Thermal Characteristics of Poly (DL-Lactic Acid) Microspheres Containing Neurotensin Analogue[1)]

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The thermal characteristics of poly (DL-lactic acid) (DL-PLA) microspheres containing a hexapeptide (NA: H(CH_3)–Arg–Lys–Pro–Trp–*tert*-Leu–Leu–OEt) with neurotensin activity were investigated. PLA microspheres with a drug content of 1.5—11.0% were prepared by a novel o/w (oil-in-water) solvent evaporation method. Both DL-PLA and NA were amorphous in form, and an increase in heat capacity at glass transition temperature (T_g) of the polymer was observed in DL-PLA microspheres containing NA. The T_g of DL-PLA (PLA2000 bulk) was 307.8 K, while T_g of microspheres containing NA (content 6.0%) shifted to 321.2 K. The T_g of PLA2000 microspheres was found to increase with an increase in the content of NA, and its increasing tendency reached a plateau at an NA content of greater than 6%. The apparent activation energy of glass transition of PLA2000 bulk and the microspheres was calculated to be 86.3 and 99.3 kcal/mol, respectively. As a result of the release test after storage at 4 °C and 40 °C for 1 month, nearly the same release profiles of NA from PLA2000 microspheres were found. The release rate of NA after the initial release became slow after storage at 45 °C for 1 month. This may be attributed mainly to a decrease in surface area caused by the formation of agglomerates of PLA 2000 microspheres under conditions near T_g.

Keywords poly lactic acid; microsphere; glass transition temperature; neurotensin analogue; strorage condition; release test

Introduction

The preparation of drug-loaded microspheres has received much attention in recent years.[2)] Especially, sustained release microspheres using a biodegradable polymer such as poly lactic acid (PLA) have been studied during the past decade.[3,4)] Recently, PLA with a low molecular weight has been used for sustained release preparation.[5–7)] It has been reported that the glass transition temperature (T_g) of PLA with a low molecular weight is near room temperature,[8)] and it decreases in the presence of small amounts of water.[9)] It is well known that T_g can affect the diffusiveness of a polymer, its mechanical properties and its stability during storage.[9–12)] Benoit *et al.*, for example, showed changes in T_g of PLA microspheres containing progesterone during storage at 22 °C, and discussed the change in the disperse state of progesterone throughout the PLA matrix.[13)] Asano *et al.* also examined the disperse state of a luteinizing hormone releasing hormone (LH-RH) agonist in PLA pellet prepared by the melt-pressing technique using differential scanning calorimetry (DSC). It was shown that a shift in T_g was observed with an increase in the preparative temperature.[14)] However, thermal characteristics such as the T_g of PLA with low molecular weight microspheres prepared by an oil-in-water (o/w) solvent evaporation method has rarely been reported.

In the previous paper,[15)] the authors reported the preparation of PLA microspheres containing a hexapeptide (NA: H(CH_3)–Arg–Lys–Pro–Trp–*tert*-Leu–Leu–OEt) with neurotensin activity[16)] using a novel o/w solvent evaporation method. It was reported that the sustained release of NA from DL-PLA of molecular weight 2000 (PLA2000) microspheres maintained drug levels in plasma for one month after subcutaneous injection.[17)] T_g of PLA2000 is about 35 °C, so close to room temperature that it is possible that the storage temperature affects the matrix of the microspheres and eventually affects the release profile of NA.

In this paper, thermal characteristics of PLA2000 microspheres containing NA were reported. Release profiles of NA from PLA2000 microspheres after storage at different temperatures were also examined.

Experimental

Materials Neurotensin analogue (NA) was synthesized at Tsukuba Research Laboratories of Eisai Co., Ltd. DL-PLA with molecular weight of 2000 as number averages was obtained from Japan Synthetic Rubber Co., Ltd., Tokyo, Japan. All other chemicals used were of reagent grade.

Preparation of Microspheres PLA2000 microspheres containing NA were prepared by the novel o/w solvent evaporation method as previously described.[15)] Briefly, the oily phase consisted of 200 mg of PLA, 20 mg of NA and 9.2 mg of sodium caprylate in 0.6 ml of methylene chloride–ethanol mixed solvents (5 : 1, v/v ratio). The oily phase was poured into a water phase containing 0.5% polyvinylalchohol through a nozzle of about 1.0 mm i.d. under dispersion with an Ultra disperser® (Yamato Kagaku, Tokyo, Japan) at 5000 rpm. The resulting o/w emulsion was stirred gently for 3 h at room temperature and under ambient pressure. The hardened microspheres were collected by centrifugation, washed with deionized water, and lyophilized into a powder, which was dried under reduced pressure for at least 48 h.

