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Physicochemical properties of high-molecular-weight poly(α,β-malic acid) synthesized by direct polycondensation

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Summary

We studied high–molecular-weight α,β-PMA synthesized by polycondensation in order to find possible applications for biomaterials. Its solubility in different solvents, its hydrolysis and its acidity were also examined. The α,β-PMA molecular weight increased markedly up to 20 h and then decreased showing that the molecular weights for synthesized α,β-PMA depends on the reaction time. We prepared high-yield α,β-PMA with a molecular weight of 3600 by direct polycondensation using tin(II) chloride as a catalyst at 130°C for 20 hours and concluded that our method is suitable to synthesize higher molecular weight compounds of α,β-PMA.

Introduction

Poly(α-hydroxy acid) is attracting attention for use as a biodegradable polymer. Poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers are commercially available for medical applications [1-3] such as scaffolds and sutures. Poly(malic acid) (PMA), known as the L-malic acid polymer contained in grapes and apples,[4] is water-soluble, biodegradable, and bioabsorbable,[5, 6] and has two remarkable advantages in medical applications: its easy metabolization in vivo through a tricarboxylic acid (TCA) cycle [7] and the presence of a modifiable pendant carboxylic group on the molecule that binds easily to other functional groups, leading to the introduction of drugs into its polymeric chain. It is thus important to develop an easy, high-yield way of producing this functional polyester.

Whereas most PMA compounds are synthetic, though PMA is also available from natural resources [8-12]. PMA is synthesized 2 ways: ring-opening polymerization and direct polycondensation. Many researchers have synthesized poly(β-malic acid benzyl ester) by polymerizing benzyl malolactonate through ring-opening polymerization [6, 13-17]. This polymer is difficult to synthesize, because of several steps involved in the reaction cycle and much time required for repeated purification.

In contrast, direct polycondensation is possible in a 1-step reaction. It also has an advantage in "green" chemistry as this method uses fewer organic solvents compared

to other polymerization such as ring-opening polymerization, with water being its only by-product. This makes this method attractive for environmental conservation. Ohtani et al. synthesized α,β-PMA with a molecular weight of 1900 by directly polycondensation of L-malic acid and detailed its physicochemical properties [12]. Such a synthesis is not yet practical because the molecular weight of the synthesized polymer is too low for medical applications and its properties such as yield are not sufficient enough for industrial use. More basic studies are needed to establish a way of synthesizing high-molecular-weight PMA. We previously investigated the reaction conditions of α,β-PMA (solvents, catalysts and temperatures) by direct polycondensation (manuscript in preparation). These results suggested possible applications of PMA for drug delivery systems (DDS) and leading to the two advantages mentioned above [18, 19].

Here, we present some new information on α,β-PMA synthesis by direct polycondensation and detail some physicochemical properties such as acidity, hydrolysis rate, and solubility in different solvents.

Experimental

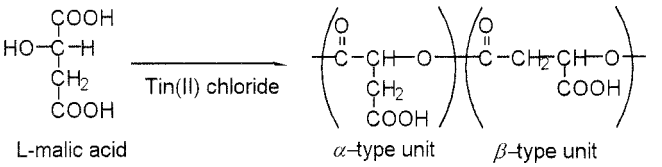
Materials

L-malic acid (Aldrich, Milwaukee, USA), citric acid (Wako Chemicals, Osaka, Japan), diethyl ether (Wako Chemicals), petroleum ether (Kanto Chemicals, Tokyo, Japan), tetrahydrofuran (THF) (Wako Chemicals), tin(II) chloride (Wako Chemicals), acetone-d_6 (Cambridge Isotope Laboratories, Andover, USA), and deuterium oxide (Aldrich) were purchased commercially and used without further purification, as were other reagents.

Methods

^{1}H NMR spectra were recorded on a Bruker ARX300 using TMS as the internal reference. The molecular weight of the obtained PMA was estimated in a citric acid 0.1M THF solution by gel permeation chromatography (GPC) (apparatus: Tohso HLC-8220GPC, column: TSK Gel SuperHz2000 and SuperHz4000, standard: polystyrenes). Polymer glass transition temperature (T_g) was measured by differential scanning calorimetry (DSC) using a Rigaku DSC8240 in air and heating at 10°C/min.

