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Enzyme-catalyzed syntheses of poly(1,6-hexanediyl isophthalate) and poly(1,6-hexanediyl terephthalate) in organic medium

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Summary

The enzyme-catalyzed synthesis of poly(1,6-hexanediyl terephthalate) and poly(1,6-hexanediyl isophthalate) is described. An unsuccessfull attempt to synthesize poly(1,6-hexanediyl *o*-phthalate) is also presented and the reactivity of those three monomers is discussed according to the enzymatic nature of the catalyst. The crude polyesters are characterized by ^{1}H and ^{13}C NMR spectroscopy and by differential scanning calorimetry. Macrocycles are isolated from poly(1,6-hexanediyl isophthalate) and characterized by steric exclusion chromatography and by mass spectrometry. The number-average molar masses and the thermal properties of the crude, linear and cyclic polyesters are also reported.

Introduction

Enzyme-catalyzed polycondensation has been extensively studied during the last decade and in particular, enzymatic polyester synthesis has received much attention from many searchers. All reactional systems corresponding to AA+BB or AB+AB-type syntheses have been reviewed using lipases instead of chemical catalysts [1].

Thus, mainly aliphatic polyesters are obtained from AB+AB-type systems using hydroxyacids [2-5], hydroxyesters [6-9] and lactones [8, 10-12] as monomer. However, Knani *et al.* [9] successfully synthesized a polyester with phenyl side groups ; they enzymatically prepared a lateral-substituted aliphatic copolyester from methyl 6-phenyl-6-hydroxyhexanoate.

On the other hand, a wide variety of polyesters is obtained from AA+BB-type syntheses. Polymers are prepared from diols-diacids [13-14], diols-diesters [15-25] or anhydrides-diols [26] and numerous polyesters with various constitutive units have been synthesized according to the monomer structures, *e.g.*, epoxyde [16-17], double bond [16, 18-24], aromatic ring [19]. Aromatic polymers were obtained from aromatic diols [19, 27] and in particular, bisphenol A was used as a monomer in the enzymatic synthesis of polycarbonates [27]. In this latter case, only oligomers were obtained.

Despite the large number of monomers and enzymes which were tested, the enzyme-catalyzed synthesis of aromatic polyesters derived from phthalic monomers has never been reported in literature up to now.

In this article, we describe for the first time the synthesis of poly(1,6-hexanediyl isophthalate) and poly(1,6-hexanediyl terephthalate) obtained by enzyme catalysis in organic medium. An attempt to poly(1,6-hexanediyl *o*-phthalate) synthesis is also described. The samples were characterized by steric exclusion chromatography (SEC), ^{1}H and ^{13}C NMR spectroscopy and differential scanning calorimetry (DSC). Cyclic oligomers were characterized by mass spectrometry.

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Experimental part

Materials

Dimethyl *o*-phthalate, dimethyl isophthalate, dimethyl terephthalate and 1,6-hexanediol were used without further purification. Novozyme is a two-components enzymatic catalyst. Both lipases (E.C. 3.1.1.3) from *Candida antartica* were immobilized on a macroporous acrylic resin. Its activity is 5500 PLU/g. One PLU corresponds to the synthesis of one μmole of propyl laurate from lauric acid and 1-propanol per min at 60 °C. Toluene was used as solvent and stored over molecular sieves (5 Å).

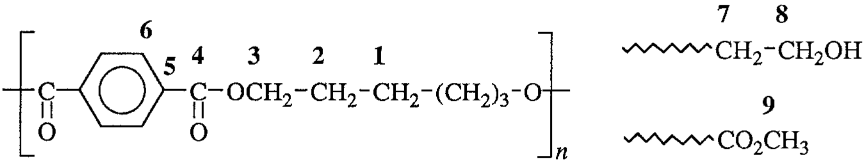
Polyester syntheses and fractionation

1,6-hexanediol (0.075 mole), diester (0.075 mol.), toluene (150 mL) and the enzymatic preparation (5 g) were introduced in a thermostated double jacket reactor. The reactor was surmounted by a thermostated tube. Methanol elimination was favored by nitrogen bubbling (0.2 L/min). The removed methanol and solvent were collected in a flask at the outlet of the tube. The volume of the solution was held constant. After reaction, the catalyst was removed by filtration and the solvent was evaporated under reduced pressure.

