature and causes a gradual devitrification of the whole molecule at higher temperatures.

(Keywords: glass transition; poly(L-methionine); poly(L-serine))

INTRODUCTION

A comprehensive study of the heat capacities of all homopoly(amino acid)s, that can be derived from the naturally occurring amino acids, was completed recently. For most of them it was found that up to about 400 K the heat capacity at constant pressure (C_p) could be represented by approximate vibrational spectra, typical for the respective rigid solids[1,2]. Exceptions were noted only for poly(L-methionine) (PMET) and poly(L-serine) (PSER). The structure of the two poly(amino acid)s is $(CHR–CO–NH–)_n$, where R is $(CH_2)_2–S–CH_3$ for PMET and $CH_2–OH$ for PSER. For these two samples, C_p increased beyond the vibration-only limit over a wide temperature range (260–400 K). The shape of the C_p curve was reminiscent of broad glass transitions seen in some semi-crystalline polymers. In this paper the heat capacities of liquid PMET and PSER will be estimated, and the question will be discussed if, indeed, PMET and PSER undergo a glass transition.

Glass transitions are very common in amorphous polymers[3]. They usually indicate the onset of segmental motion of the polymer chains. In the case of nylon 6.6, 15 amide groups have been suggested to be involved in the motion, the number being based on crosslinking studies[4]. Thermally, the glass transition is characterized on heating by a step-like increase in C_p and an increase in the slope of the specific volume *versus* temperature plot. Normally, these changes occur over a temperature range of 5–20 K. Empirical rules have been developed for the increase in C_p at the glass transition (ΔC_p)[5]. Typically, 11 J K^{-1} mol^{-1} are expected per *bead* that becomes mobile. Beads can be thought of as groups of atoms that can move as a whole, e.g. $–CH_2–$, –CONH–, etc. For many amorphous polymers that acquire full mobility at their glass transition this empirical rule is satisfied. In semi-crystalline polymers the amorphous parts of the molecules still undergo a glass transition. The glass transition is, however, often substantially broadened when compared to fully amorphous polymers, probably due to the constraining effect of the surrounding crystallites. The ratio of measured to fully amorphous ΔC_p should correspond to the amorphous fraction in the semi-crystalline polymer. Complications arise from larger beads, such as phenyl groups, that may show a higher ΔC_p, and from *rigid amorphous* fractions that do not participate in the glass transition and reduce ΔC_p[6].

The temperature of the glass transition (T_g) decreases, naturally, with increasing flexibility of the main chain. Polyethylene has a T_g of 237 K[7] while aliphatic polyamides of commercial interest (nylons) have T_gs just above room temperature[8]. This increase in T_g by about 90 K is largely due to the hydrogen-bonding ability of the polyamides[9]. Poly(amino acid)s have a similar chemical structure as the nylons (note that polyglycine is nylon 2), but with a potential for one hydrogen bond every third carbon (second bead) along the chain. Another difference with nylons is that most poly(amino acid)s are capable of *intra*chain H-bonding, largely in α helices, characteristic of the polypeptide structure, resulting in rod-like chain segments. The chains of poly(amino acid)s are therefore much more rigid, and a high T_g should be expected.

Proteins, although not homopolymers, are chemically similar to poly(amino acid)s and the same considerations as to chain rigidity apply to both classes of macromolecules. The expectation of a high T_g is indeed borne out in the literature data for three fibrous, amorphous proteins. Highly dehydrated gelatin films (i.e. the amorphous form of collagen, consisting of 30 mol% glycine, 20 mol%

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*To whom correspondence should be addressed

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proline and hydroxyproline, and 10 mol% alanine) were found to have a glass transition at 469 K[10]. The transition shifts to much lower temperatures for wet samples, due to disruption of the hydrogen-bonded network by the water molecules, as is also noted for the nylons[9]. Similarly, for films of amorphous silk fibroin[11] and silk sericin[12] a T_g was determined at 446 K and 443 K, respectively, followed by irreversible crystallization to the β-crystalline form.