Measurement of T_g Thermal analysis was performed with a Perkin Elmer DSC 7 equipped with an intracooler. The instrument was calibrated at 10 K/min against Indium. The samples were dried for more than 24 h under reduced pressure, and 5—6 mg of the sample powder was sealed into high pressure steel pans. The measured T_g value of the polymer is generally known to be affected by its thermal history, which requires it to be canceled in the measurement of T_g. Especially, when the T_g of a polymer is measured, the polymer is heated to a temperature of 30 K higher than its T_g, and cooled at a constant rate followed by heating to this temperature again to cancel its thermal history.[18)] In PLA2000 microspheres, the following temperature program was used. Samples were heated to 353 K with 10 K/min and cooled with 20 K/min to 213 K followed by heating to 353 K with 2—50 K/min again. The mid and onset point T_gs were obtained using the standard program supplied by Perkin Elmer.

Release Test of NA from Microspheres Five mg of microspheres were suspended in 5 ml of the release medium consisting of 1/30 M phosphate buffer containing 0.02% Tween 80 at pH 7.4 and incubated at 37 ± 1 °C (50 l/min). Since NA was hydrolyzed in a buffer solution, the residual NA in the microspheres was periodically determined after filtering the microspheres through a 0.45 μm Millipore® filter. NA in microspheres were extracted with a mixture of 1 ml of chloroform and 5 ml of 0.2 M $NaClO_4$–$HClO_4$ buffer at pH 2.0. The aqueous layer was submitted to high performance liquid chromatography (Shimazu LC-6A equipment, Kyoto, Japan) with fluorescent detection (Shimazu RF-530, Kyoto, Japan). Chromatography conditions for the determination of NA were as follows: column, an ODS (5 μm) column (150 mm × 6.0 mm i.d., YMC AM 312 packed column, YMC Co., Ltd., Kyoto, Japan); a mobile phase, a mixture of acetonitrile and 0.1% perchloric acid aqueous solution (3 : 5); flow rate of the mobile phase, 1.0 ml/min; excitation and emission wave-

lengths, 280 and 350 nm, respectively.

Observation of Microspheres The shapes of microspheres before and after the storage were examined by the use of a scanning electron microscope (model Alpha 30A Topcon Co., Ltd., Tokyo, Japan).

Results and Discussion

The T_g of PLA2000 Microspheres Containing NA The influence of heating rate on the DSC curves of PLA2000 bulk and microspheres containing NA is shown in Fig. 1. Both DL-PLA and NA are amorphous.[16] NA showed no assignment to T_g in DSC, and thus the increase of heat capacity at the T_g of PLA was observed. The T_gs (mid points) of PLA2000 bulk ranging from 305.8 to 312.7 K observed at all the heating rates were extremely near room temperature. The observed T_gs of PLA2000 microspheres containing NA, which was higher than that of PLA2000 bulk at all heating rates, ranged from 318.8 to 325.0 K. Since glass transition is a kind of relaxation phenomenon, the dependency of relaxation time on temperature is generally considered to obey Arrheniuse's law.[19] Therefore, the apparent activation energy of transition can be obtained according to the method described by Barton.[20] A linear relationship was observed when the logarithm of the heating rate was plotted against the reciprocal of glass transition temperatures ($1/T_g$) as shown in Fig. 2. The apparent activation energy of the glass transition of PLA2000 bulk and PLA2000 microspheres containing NA were calculated to be 86.3 and 99.3 kcal/mol, respectively, indicating that the dependency of glass transition in PLA2000 microspheres containing NA on temperature tended to be larger than that in PLA2000 bulk. Since the measured T_g value using DSC was changed by the heating rate, further measurements of T_g in DSC were performed with a heating rate of 10 K/min as a typical heating rate.[9]

The DSC curves of PLA2000 microspheres with a different content of NA are shown in Fig. 3A, and the relationship between the T_gs of PLA 2000 microspheres and the content of NA in microspheres is shown in Fig. 3B. The T_g of PLA2000 microspheres increased linearly to about 320 K with an increase in the content of NA. However, this increasing tendency of T_g was found to stop with an NA content of greater than 6%.

