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Phase Transformations of Glutamic Acid and Its Decomposition Products

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ABSTRACT: The thermal behavior and phase transformations of the α and β polymorphs of L-glutamic acid were studied by differential scanning calorimetry, thermogravimetry, powder X-ray diffraction, gel permeation chromatography, and mass spectrometry. Solid-state transformation of α to β polymorph was observed at temperatures above 140 °C, together with, in sequence, cyclization to pyroglutamic acid and polymerization to polyglutamic acid. The resulting polyglutamic acid is a high molecular weight amorphous polymer, with a glass transition temperature at ∼20 °C.

1. Introduction

Amino acids are the fundamental building blocks of proteins and have applications in the food, agricultural, cosmetics, and pharmaceutical industries.[1−4] They are speculated to be components of the origin of life and may exist in interstellar space. This means that amino acid molecules are likely to be stable under a diverse range of conditions including temperature and water vapor pressure, but also, under certain conditions, transform into large biological molecules. In spite of this, only a few studies have been reported on their thermal stability and decomposition/transformation. Thermal polymerization of a number of mixed amino acids have been studied;[5−10] most amino acids (e.g., glutamic acid (Glu) and phenylalanine) do not homopolymerize to linear peptides under thermal treatment, while a few others (e.g., glycine and lysine) do lead to linear peptides when heated alone.[9,10] It is also suggested that the amino acids that do self-polymerize can modify the behavior of those that do not, resulting in copolymerization of various amino acids (e.g., copolymerization of Glu and glycine).[10]

We are interested in L-glutamic acid (L-Glu) and its derivatives due to a wide spectrum of commercial applications including their incorporation in drugs for the treatment of ulcers,[11,12] hypoglycemia,[13] epilepsy, Parkinson's, and other neurological disorders.[14−17] They are used extensively as food additives (codes E620−629) and flavor enhancers in their sodium salt forms; L-Glu provides a necessary nutrient to metabolize sugar and fats and to aid in transporting potassium through the blood−brain barrier.[18] The thermal stability of L-Glu in the food and pharmaceutical industries is important because of its possible degradation, which may impact product safety and quality.[19]

L-Glu is a typical dimorphic system with metastable α and stable β polymorphs; it is well-known from previous studies[20−23] that the α to β transformation is solution-mediated and if α crystals are separated from their mother liquor, the dry crystals are indefinitely stable;[22,24,25] indeed, it was claimed that "there is evidently no solid state route to the β structure".[24] This claim was based on observations at room temperature; the lack of experimental data on the thermal behavior of this system near and beyond the melting points of the two polymorphs were key motivations to understanding temperature-induced changes in these materials. An unexpected outcome has been that a solid state α to β transformation does occur at high temperature, in contrast to literature expections.[24]

Previous studies on the thermal behavior of L-Glu have been limited to the β polymorph.[26−28] It was suggested[26] that the first decomposition step of β L-Glu involved dimerization to form a diketopiperazine, while an alternative proposal[27] was that pyroglutamic acid (P) formed as a decomposition intermediate instead of diketopiperazine. The overall objective of this work has been to investigate the temperature-dependent structural and chemical evolution on heating and cooling both L-Glu polymorphs in the range −40 to 200 °C. In order to capture the relevant phase transformations and chemical changes that occurred, a set of complementary techniques, X-ray diffraction (XRD), thermogravimetry (TG), differential scanning calorimetry (DSC), gel permeation chromatography (GPC), and mass spectrometry (MS), was used.

2. Experimental Methods

2.1. Materials and Sample Preparation. All experiments were performed with high-purity L-Glu (purity >99%, VWR International Ltd., UK). To obtain pure α and β forms, the following procedure was used. A saturated 38 g/L aqueous solution of L-Glu was filtered at 70 °C with a membrane filter (pore size = 0.2 μm). Then, without mixing, the solution was cooled to a crystallization temperature of either 15 °C via quench cooling for the α form or 53 °C via controlled cooling at the rate of 1 °C/min for the β form: It is known that at temperatures below 25 °C the formation of α dominates the crystallization process.[22] Once the desired crystallization temperature was reached, a vigorous mixing of the solution by a three-blade marine impeller at 800 rpm was applied. Under the selected conditions, crystallization of α took approximately 15 min and an hour for β. The resulting slurry was filtered with a membrane filter (pore size = 0.2 μm) and dried at 40 °C for 2 days by allowing slow evaporation of solvent to avoid transformation or decomposition of the crystals. The dried powder of each polymorph and its phase purity was identified by XRD using a STOE STADI P X-ray powder diffractometer equipped with an image plate position sensitive detector (IP-PSD), Cu $K\alpha_1$ radiation (λ = 1.54051 Å) over the 2θ range 6.0° to 125.0° at room temperature.