Synthesis of α,β-PMA



(1)

A mixture of 0.2 mol L-malic acid (28 g) and 0.36 wt% Tin(II) chloride (0.1 g) was stirred at 110-140°C for 20 h in a vacuum under a N_2 stream (1 mmHg). The resultant polyester was dissolved in 150 ml of THF solution and precipitated in a mixture of 1,000 ml of diethyl ether and 1,000 ml of petroleum ether. ^{1}H NMR (acetone-d_6,

δppm): 5.5-5.4(*d*, 1 H, methylidine unit), 3.0-2.9(*d*, 2 H, methylene unit) In regard to the obtained polymer, although not described as a ^{1}H NMR result since the peak was slightly seen to be in the neighborhood of 6.6 ppm-7.0 ppm, some terminal groups are considered to be double bonds. As well, we conclude that these polymers have random sequences of both α-and β-type units in the equal ratio as reported by Ohtani et al.[12] ; T_g=1°C (reaction time for 20 h).

Results and discussion

Effects of reaction time

Table 1 shows the features of α,β-PMAs synthesized at 130°C such as weight average molecular weight (M_w), number average molecular weight (M_n), molecular weight distribution (M_w/M_n), and yield (amount of recovery). M_w was found between 1700 and 3600, M_n between 1100 and 2000, M_w/M_n between 1.5 and 1.8, and the yield between 71 and 99%.

Table 1. Direct polycondensation of L-malic acid for synthesizing α,β-PMA

Reaction time (h)	M_w	M_n	M_w/M_n	Yield (%)
5	1700	1100	1.5	>99
10	3200	1800	1.8	89
15	3300	1800	1.8	75
20[a]	3600	2000	1.8	75
25	2800	1600	1.8	71

Molecular weights were determined by GPC (column: TSK Gel SuperHz4000 + SuperHz2000 + SuperHz2000) in citric acid 0.1M THF solution using polystyrene as the standard. [a] Taken from our previous study (manuscript in preparation).

Fig. 1 shows the relationship between M_w and reaction time based on the results in Table 1. M_w increased markedly to 3600 up to 20 h, then decreased. In this reaction system, when the reaction time exceeded 20 h, the already formed polymers decomposed, suggesting that the molecular weight of α,β-PMA did not increase even if the reaction time exceeded 20 h. Fig. 2 shows the relationship between the yield and the reaction time based on Table 1. In the early stages of the reaction, although the molecular weight was low, the yield was high. As the reaction progressed over time and the molecular weight became high, the yield gradually decreased, indicating that the side reactions, such as, depolymerization and intramolecular dehydration, for polycondensation reaction of L-malic acid increase with the reaction time.

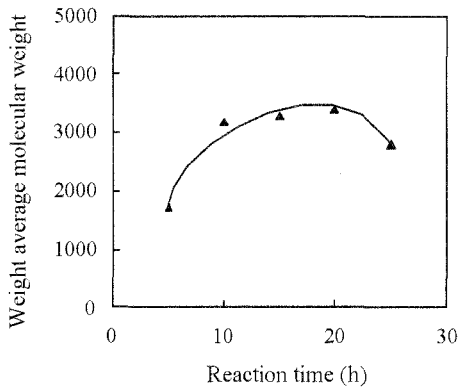


Fig. 1. Relationship between reaction time and molecular weight. The reaction temperature was 130°C. The molecular weight was estimated by GPC.

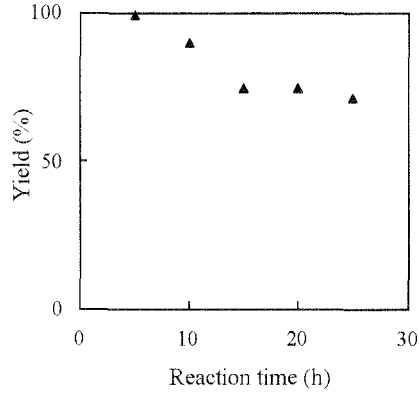


Fig. 2. Relationship between reaction time and yield. The reaction temperature was 130°C. The yield is equal to the amount of recovery.

Chromatograms of α,β-PMAs

Chromatograms of α,β-PMA synthesized at different reaction times (5, 10, 15, 20 and 25 h) are shown in Fig. 3. Focusing on the main peak, we can observe that the peak at 10 h or later showed faster retention time than that at 5 h. The rapid increase in the molecular weight of the main α,β-PMA fraction occurred at 5-10 h.