Change in T_g of PLA2000 in the Presence of NA and Other Drugs Changes in T_g of PLA2000 bulk in the presence of some drugs, especially in a physical mixture, were examined in comparison to PLA2000 microspheres containing NA (Table I). If a drug is dissolved in PLA, the T_g of PLA will be shifted to a lower temperature.[13] There was a remarkable decrease in the T_g of PLA2000 in the presence of salicylic acid and *p*-hydroxy methyl benzoate, as shown in Table I. Nicotinic acid and cholesterol decreased the T_g of PLA2000 bulk slightly. The T_g of PLA2000

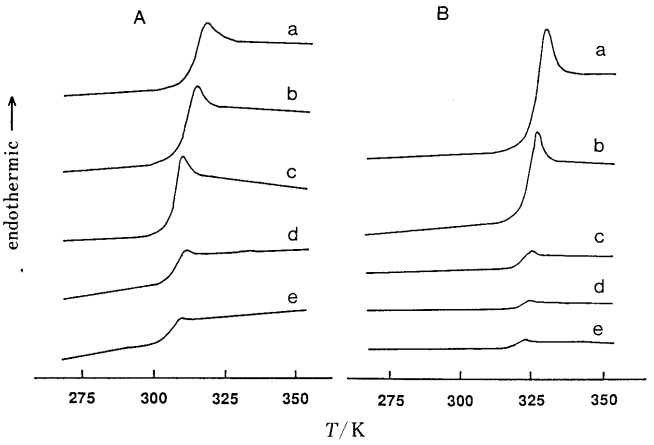

Fig. 1. Influence of Heating Rate on the DSC Curves of PLA2000 Bulk and PLA2000 Microspheres Containing NA (Content 6.0%)

A, PLA2000 bulk; B, PLA2000 microspheres containing NA. Heating rate; a, 50; b, 20; c, 10; d, 5; e, 2 K/min.

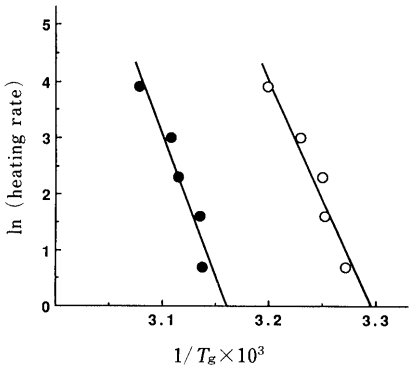

Fig. 2. Plots of Logarithm of Heating Rate *versus* Reciprocal of Glass Transition Temperature of PLA2000 Bulk and PLA2000 Microspheres Containing NA

○, PLA2000 bulk; ●, PLA2000 microspheres containing NA.

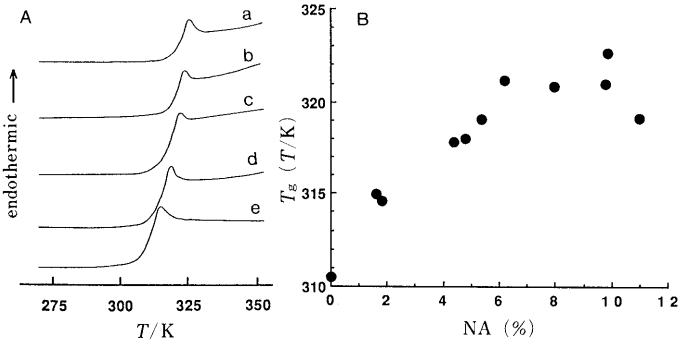

Fig. 3A. DSC Curves of PLA2000 Microspheres Containing Various Amounts of NA

NA content; a, 9.8; b, 6.2; c, 4.4; d, 1.8; e, 0%.

3B. Relationship between T_g (Mid-Point) and the Content of NA in PLA2000 Microspheres

TABLE I. Glass Transition Temperatures of PLA2000 in the Presence of NA and Other Drugs

Drugs in physical mixture[a]	T_g (K)[b]	
	Onset	Midpoint
PLA2000 bulk	304.7	307.8
NA (neurotensin analogue)	304.4	307.4
Insulin bovine	305.8	308.8
Nicotinic acid	302.9	305.9
Salicylic acid	292.9	297.0
p-Hydroxy methyl benzoate	285.7	294.7
Cholesterol	300.5	303.7
PLA 2000 microsphere		
Drug-free	308.0	310.5
NA content 8.0%	317.0	320.9

a) drug–PLA2000 bulk = 1 : 10 (w/w). *b*) The mid- and onset point T_gs were obtained in the second heating run with a heating rate of 10 K/min.