2.2. Instruments and Methodologies. The thermal transformation of α and β polymorphs was analyzed by DSC using a Perkin-Elmer Pyris1 DSC under dry N_2. The heat flow was calibrated using

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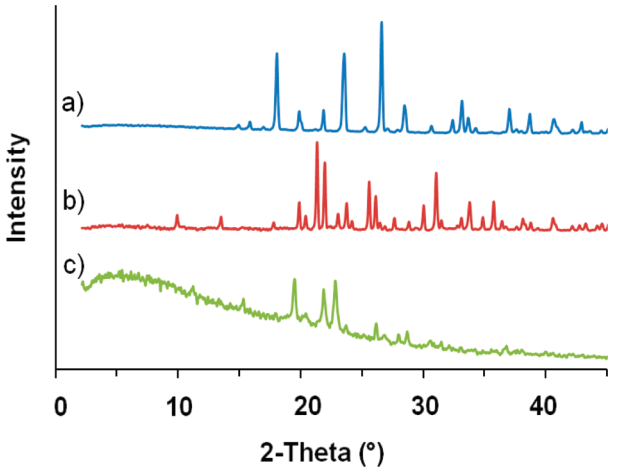


Figure 1. XRD patterns of (a) α L-Glu, (b) β L-Glu, and (c) the product of melting α L-Glu at 200 °C and cooling.

In (M_{Pt} = 156.6 °C, ΔH = 28.45 J/g) and the temperature with In and Zn over the range 150−425 °C. The powder was weighed in a 50 μL Al sample pan with a perforated lid before and after thermal treatment. Each sample was heated from 40 to 200 °C, followed by cooling to −40 °C and reheating to 200 °C. The heating rate varied from 0.5 to 20 °C/min and the cooling rate was 10 °C/min.

The weight loss during heating was quantified by TG using a Perkin-Elmer Pyris1 TG system. Samples were heated from 40 to 550 at 20 °C /min, in flowing dry N_2. To characterize the changes occurring at each thermal event, samples were heated to various temperatures, including 165, 176, 180, 185, and 200 °C (prior to and after melting) by DSC, cooled to room temperature, and analyzed by XRD. The samples were also heated in a furnace at different temperatures: 140, 150, 160, and 170 °C, for times ranging from 1 to 60 h so that time-dependent thermal behavior of L-Glu could be monitored and analyzed by XRD. Products obtained from heating each polymorph at 200 °C and subsequent cooling were analyzed by GPC and EI-MS.

GPC used a tetrahydrofuran (THF) - GPC system equipped with a Knauer K-2301 RI Detector. The polymers were separated by size inside a Varian PL Gel 5 μm Mixed C - Column. The retention time revealed the average molecular weight (MW) which was calibrated against polystyrene standards. Electron impact ionization mass spectrometry (EI-MS) was used to identify mass fragments with molecular weight < 1000. The spectra were recorded on an Auto-Spec E spectrometer.

3. Results and Discussion

The α and β forms of L-Glu can be readily distinguished by their powder XRD patterns, as shown in Figure 1.

The patterns (a, b) match those reported in the powder XRD file, cards, 30-1740 and 32-1701, respectively. The third type of XRD pattern encountered resulted from dehydration and polymerization of the α and β forms on heating to 200 °C and subsequent cooling, Figure 1c. This pattern contains a set of sharp peaks, which match the powder XRD pattern of L-P,[29] card 37-1706, superposed on a broad diffraction peak attributed to the presence of an amorphous phase which, in this case, is high molecular weight, amorphous polyglutamic acid, PGA.

DSC results for α L-Glu over the temperature range −40 to 200 °C are shown in Figure 2. On initial heating, apart from a baseline glitch at 160 °C associated with the change in heating rate, a large endotherm peaking at 185 °C is seen, which is not reversible on cooling. Instead, the cooling cycle is rather featureless and shows only a broad hump at ∼20 °C. On second heating, the baseline discontinuity at ∼20 °C is reproduced as a broad endotherm and there are no further

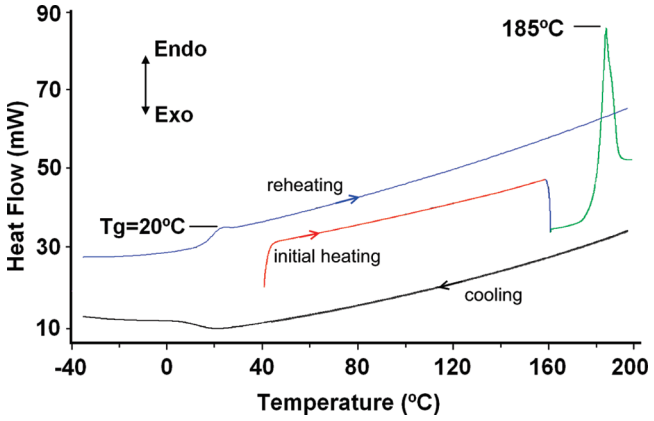


Figure 2. DSC curves on heating, cooling, and reheating α L-Glu. Heating rate on first cycle 20 °C/min from 40 to 160 °C, then at 2 °C/min to 200 °C. Cooling and second heating rates, 10 °C/min. The baseline discontinuity on first heating at 160 °C is due to the change in heating rate. T_g = glass transition temperature.