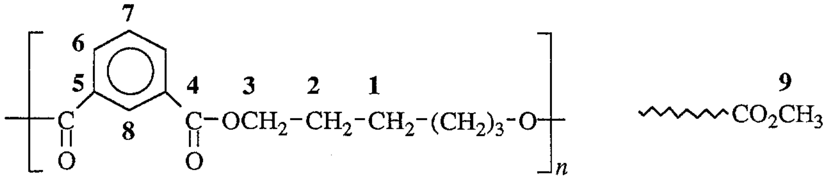
The crude poly(1,6-hexanediyl isophthalate) (5 g) was mixed with methanol (150 mL) for 6 hours at 50 °C. After cooling, the insoluble fraction was removed by filtration and methanol was eliminated by evaporation under reduced pressure. Both fractions were dried under vacuum at room temperature.

Measurements

^1H and ^{13}C NMR spectra were recorded on Bruker spectrometers in $CDCl_3$ solution [^1H NMR, 300 MHz, Reference : δ ($CHCl_3$) = 7.26 ppm. ^{13}C NMR, 75.5 or 62.9 MHz, Reference : δ ($CHCl_3$) = 77.0 ppm].



δ = 1.53 (m; H^1), 1.80 (m; H^2), 3.64 (t; H^8), 3.92 (s; H^9), 4.32 (t; H^3), 8.06 (s; H^6) ppm; 25.4 (C^1), 28.3 (C^2), 32.3 (C^7), 52.1 (C^9), 62.2 (C^8), 65.0 (C^3), 129.2 (C^6), 133.8 (C^5), 165.4 (C^4) ppm for **1**.



δ = 1.52 (m; H^1), 1.80 (m; H^2), 3.93 (s; H^9), 4.34 (t; H^3), 7.50 (t; H^7), 8.19 (d; H^6), 8.65 (s; H^8) ppm ; 25.5 (C^1), 28.4 (C^2), 65.0 (C^3), 128.3 (C^7), 130.4 (C^5), 130.6 (C^8), 133.4 (C^6), 165.5 (C^4) ppm for **2**.

I_3 and $I_{arom.}$ are the relative intensities of H^3 and of the whole aromatic protons, respectively. $I_3/2$ and $I_{arom.}/2$ are proportional to the number of ester functions formed $[-CO_2CH_2-]_t$ and to the initial number of methyl ester functions $[-CO_2CH_3]_0$ so that the extent of reaction p can be calculated according to the following relation :

$$p = \frac{[-CO_2CH_2-]_t}{[-CO_2CH_3]_0} = \frac{I_3}{2} x \frac{2}{I_{arom.}} = \frac{I_3}{I_{arom.}}$$

Number-average molar masses were determined using Waters Model 410 steric exclusion chromatography (SEC) at room temperature with a differential refractometer. Tetrahydrofuran (THF) served as mobile phase with a flow rate of 1 mL/min using μStyragel columns (10^4, 10^3, 500 Å) and 0.5 mL/min using PLgel columns (50, 100, 500 Å). The average molar masses were determined using a calibrated curve with polystyrene standards.

Thermograms were recorded on a DuPont 9900 DSC 910 instrument. Samples were heated at 20°C/min and cooled at 5°C/min under nitrogen atmosphere. Glass transition temperature was determined at the inflexion point of the DSC curve.

Mass spectrum was obtained by using MALDI TOF Laser (VSL 337 ND Laser Science Inc.), $\lambda = 337$ nm, 3 ns pulse, 200 μJ/pulse. 1 mL of a solution containing the sample and the matrix (5-chlorosalicylic acid) dissolved in THF was deposited onto a polished stainless steel substrate (5 mm in diameter) and allowed to dry in air. **2b** : *m/z* (M_yH^+, M_yNa^+, M_yK^+, respectively) = 498, 522, not considered (y=2), 747, 769, 785 (y=3), 996, 1018, 1035 (y=4), 1244, 1267, 1284 (y=5), 1492, 1515, 1533 (y=6), not considered, 1763, not considered (y=7).

Results and discussion

Polyester syntheses and characterization of the crude samples

As we previously showed [23, 24], Novozyme has been successfully used as catalyst for the preparation of aliphatic or unsaturated polyesters from diols and diesters. Thus, the specificity of this powerfull catalyst seems to be very broad and we take avantage of it in order to synthesize aromatic polyesters from 1,6-hexanediol and phthalic diesters. We attempted to prepare poly(alkylene phthalate)s from the dimethyl esters of *o*-, *m*- and *p*-phthalic acids (dimethyl phthalate, dimethyl isophthalate and dimethyl terephthalate, respectively).