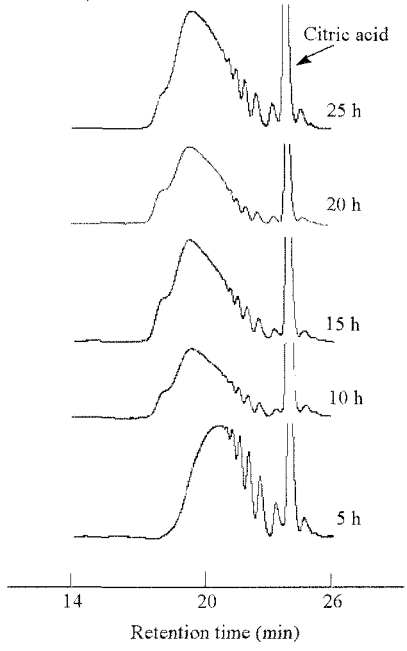


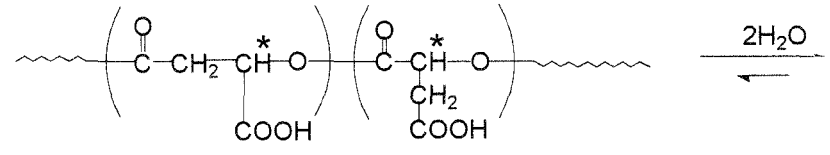
Figure 3. Chromatograms of synthesized α,β-PMA (column: TSK Gel SuperHz4000 + SuperHz2000 + SuperHz2000). The eluent contained citric acid.

In a previous study, we found that α,β-PMA synthesis depended on the reaction temperature (manuscript in preparation). We also found that the reaction depended on the reaction time.

Acidity test

The pH of the 0.1M α,β-PMA solution was 2.6, slightly higher than the 2.2 reported by Ohtani et al. [17] but lower than the 4.2, reported by Braud et al. [20]. Regarding to the application of α,β-PMA to biomaterials, the question remains on how to increase the pH to neutral.

Hydrolysis of α,β-PMA



(2)

α,β-PMA was dissolved in a 1% deuterium oxide solution. We studied the hydrolysis of this molecule at 37°C using ^{1}H NMR.

It is important to clarify hydrolysis at this temperature (37°C) because of in vivo use. When the ester compound of the polymer main chain was hydrolyzed, the methylidine signal shifts from 5.5 ppm (*) to 4.45 ppm (**) as the main chain ester was decomposed. The hydrolysis rate is estimated from the point at which it becomes an alcoholic hydroxyl group and the groups increase using the following formula:

$$\frac{[5.5\text{ ppm ratio}]}{[5.5\text{ ppm ratio}] - [4.45\text{ ppm ratio}]} \times 100 = [\text{Ester unit}]/[\text{Total malic acid unit}]\ \% \qquad (3)$$

The half-life of the ester compound was 15 days, and was 80% hydrolyzed after 40 days (Fig. 4). This hydrolysis was faster than the ones of commercially available PLA [21-30], PGA [23], and their copolymers [23,26,31,32] or bacterial polyesters, such as poly(hydroxybutyrate) (PHB) and poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) [33,34]. To make its use more attractive, α,β-PMA should be degradable in about one month.

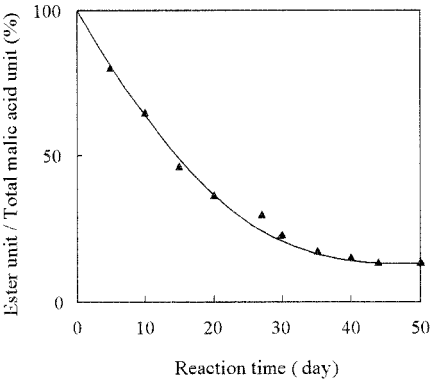


Fig. 4. Hydrolysis of α,β-PMA at 37°C in deuterium oxide.

Solubility test

α,β-PMA was dissolved in solvents at a concentration of 1% to determine its solubility. Interestingly, α,β-PMA was not dissolved in the halogen solvents in which polyesters are generally dissolved. Its easy reaction and fabrication suggest that this compound may be applicable in many fields in addition to biomedical materials.

Table 2. Solubility of α,β-PMA

Solvent	Incubation time	
	8 h	3 days
Hexane	--	NC
Toluene	--	-
m-cresol	--	NC
1,3-dimethoxybenzene	--	NC
1,3-dichlorobenzene	--	NC
Diphenyl ether	--	NC
Diethyl ether	+	++
Ethyl acetate	+	NC
Chloroform	--	NC
Methylene chloride	--	NC
Ethylene chloride	--	NC
THF	++	NC
1,4-dioxane	++	NC
Acetone	++	NC
Dimethylformamide	++	NC
Dimethyl sulfoxide	++	NC
Acetonitrile	--	NC
Ethanol	++	NC
Methanol	++	NC
Water	++	NC

++: completely dissolved, +: partially dissolved, -: swelled, --: insoluble, NC: no change.

Conclusions

We synthesized high-yield and high-molecular-weight α,β-PMA, determined the acidity and hydrolysis rate of the α,β-PMA solution and its solubility in different solvents. Given its biodegradable, bioabsorbable properties, synthesized α,β-PMA is applicable in such uses as DDSs and the fabrication of scaffolds.

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