A glass-type transition in globular proteins (e.g. metmyoglobin) around 200 K has received considerable attention recently[13,14]. It only involves surface residues of the globule and is largely driven by surrounding solvent molecules. In that respect it is peripheral to the current discussion.

For the more highly organized poly(amino acid)s the limited literature data concerning a glass transition invariably associate it with side-group relaxations. Proton n.m.r. spectra of poly(L-alanine), poly(phenyl-L-alanine), poly(L-leucine), poly(γ-benzyl-L-glutamate) and poly(sodium α-L-glutamate) were measured from 77 K to 480 K[15]. The temperature dependence of the second moment could be wholly accounted for in terms of side-group motion. Only rotational motion of the side groups, with little or no participation of the helical backbone, was found up to 500 K for many poly(γ-alkyl-L-glutamate)s, using dynamic mechanical measurements[16]. The most prominent relaxation is around room temperature and has the characteristics of a glass transition. The only d.s.c. study of poly(amino acid)s included homopolymers and copolymers with (N^{ε}-carbobenzoxy-L-lysine) and (*O*-carbobenzoxy-L-tyrosine) as side groups[17]. A clear glass transition is seen in the d.s.c. traces around 310 K, with ΔC_p values that are proportional to the side group length, indicating again motion in the side groups.

It is thus not clear what type of motion the poly(amino acid)s exhibit, and whether a glass transition, as seen in semi-crystalline polymers, could explain the observations. In this paper we propose that PMET and PSER may undergo a glass transition around room temperature, and speculate on the possible motion involved, based on our d.s.c. data and additional qualitative experiments. Before such discussion is possible it was necessary to develop an estimate of the heat capacity of liquid poly(amino acid)s not available from experiments.

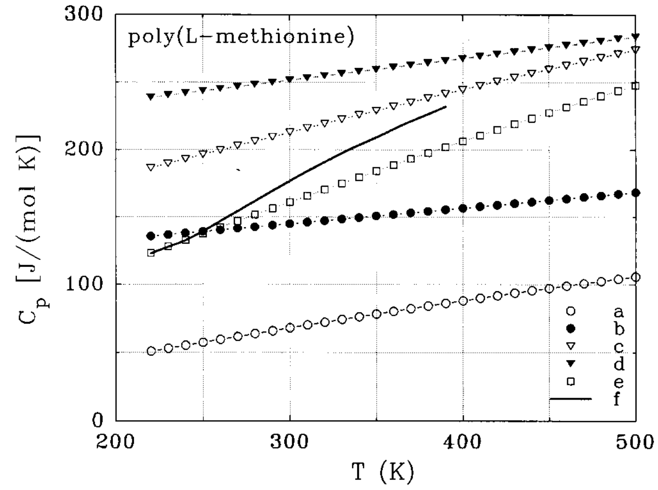

Figure 1 Heat capacities at constant pressure (C_p) for poly(L-methionine), PSER: a, calculation for a rigid backbone only, based on the adjusted vibrational spectrum of polyglycine and the Θ temperatures determined for PMET; b, calculation for mobile side chains only, based on the addition scheme for liquids; c, calculation for a repeat unit where the backbone is rigid and the side chains are mobile (sum of a and b); d, calculation for the liquid polymer, from addition schemes; e, calculation for the solid polymer from approximate vibrational spectrum; f, experimental measurements from differential scanning calorimetry

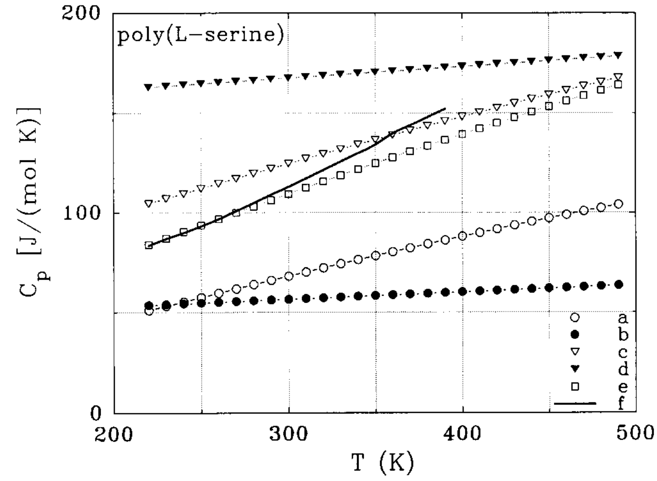

Figure 2 Heat capacities at constant pressure (C_p) for poly(L-serine), PSER: labels same as for *Figure 1*