Dimethyl phthalate and 1,6-hexanediol did not react in toluene at 60°C neither in the presence of the enzymatic preparation since no polymer was obtained after 9 days. This result is confirmed by ^1H NMR analysis ; only unchanged monomers remain after removing the enzyme by filtration and evaporating toluene under reduced pressure. According to the experimental conditions, Novozyme does not catalyze the polytransesterification of dimethyl phthalate with 1,6-hexanediol.

This lack of reactivity does not result from experimental parameters as they were successfully applied to the above-mentioned syntheses [23, 24]. On the other hand, dimethyl isophthalate and dimethyl terephthalate were transesterified by 1,6-hexanediol as it will be shown in the following lines. The relative *ortho* position of methyl ester groups is therefore responsible of this lack of reactivity ; it probably hinders the formation of the enzyme-substrate complex which corresponds to the first step of the enzymatic mechanism.

Crude poly(1,6-hexanediyl terephthalate) **1** and poly(1,6-hexanediyl isophthalate) **2** were obtained with different yields according to their respective solubility in toluene.

In the case of **1**, the reaction was stopped after 3 days because the oligomers formed in toluene precipitated making the stirring inefficient. The insoluble and soluble fractions were recovered by solubilization in chloroform and analyzed after removing the supported enzyme by filtration and evaporating the solvents.

The ^1H NMR spectrum of **1** is shown in Fig. 1a. The presence of the triplet (H^3, 4.34 ppm) clearly indicates that the enzymatic polytransesterification took place. As expected however, the rather high intensities of the singlet (H^9, 3.92 ppm) and of the triplet (H^8, 3.64 ppm) corresponding to methyl ester and hydroxy end groups respectively, show that the conversion is not very high. The extent of reaction was calculated according the relation proposed in the experimental part and its value is 0.93. Thus, the number-average polycondensation degree of **1** is equal to 14 if no cycle is present in the crude sample. This latter point is discussed in the molecular weight determination part.

The preparation of 2 was performed with a good achievement as shown by its ^1H NMR spectrum (Fig. 1b) where the singlet (H^9, 3.93 ppm) corresponding to the methyl ester end groups has a very weak intensity. Furthermore, no signal corresponding to the hydroxy end groups is observed. The extent of reaction is more than 0.99 according to the same calculation done previously.

Characterization of **2b** *by MS and by SEC*

We previously showed that aliphatic or unsaturated polyester synthesis by enzyme-catalyzed polycondensation is accompagnied by cyclization [23, 24]. Linear polymer **2a** and macrolactones **2b** result from the polytransesterification of dimethyl isophthalate with 1,6-hexanediol in toluene at 60°C using Novozyme as catalyst. Macrolactones **2b** were extracted from the crude sample **2** using methanol (see experimental part for conditions) and the unextracted fraction corresponds to **2a**.