Table 1. Variation of Endotherm Maximum Temperature of L-Glu Polymorphs at Different Heating Rates

sample	heating rate (°C/min)	endotherm maximum temperature (°C)
α	0.5	174
	1	182
	2	185
	10	202
β	2	194
	10	210

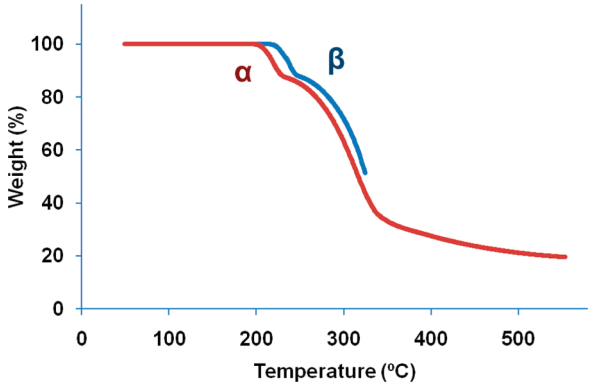


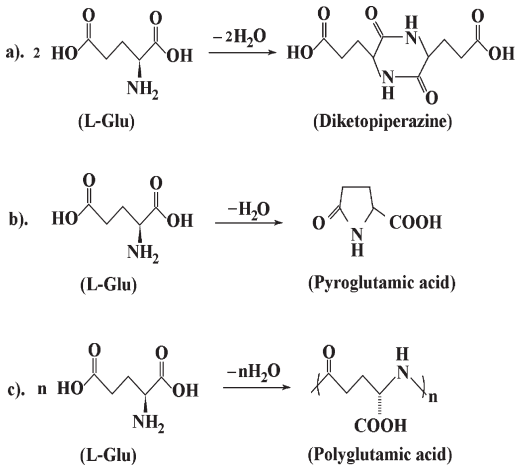
Figure 3. TG curves of α and β L-Glu.

heat effects up to 200 °C. The baseline change at ∼20 °C on cooling and subsequent reheating has the characteristic appearance of a glass transition from a rigid amorphous solid below T_g to a highly viscous liquid above T_g. In addition, the absence of a melting/crystallization heat effect on the second heat/cool cycle is further evidence that the heat effect at ∼20 °C is a glass transition.

Very similar behavior was seen in the DSC results on β L-Glu. These are not shown, since the only difference is a small change in the temperature of the large endotherm on first heating, which increases with increased heating rate, as summarized in Table 1 for both α and β polymorphs heated at different rates. This phase transformation in β occurs at ∼8−10 °C higher than that in α.

In order to investigate further the nature of the changes seen at ∼180 to 200 °C, TG data were recorded on heating and are shown for both α and β polymorphs in Figure 3.

Scheme 1. Possible Transformations of L-Glu on Heating to Yield (a) Diketopiperazine, (b) P and (c) PGA



The general shape of the TG profiles is similar in both cases, but the weight loss of β occurs a few degrees higher than that of α; at temperatures above ~200 °C, an initial weight loss totalling about 12% occurs, which is followed by a poorly resolved plateau and a subsequent large loss in weight above ~250 °C; similar results have been reported previously for TG on β[8]. The initial weight loss of ~12% corresponds to loss of one molecule of water per L-Glu formula unit (theoretical value 12.24 wt %). The loss in weight on heating is not regained on cooling (not shown). There appears to be an approximate correlation between the DSC endotherm seen in Figure 2 and the weight losses in the range of 200−220 °C, seen in Figure 3. The samples also appear to melt in the temperature range of 180−200 °C, and therefore the DSC endotherm may represent a combination of dehydration/transformation and melting (see later).

Three candidate reactions, each associated directly with the loss of one molecule of H_2O per formula unit of L-Glu, are shown in Scheme 1, two of which have received consideration in the literature.[26,27] First, dimerization of L-Glu to give a diketopiperazine.[26] Second, internal cyclization to produce P.[27] Third, polymerization to produce PGA.

In order to gain further information on the nature of the dehydration product(s) and mechanism, a combination of XRD, GPC, and MS was used on a sample of L-Glu that had been heated to 200 °C and cooled to room temperature. By XRD, the phases present were P and an amorphous material. By GPC, Figure 4, a broad molecular weight distribution peaking at M_p = 26363 was observed and with molecular weight up to 30 000. These results are characteristic of a high molecular weight polymer, with a significant molecular weight distribution and are therefore attributed to PGA.

The MS spectrum, Figure 5, shows a peak at mass/charge 129, which is attributed to P ($C_5H_7NO_3$), together with a peak at 84, which corresponds to the loss of CO_2H. This result confirms the presence of P in the P/PGA mixture.

The combined XRD, GPC, and MS results, on this and other samples, show the presence, depending on conditions, of up to three crystalline phases: α L-Glu, β L-Glu, and P and an amorphous phase, attributed to PGA. We found no evidence for the formation of diketopiperizine, Scheme 1a, but clear evidence for Scheme 1b.