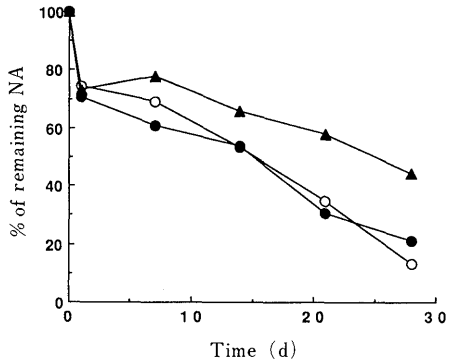

Fig. 4. Release Profiles of NA from PLA2000 Microspheres after Storage at Different Temperatures for 1 Month

○, 4 °C; ●, 40 °C; ▲, 45 °C.

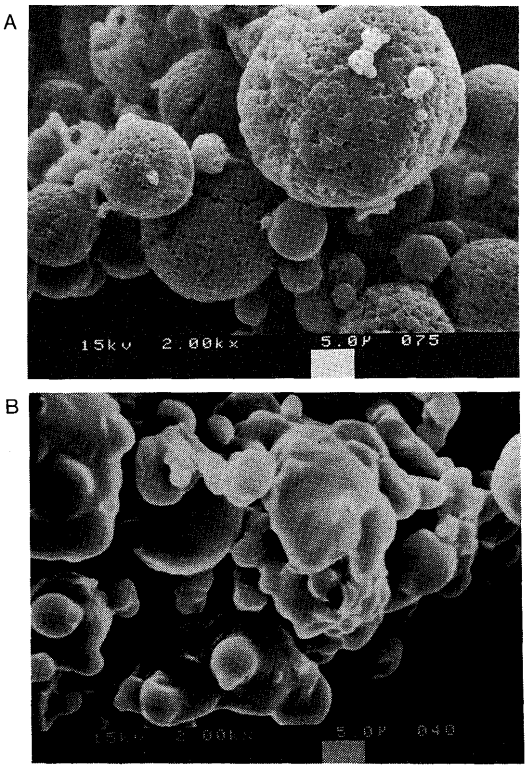

Fig. 5. Scanning Electron Photomicrographs of PLA Microspheres Before and After Storage at 45 °C for 1 Month

A) before storage; B) after storage at 45 °C.

remained almost unchanged in the presence of NA and insulin. No increase in the T_g of PLA2000 was observed in PLA2000–drug physical mixtures, as was the case with PLA2000 microspheres containing NA. The T_g of drug-free microspheres prepared with PLA2000 alone was slightly higher than that of PLA2000 bulk. The T_g of PLA2000 microspheres containing NA (8% content) further increased to higher than 10 K compared with that of drug-free PLA2000 microspheres. T_g is generally defined as the temperature at which the segment motion of a polymer chain can start. Both PLA and NA were dissolved completely in a mixed solvent (CH_2Cl_2–EtOH) in the preparation of microspheres, in which it is possible that NA would be molecularly dispersed throughout the PLA matrix. The increase of T_g in PLA2000 microspheres containing NA could suggest that the start of the segment motion of a PLA2000 polymer can be delayed by the interaction between cationic NA and an anionic PLA polymer.

Change in Release Profiles of NA from PLA2000 Microspheres after Storage at Different Temperatures Figure 4 shows the release profiles of NA from PLA2000 microspheres (T_g: 47.7 °C) in a buffer solution at pH 7.4 after storage at different temperatures. Almost the same release profiles of NA were found after storage at 4 °C and 40 °C for 1 month. However, the release rate of NA after the initial rapid release became slow after the storage near T_g (45 °C) for 1 month. It has been reported that the diffusiveness of a drug in a polymer matrix increases due to the large increase in polymer chain mobility which occurs near T_g.[13] If it should be the main effect on PLA2000 microspheres containing NA during the storage, the release rate of NA from the microspheres would become fast after the storage near T_g. PLA polymer generally tends to soften and adhere under conditions near T_g. The change in release profiles of NA after storage at 45 °C may be attributed mainly to the decrease in suface area caused by the formation of agglomerates of PLA2000 microspheres as shown in Fig. 5.

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