EXPERIMENTAL RESULTS

The two poly(L-amino acid)s were purchased from Sigma Chemical. The samples were kept over a drying agent, and heated to 390 K to remove residual water before cooling and measuring. Details of the measurements and experimental data are given in reference 2.

Heat capacities

For PMET good agreement of the measured heat capacity (curve f in *Figure 1*) with a computation using an approximate vibrational spectrum (curve e in *Figure 1*) was observed only from 220 to 250 K. The observed RMS deviation in this limited temperature range was ±0.7%. At approximately 260 K the experimental C_p begins to rise above that calculated. For PSER similar results were obtained and are shown in curves e and f of *Figure 2*. Again, agreement between calculated and experimental C_p is observed at only the lowest temperatures (220–280 K) with a RMS deviation of ±0.7%. At 280 K the experimental C_p begins to rise above the calculated values. The rise becomes even sharper at approximately 360 K. The shape of the C_p curve shows that no peak is present.

If a glass transition is causing the observed C_p increase, the ultimate value reached should be compared to the C_p of the liquid. Decomposition occurs, however, before poly(amino acid)s reach the liquid state. We have, thus, estimated the expected C_p of liquid polymers using addition schemes developed earlier in our laboratory (the estimated heat capacities are shown as curves d in *Figures 1* and *2*). The concept of additivity of properties, based on the groups contained in the repeating unit, has been used extensively[18]. For C_p, the accuracy of such prediction is typically ±5.0%. Indeed, it has already been shown that over the range of the experiment (220–390 K) the C_p of the copolymers of the poly(amino acid)s can be predicted to within ±3.0% based on additivity of the individual poly(amino acid)s making up the copolymer[19]. Analysis of more than one hundred linear macromolecules contained in our ATHAS data bank has revealed that the C_p of molten or liquid polymers is even more additive in terms of small groups of atoms[20]. For both PMET and PSER the liquid C_p was therefore calculated, based on experimentally determined group contributions. The groups used were $-CONH-$ (from nylons), $-CH_2-$ (from various polymers), $-S-$ [from poly(thio-1,4-phenylene)], CH_3- (from paraffins), and $-CH<$ (from polypropylene). No suitable $-OH$ group was available in our prior collection of group contributions, so that the $-O-$ group from aliphatic polyoxides inserted in the appropriate C–H bond was used as an approximation. Note that, whenever possible, different combinations of atoms were also checked and usually gave results within 1%[21].

Qualitative experiments

Following the thermal analysis work, some qualitative experiments that are commonly linked to the glass transition (brittle point, softening temperature, scratch point determination) were performed. Referring to *Figures 1* and *2*, the increase in C_p begins as early as 240 K for PMET, while for PSER the increase is somewhat later at 270 K. The physical nature of the two samples at room temperature should reveal something about the state they are in. The PMET sample can be characterized as consisting of rather stiff pieces at 253 K. At room temperature, the pieces become sticky and softer and can be easily finger-moulded into any shape. Little can be inferred from the PSER sample, though, as it is a powder at both 253 K and 290 K.

In order to assess the effects of colder than room temperatures a small sample of PMET was shaped into a ball at room temperature and then pressed into a flat rectangular-like piece, approximately 2 mm in thickness. No change in C_p was observed for the pressed sample to within ±3.0%. The dried sample was then left inside a freezer at 253 K for 24 h over a drying agent. An attempt was then made to cut the cold sample and it was found to be hard and brittle at this temperature. The PMET sample was then moved to a refrigerator at 278 K and left for 24 h. Another attempt was made to cut the cold sample. This time a cut could be made rather easily. In addition, the sample was observed to be malleable. Similar observations were made at room temperature.