To further study the transformation sequence, a series of DSC experiments was carried out; samples were heated to various temperatures, cooled, and analyzed by XRD. In addition to melting and decomposition/transformation of L-Glu to P and PGA on heating, the α to β polymorphic transformation of L-Glu was found to occur; for example, on heating α L-Glu at 165 and 170 °C, Figure 6a, mixtures of α and β were obtained.

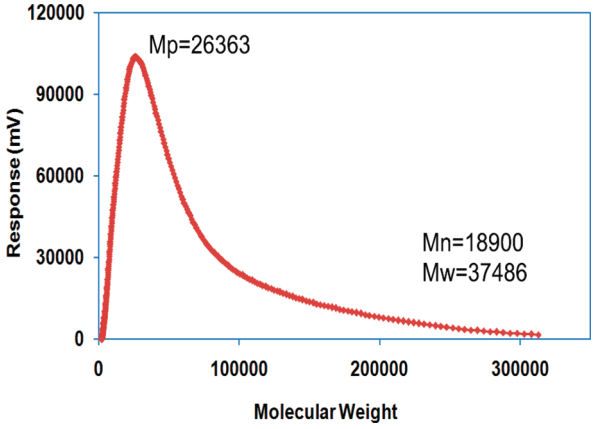


Figure 4. Gel permeation chromatogram of product of heating α L-Glu to 200 °C and cooling.

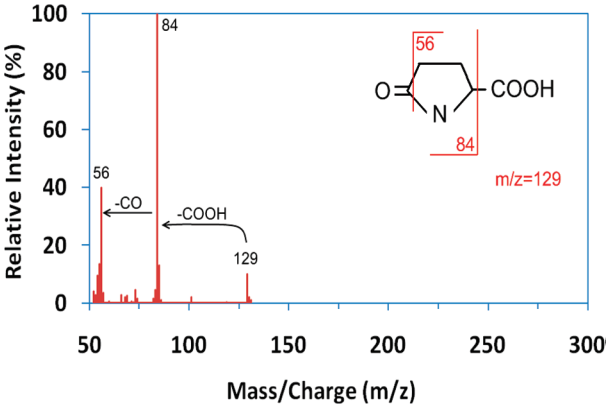


Figure 5. EI-MS m/z spectrum of product of heating L-Glu to 200 °C and cooling.

The α → β transformation is not reversible and has been observed only in the α → β direction. The α → β transformation occurs relatively slowly and, on the time scale of these experiments, appears not to proceed to completion. This may be because the α → β transformation appears to overlap with subsequent transformation of β to P and PGA, Figure 6a,b. The irreversibility of the α to β transformation indicates that the α polymorph is metastable and transforms to the thermodynamically stable β polymorph under appropriate conditions, consistent with expectations from the literature.[22] The product of heating either α or β L-Glu to ~200 °C by DSC was a mixture of crystalline P and amorphous PGA, Figure 6a,b, which is in a good agreement with GPC and MS studies of the same sample.

From these preliminary results, it was believed that given enough time, α will transform to β completely prior to melting and dehydration. However, we were unable to obtain pure β polymorph by simply heating α polymorph using DSC under different conditions (a range of heating rates and isothermal "hold" temperatures was investigated). Part of the difficulty lies in the fact that the α → β transformation occurs at temperatures close to the melting point of α. Also,

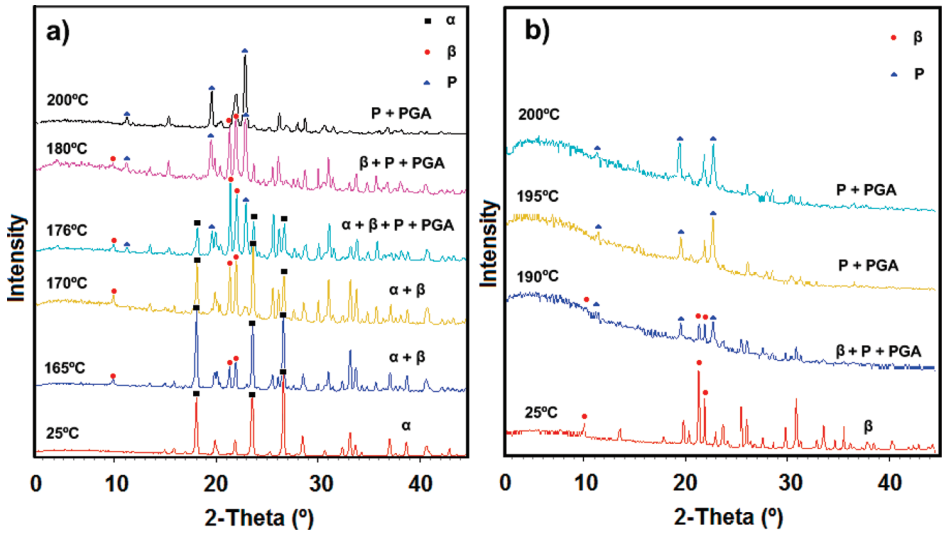


Figure 6. XRD patterns of (a) α L-Glu and (b) β L-Glu heated to different temperatures and recorded at room temperature after cooling.