For the PSER sample no physical changes were observed on transferring the sample from the freezer to room temperature, because of its powdery nature. When both samples were brought to room temperature under dry nitrogen (after the freezing and refrigeration), no appreciable change in sample weight was noted. It appears that after drying the samples in the d.s.c., they do not pick up atmospheric moisture unless left at room temperature in air for some time (2–3 h).

The next qualitative study involved observation of changes in the two samples during heating. Dried samples of both poly(amino acid)s were placed in open aluminium pans in a DuPont Differential Thermal Analyser (DTA) and heated from room temperature to 673 K. Prior to placing the PMET sample in the DTA, two cuts were made in the sample. The first was a scratch across the surface, the second a deep cut through the entire thickness of the sample. Upon heating, the scratch became less noticeable until it was no longer visible at 473 K. The deep cut also began to 'heal' as the sample was heated, and was no longer visible at 573 K. Slightly below 623 K, the sample began to blacken. At 623 K, the sample had severely decomposed, turning black.

The PSER powder sample began to exhibit stickiness at approximately 323 K. At higher temperatures only colour changes were observed, with the sample turning yellow at 473 K, brownish-yellow at 523 K and orange-yellow at 548 K. At 573 K it began to decompose.

DISCUSSION

There is no doubt that the C_p of dry poly(amino acid)s can be successfully linked to their vibrational spectra. Such calculations were done for 21 poly(amino acid)s and copoly(amino acid)s, and the average RMS deviation from the experiment for all samples (excluding PMET and PSER) was approximately ±1.5% over the whole temperature range of measurements[1,2]. Only for PMET and PSER were deviations found, as shown in *Figures 1* and *2*. These deviations cannot be associated with the presence of residual water, for two reasons. First, the experimental C_p is repeatable. As was the case with all the other poly(amino acid)s, the two samples were subjected to three heating runs. Once the water was removed in the first heating run (up to 400 K), both the sample weight and C_p remained essentially the same for the following runs, the C_p being reproducible to ±3.0%. It was shown by measurement of the heat of evaporation and mass loss that such treatment removes all water present[2]. Second, the experimental C_p of both samples is observed to be in complete agreement with the calculated C_p in the low-temperature range. One would expect a positive deviation in the experimental C_p even at the low-temperature range of the experiment if water were involved, as was seen in other wet poly(amino acid)s analysed.

Inspection of *Figures 1* and *2* shows that the measured C_p does not fully reach the liquid values, i.e. a liquid-like mobility for the polymer is not achieved. Assuming that the liquid C_p value would eventually be reached at higher temperatures, in the absence of decomposition, the obtained '100% amorphous' $\Delta C_p(\Delta C_p^a)$ is $61\ J\ K^{-1}\ mol^{-1}$ for PMET and $30\ J\ K^{-1}\ mol^{-1}$ for PSER. The midpoint of the jump is approximately 400 K for both polymers and provides an estimate of a predicted T_g for the main chain. This high value is in line with the literature observations and the intuitive expectations, outlined in the Introduction. If we assume, on the other hand, that the glass transition were complete within the range of measurements, the thus-obtained 'measured' $\Delta C_p(\Delta C_p^m)$ is $23\ J\ K^{-1}\ mol^{-1}$ (at 280 K) for PMET and $9\ J\ K^{-1}\ mol^{-1}$ (at 310 K) for PSER. For both ΔC_p^m and ΔC_p^a estimates the value for PSER is about half that of PMET.

If the difference of the 'measured' from the '100% amorphous' ΔC_p were due to crystallinity the ratio $[1-(\Delta C_p^m)/(\Delta C_p^a)]$ could be used for its estimation. This simple calculation would suggest a crystallinity of 70% for both PMET and PSER. Powder X-ray diffraction data taken on our samples yield, however, only low levels of crystallinity, certainly less than 25%, with PMET appearing even less crystalline than PSER. Therefore crystallinity cannot be used to explain the lower ΔC_p.

The presence of small crystallites can, nonetheless, explain the broadness of the assumed glass transition.

The ΔC_p values are next compared with values derived from the empirical rules[5]. Assuming two backbone beads, to account for the –CHR– and –CONH– groups, we would expect a ΔC_p of $22\ J\ K^{-1}\ mol^{-1}$ for both polymers. The higher estimated values of 61 and $30\ J\ K^{-1}\ mol^{-1}$ for PMET and PSER thus point to a contribution of the side group. Adding three beads for the side group of PMET (note that the methyl group is not counted as a bead because its motion does not change the geometry of the molecule and is already excited below 100 K), and one bead in the case of PSER (assuming no contribution of the small hydroxy group to the ΔC_p, as in the case of the methyl group), we reach estimates of $55\ J\ K^{-1}\ mol^{-1}$ for PMET and $33\ J\ K^{-1}\ mol^{-1}$ for PSER, close to the estimated ΔC_p^a for PMET and PSER, respectively.

If we next assume, as suggested in the literature, that motion of the side groups alone may occur at the glass transition of poly(amino acid)s, keeping the backbone essentially rigid, the empirical values of ΔC_p are $33\ J\ K^{-1}\ mol^{-1}$ for PMET and $11\ J\ K^{-1}\ mol^{-1}$ for PSER, close to the measured ΔC_p^m.

To improve the estimate of a ΔC_p for a transition to a polymer that has a rigid backbone and mobile side groups, the corresponding C_p should be calculated. For the computation of the backbone C_p, the approximate vibrational spectrum of rigid polyglycine can be used, with the Θ temperatures determined previously for PMET ($\Theta_1 = 691$ K, $\Theta_3 = 68$ K) and PSER ($\Theta_1 = 685$ K, $\Theta_3 = 68$ K)[2]. The results are shown as curves a in *Figures 1* and *2*. For the mobile side groups, the heat capacity contributions can be estimated from the heat capacities of the liquids, as outlined above. The results of this computation are shown as curves b in *Figures 1* and *2*.

The sum of curves a and b should correspond to a polymer with a rigid backbone and mobile side groups. Referring to *Figure 1*, we can see that the agreement of curve c (the sum of curves a and b) with the measurements for PMET is good, given the nature of the approximations used. In other words, the beginning of the glass transition at room temperature for PMET may, indeed, involve primarily the side groups. This result is in agreement with X-ray fibre diffraction experiments on PMET that indicated a hexagonal structure with significant disorder in the side groups at room temperature[22]. It is also in agreement with dielectric measurements on PMET, that show a loss peak in the temperature range of 200–270 K at frequencies from 30 to 10^6 Hz, interpreted by the authors as motion in the side groups[23].

For PSER, shown in *Figure 2*, the agreement is also acceptable for the first increase in C_p but at about 350 K one can see a second upturn that cannot be explained by motion of the side groups alone. Gradual unfreezing of the backbone may begin after the initial unfreezing of the side groups. A similar increase in the C_p of PMET could occur at higher temperatures, above our measurement range. Compared to PMET, the side-group motion in PSER starts later but the backbone motion may occur earlier. This difference in behaviour can be explained by the size of the side groups. In addition to the size difference (the side group of PSER is smaller than that of PMET), the –OH group of PSER should be involved in H-bonding. For PMET, with its longer side group and much weaker intermolecular interactions (no H-bonding is possible), the two types of motion can persist uncoupled for a wider temperature range, and the onset of backbone motion may be more gradual.

The qualitative experiments can now be understood, in agreement with the quantitative thermal data. The change in brittleness of the PMET sample on warming from 253 K to 278 K corroborates the existence of a glass transition around 280 K, obtained by d.s.c. The softening in the PSER sample around 323 K is in accord with a glass transition at 310 K, also seen by d.s.c. The 'healing' of the scratches made on the PMET sample at temperatures higher than our measurement range suggests that the PMET backbone is also gradually gaining flexibility, similar to the observation made for PSER, albeit at higher temperatures.