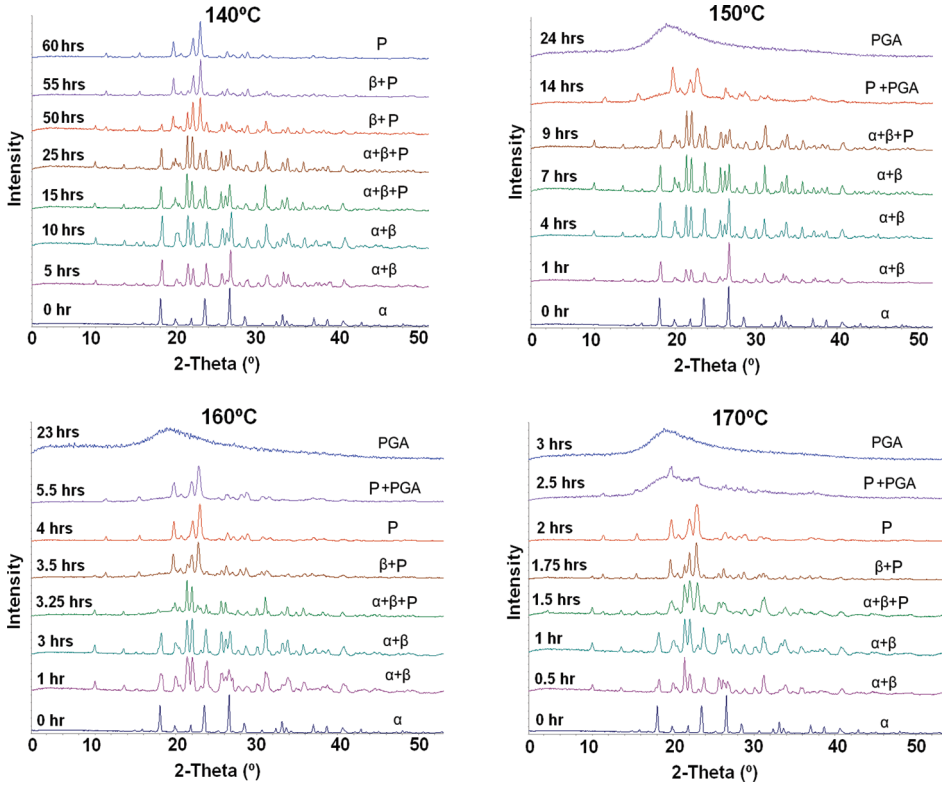


Figure 7. Powder XRD patterns of α L-Glu heated to different temperatures for various times.

melting appears to be associated with two dehydration reactions - cyclization to form P (highlighted in blue triangles) and polymerization to form PGA (amorphous). In addition, it is unclear whether it is possible to melt L-Glu before the onset of simultaneous dehydration and transformation.

In an attempt to clarify the sequence of transformation steps, isothermal heat treatments were carried out by heating samples in an oven at lower heating temperatures (140−170 °C) and for longer times (1−60 h) followed by rapid cooling to room temperature. As a result, improved separation of the transformations was achieved, as shown in Figure 7. In particular, this revealed the stepwise nature of the α → β transformation, internal cyclization, and polymerization.

At three temperatures, 140, 160, and 170 °C, patterns of pure P were obtained after certain times, indicating that this is a key reaction intermediate before the final polymerization step. The gradual disappearance of the various crystalline XRD peaks and the appearance of the broadened amorphous diffraction patterns of the products of heating L-Glu to 150, 160, and 170 °C indicates that, after sufficient time, the original sample is fully melted and has transformed to amorphous PGA.

Although it has been possible to isolate samples of pure P and PGA, it has not been possible to obtain pure β commencing with α. The interpretation of this observation is that once formed, the β polymorph readily transforms to P. Since the

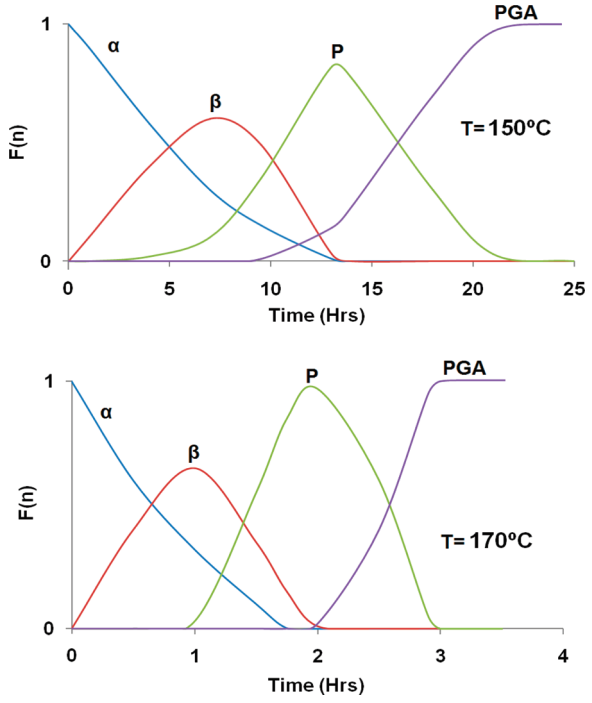


Figure 8. Evolution of estimated fractional amounts, $F(n)$, of phases as a function of time at 150 (top panel) and 170 °C (bottom panel).

$\beta \rightarrow$ P transformation includes loss of H_2O, possibly this transformation could be suppressed in an atmosphere of high water vapor pressure.

To demonstrate graphically the transformations of L-Glu on heating, the evolution of fractional amounts of each phase is shown schematically in Figure 8. The amounts were estimated approximately, using the relative integrated intensity (area) of the strongest peak (for α, the second strongest peak was used due to the overlapping of its strongest peak with a β peak) in the XRD patterns of each phase (α, β, P, PGA), in Figure 7. Without any attempt at calibration, the resulting curves are at best semiquantitative but nevertheless show clearly the sequence of steps in the overall transformation of L-Glu to PGA, Figure 8.

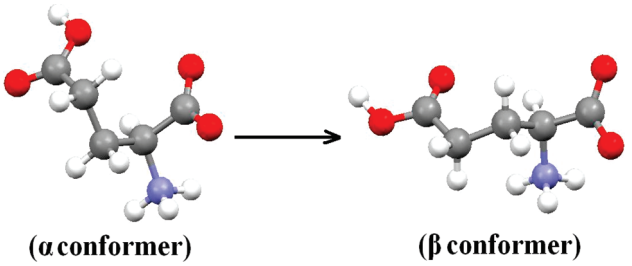
The main transformation sequence is

$$\alpha \rightarrow \beta \rightarrow \text{P} \rightarrow \text{PGA}$$

for temperatures in the range of 140−170 °C. Transformation rates are very temperature dependent. For instance, ~14 h are required for transformation of L-Glu to P at 150 °C, but only 2 h at 170 °C; the transformation sequence is complete, giving pure PGA, after ~24 h at 150 °C but after only 3 h at 170 °C. Under these conditions, pure β polymorph was not obtained because, once formed, it transformed readily to P.

From these various sets of results, we demonstrate the high temperature behavior of L-Glu polymorphs and the sequence of their transformation, for the first time. It is clear that both α and β polymorphs undergo dehydration with loss of one molecule of water per formula unit, together with melting at similar temperatures in the range 140−220 °C. From the TG results, Figure 3, the β polymorph loses H_2O at a slightly higher temperature than the α polymorph and the DSC endotherm occurs at a slightly higher temperature for a given heating rate, Table 1. These observations are consistent with the thermodynamic stability of β, compared to the thermodynamic metastability of α.

Scheme 2. The Polymorphic Transformation of L-Glu from Conformation α to Conformation β



We do not know if it is possible to melt either, or both, α and β polymorphs before dehydration occurs. Dehydration occurs at temperatures as low as 140 °C during long-time annealing but occurs at ≥180 °C during the rapid heating rates used in DSC and TG. Associated with the loss of water, a two-step reaction occurs: internal cyclization to give P followed by polymerization to give PGA.

The P that forms is crystalline and is readily detected by XRD and MS. The PGA is amorphous, as shown by XRD. It exhibits a glass transition at ~20 °C, which corresponds to the transformation from a highly viscous liquid above 20 °C to a rigid, amorphous glass below 20 °C. Its molecular weight distribution is demonstrated by GPC.

The formation of pure P as an intermediate confirms the cyclization reaction to be rapid and complete, consistent with certain literature findings obtained under different heating conditions,[8,30] thus resolving the discrepancy in the identification of dehydration intermediates[26,27] by using XRD and MS.

We observe, for the first time, the polymerization of L-Glu on heating without the aid of other amino acids, whereas this was claimed to be an unlikely event.[31] The known methods for thermal polymerization of L-Glu are restricted to copolymerization with other amino acids to form linear peptides.[32,33] Our results show that direct polymerization of L-Glu is possible. Such polymerization is initiated in the absence of other amino acids, although the intermediate P may act as an active acid and play a significant role in aiding polymerization.[30,31]

There is much interest in the $\alpha \rightarrow \beta$ transformation because of its relevance to the food and pharmaceutical industries, and prior to this study, it was not thought possible to carry out this transformation simply by heating.[24] While we do not dispute that α crystals, once formed, are stable indefinitely at room temperature, our results indicate that at high temperatures the $\alpha \rightarrow \beta$ transformation does occur at a rate that is very temperature dependent. In order to understand why the $\alpha \rightarrow \beta$ transformation is a difficult process, and does not occur in the solid state at room temperature, we show in Scheme 2 that the conformations of α and β monomers are very different; consequently, as shown in Figure 9, the molecular packing arrangements in the two crystalline structures are also very different.[34,35]

The $\alpha \rightarrow \beta$ transformation in the solid state must therefore be reconstructive in nature: it is difficult to see how homogeneous nucleation of β within the α crystal lattice could occur because of the structural constraints imposed by the surrounding α crystal lattice. Heterogeneous nucleation on the surface of α crystal would not have this same structural constraint. The β structure is thermodynamically stable and therefore has lower free energy than the α structure. However, the structural transformation from $\alpha \rightarrow \beta$ involves a high activation barrier