CONCLUSIONS

Heat capacity data, coupled with qualitative experiments, indicate a side-group glass transition for PMET at 280 K and for PSER at 310 K. This motion, once active, may gradually induce backbone flexibility. For PSER the onset of the latter motion may be around 350 K, while for PMET it should be above 390 K.

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REFERENCES

1 Roles, K. and Wunderlich, B. *Biopolymers* 1991, **31**, 477
2 Roles, K., Xenopoulos, A. and Wunderlich, B. *Biopolymers* in press
3 Ferry, J. D. 'Viscoelastic Properties of Polymers', 3rd Edn, Wiley, New York, 1970; McCrum, N. G., Read, B. E. and Williams, G. 'Anelastic and Dielectric Effects in Polymeric Solids', Wiley, Chichester, 1967
4 Boyd, R. H. *J. Chem. Phys.* 1959, **30**, 1276
5 Wunderlich, B. *J. Phys. Chem.* 1960, **64**, 1052
6 Wunderlich, B. 'Thermal Analysis', Academic Press, Boston, 1990
7 Gaur, U. and Wunderlich, B. *Macromolecules* 1980, **13**, 445
8 'Polymer Handbook' 3rd Edn (Eds J. Brandrup and E. H. Immergut), Wiley-Interscience, New York, 1989, Ch. VI
9 Starkweather, H. W. Jr in 'Nylon Plastics' (Ed. M. I. Kohan), Wiley-Interscience, New York, 1973, Ch. 9
10 Yannas, I. V. *J. Macromol. Sci.-Rev. Macromol. Chem.* 1972, **C7**, 49
11 Magoshi, J., Magoshi, Y., Nakamura, S., Kasai, N. and Kakudo, M. *J. Polym. Sci., Polym. Phys. Edn* 1977, **15**, 1675
12 Tsukada, M., Magoshi, J. and Miyauchi, K. *Kobunshi Robunshu* 1977, **34**, 71
13 Frauenfelder, H., Petsko, G. A. and Tsernoglou, D. *Nature* 1979, **280**, 558
14 For a recent review: Young, R. D. and Scholl, R. *J. Non-Cryst. Solids* 1991, **131–133**, 302
15 Kail, J. A. E., Sauer, J. A. and Woodward, A. E. *J. Phys. Chem.* 1962, **66**, 1292
16 Sugai, S., Kamashima, K., Makino, S. and Noguchi, J. *J. Polym. Sci. A-2* 1966, **4**, 183; Hiltner, A., Anderson, J. M. and Borkowski, E. *Macromolecules* 1972, **4**, 446; Tsutsumi, A., Hikichi, K., Takahashi, T., Yamashita, Y., Matsushima, N., Kanke, M. and Kaneko, M. *J. Macromol. Sci.-Phys.* 1973, **B8**, 413

17 Pezzin, G., Ceccorulli, G., Pizzoli, M. and Peggion, E. *Macromolecules* 1975, **6**, 762
18 van Krevelen, D. W. 'Properties of Polymers', 3rd Edn, Elsevier, Amsterdam, 1990
19 Roles, K. and Wunderlich, B. *J. Polym. Sci., Polym. Phys. Edn* 1993, **31**, 279
20 Gaur, U., Cao, M.-Y., Pan, R. and Wunderlich, B. *J. Thermal Anal.* 1986, **31**, 421; Pan, R., Cao, M.-Y. and Wunderlich, B. *J. Thermal Anal.* 1986, **31**, 1319
21 All data on group contributions are included in the ATHAS data bank, available from the authors
22 Colonna-Cesari, F. and Premilat, S. *Polymer* 1976, **17**, 267
23 Tsutsumi, A., Hikichi, K., Mitsumaki, M., Kaneko, M. and Furichi, J. *J. Phys. Soc. Japan* 1967, **22**, 1120