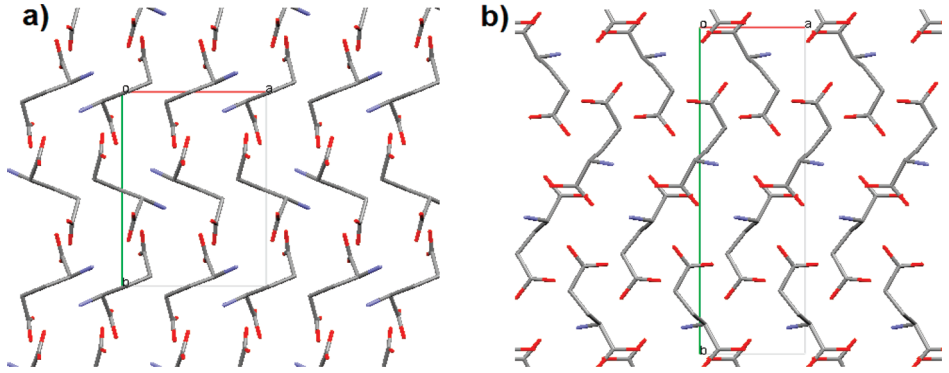


Figure 9. Molecular packing of L-Glu in crystalline structure of (a) α form and (b) β form along *c* axis.[33,34]

which presumably explains why the transformation is not observed in the solid state at room temperature. At high temperatures ≥140 °C, the thermal energy of the molecules effectively reduces the kinetic barrier to nucleation and subsequent growth of the stable β polymorph.

We do not have any detailed information on the α to β transformation mechanism. Clearly, at high temperatures, it is not solution-mediated. It may be an entirely solid-state process. Or possibly, partial melting of α at the α−β interface may be involved. Further studies on this are required.

4. Conclusions

Using a combination of DSC/TG, GPC, MS and XRD, it is found that both α and β polymorphs of L-Glu on heating in the range 140−220 °C undergo a complex and overlapping set of transformations involving dehydration, melting, and structural reorganization. Both XRD and MS showed the formation of pyroglutamic acid prior to formation of high molecular weight polyglutamic acid, resolving the disagreement between two groups[26,27] in which the IR technique was not adequate to differentiate pyroglutamic acid from diketopiperazine. Additionally, various product mixtures are possible, depending on the heating condition of temperature and time.

We observed, for the first time, a partial and irreversible transformation of α to β polymorph at temperatures ≥140 °C, thus confirming the metastability of the α polymorph. DSC results show a single broad endotherm peaking at ∼180 °C, whose peak temperature increased somewhat with heating rate. At least three processes may occur simultaneously and contribute to this endotherm: α → β transformation, melting, and decomposition/dehydration/transformation. The peak temperature was observed to depend on heating rate; this is probably because a thermally activated and complex sequence of overlapping reactions and transformations occur and are represented by this endotherm. The isothermal studies of L-Glu at different temperatures reveal the sequence of the reaction to be α → β transformation, followed by internal cyclization to give pyroglutamic acid and subsequent polymerization to give polyglutamic acid.

The isothermal heat treatments as a function of time have shown that it is possible to at least partially separate the different steps in the transformation sequence, allowing isolation of pure pyroglutamic acid as an intermediate. These studies also showed that, with sufficient time, the various transformations occur at temperatures as low as 140 °C, which is well below the endotherm seen during the dynamic DSC experiments. Further studies are required to find conditions to separate the α → β transformation from subsequent dehydration/melting.

This work, on the thermal transformations of L-Glu to pyroglutamic acid and polyglutamic acid, may be of relevance in three wider areas. First, although the transformations occur at high temperatures under our experimental conditions, the formation of pyroglutamic acid and polyglutamic acid involves dehydration, and therefore, under high vacuum conditions, such as in outer space, the transformations may occur at much lower temperatures. Biochemical processes, both natural and laboratory-based, are, of course, conducted at or below body temperature, and are usually solution-mediated. Our results show that temperature may be an additional experimental variable that can be used to investigate solid-state biochemical transformations under laboratory conditions.

Second, the formation of high molecular weight polyglutamic acid by thermal transformation and polymerization of L-Glu may be regarded as a prototype amino acid polymerization that gives a product of likely biochemical compatibility. Similar thermally induced transformations of other amino acids and their derivatives may be utilized for the design and fabrication of scaffold materials for tissue engineering applications.[36,37]

Third, we have shown, for the first time, that the α to β transformation of L-Glu is, indeed, possible in the solid state. Polymorphism, and the stability of polymorphs with different pharmacological activity, is important in many amino acid-based substances. The results and methodology presented here, with temperature (and potentially water vapor pressure) as a key variable under dry conditions, may be applicable to a better understanding of the thermal stability of a wide range of amino acid-related materials.

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References

(1) Wee, Y. J.; Kim, J. N.; Ryu, H. W. *Food Technol. Biotechnol.* **2006**, *44* (2), 163–172.
(2) Wu, G. *Amino Acids* **2009**, *37*, 1–17.
(3) Wang, W. W.; Qiao, S. Y.; Li, D. F. *Amino Acids* **2009**, *37*, 105–110.
(4) Kiwada, H; Akimoto, M; Araki, M.; Tsuji, M.; Kato, Y. *Chem. Pharm. Bull.* **1987**, *35* (7), 2935–2942.
(5) Rohlfing, D. L. *Science* **1976**, *193* (4247), 68–70.
(6) Phillips, R. D.; Melius, P. *Int. J. Peptide Protein Res.* **1974**, *6* (5), 309–319.
(7) Fouche, C. E., Jr; Rohlfing, D. L. *Biosystems* **1976**, *8* (2), 57–65.
(8) Fox, S. W.; Harada, K.; Vegotsky, A. *Experientia* **1959**, *15* (2), 81–84.
(9) Heinrich, M. R.; Rohlfing, D. L.; Bugna, E. *Arch. Biochem. Biophys.* **1969**, *130*, 441–448.

(10) Fox, S. W.; Vegotsky, A.; Harada, K.; Hoagland, P. D. *Ann. N.Y. Acad. Sci.* **1957**, *69*, 328–337.
(11) Chen, S. H.; Lei, H. L.; Huang, L. R.; Tsai, L. H. *Dig. Dis. Sci.* **2001**, *46* (10), 2285–2291.
(12) Tsai, L. H. *J. Biomed. Sci.* **2005**, *12*, 255–266.
(13) Gomez, M. R.; Gotham, J. E.; Meyer, J. S. *Pediatrics* **1961**, *28* (6), 935–942.
(14) Lloyd, K. G.; Hornykiewicz, O. *Nature* **1973**, *243*, 521–523.
(15) Isacson, O.; Kordower, J. H. *Ann. Neurol.* **2008**, *64* (Suppl), S122–S138.
(16) Gubellini, P.; Pisani, A.; Centonze, D.; Bernardi, G.; Calabresi, P. *Prog. Neurobiol.* **2004**, *74*, 271–300.
(17) Cuomo, D.; Martella, G.; Barabino, E.; et al. *J. Neurochem.* **2009**, *109*, 1096–1105.
(18) Smith, Q. R. *J. Nutr.* **2000**, *130*, 1016S–1022S.
(19) Bernstein, J.; Henck, J. O. *Cryst. Eng.* **1998**, *1*, 119–128.
(20) Scholl, J.; Bonalumi, D.; Vicum, L.; Mazzotti, M. *Cryst. Growth Des.* **2006**, *6*, 881–891.
(21) Dharmayat, S.; Carderon De Anda, J.; Hammond, R. B.; Lai, X.; Roberts, K. J.; Wang, X. Z. *J. Cryst. Growth* **2006**, *294*, 35–40.
(22) Kitamura, M. *J. Cryst. Growth* **1989**, *96*, 541–546.
(23) Ferrari, E. S.; Davey, R. J. *Cryst. Growth Des.* **2004**, *4*, 1061–1068.
(24) Davey, R. J.; Blagden, N.; Potts, G. D.; Docherty, R. *J. Am. Chem. Soc.* **1997**, *119*, 1767–1772.
(25) Ono, T.; Horst, J. H.; Jansens, P. J. *Cryst. Growth Des.* **2004**, *4*, 465–469.
(26) Valse, T.; Valse, G.; Doca, M.; Doca, N. *J. Therm. Anal. Calorim.* **2003**, *72*, 597–604.
(27) Nunes, R. S.; Cavalheiro, E. T. G. *J. Therm. Anal. Calorim.* **2007**, *87*, 627–630.
(28) Valse, T.; Valse, G.; Doca, N. *J. Therm. Anal. Calorim.* **2005**, *80*, 425–428.
(29) Van Zoeren, E.; Oonk, H. A.; Kroon, J. *Acta Crystallogr.* **1978**, *B34*, 1898–1900.
(30) Hartmann, J.; Brand, M. C.; Dose, K. *Biosystems* **1981**, *13*, 141–147.
(31) Harada, K.; Fox, S. W. *J. Am. Chem. Soc.* **1958**, *80*, 2694.
(32) Fox, S. W.; Harada, K. *J. Am. Chem. Soc.* **1960**, *82*, 3745–3751.
(33) Harada, K.; Fox, S. W. *Arch. Biochem. Biophys.* **1960**, *86*, 274.
(34) Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. *J. Cryst. Mol. Struct.* **1972**, *2*, 225–233.
(35) Lehmann, M. S.; Nunes, A. C. *Acta Crystallogr.* **1980**, *B36*, 1621–1625.
(36) Matsusaki, M.; Yoshida, H.; Akashi, M. *Biomaterials* **2007**, *28*, 2729–2737.
(37) Kyle, S.; Aggeli, A.; Ingham, E.; McPherson, M. J. *Trends Biotechnol.* **2009**, *27* (7), 423–433.