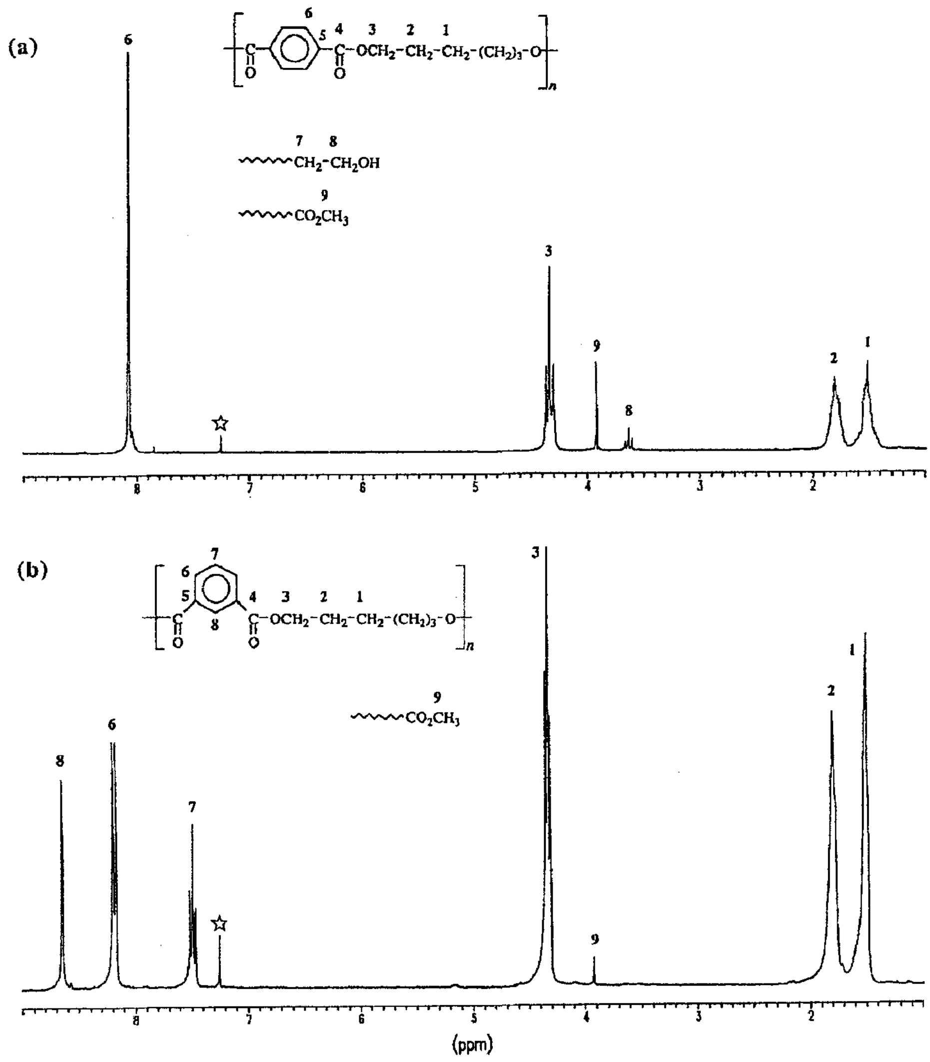


Fig. 1. *^1H NMR spectra of* **1** *(a) and* **2** *(b) in $CDCl_3$ solution. Attributions are given on the figure and the chemical shifts are reported in the experimental part.* (☆) *$CHCl_3$.*

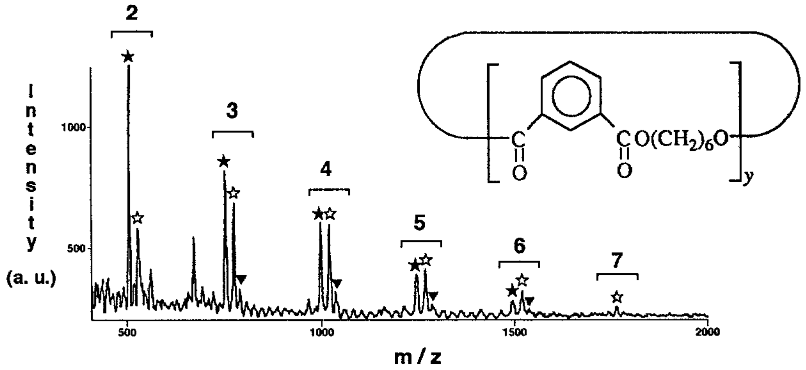


Fig. 2. *Positive ion MALDI-TOF mass spectrum of* **2b** *(matrix : 5-chlorosalicylic acid; 200 μJ/pulse). y values are assigned above each signal.* (★) *M_yH^+ (or M_y^+),* (☆) *M_yNa^+,* (▼) *M_yK^+. The detailed conditions of the analysis are described in the experimental part.*

Their mass fractions are 91 % and 9 %, respectively. The mass spectrum of **2b** is shown in Fig. 2. Each signal is made up of three peaks relative to M_yH^+, M_yNa^+ and M_yK^+ corresponding to the same cyclic species. Thus, macrocycles with DP ranging from 4 to 14 (y = 2 to 7) are detected. It was not possible to identify the cyclic dimer (y = 1, DP = 2). As corroborated by SEC analysis (Fig. 3), this latter was not formed. On the other hand, macrocycles with DP ranging from 4 to 14 were also characterized by SEC and the good resolution of each species makes it an internal calibration possible (see Tab. 1).

$\overline{M}_n$ determination of **1**, **2**, **2a** *and* **2b**

The low extent of reaction (0.93) obtained during the preparation of **1** explains its relatively low number-average molar mass (Tab. 1). Insolubility of the longest chains in toluene limits their own growth ; this can explain why the polydispersity index is below the value calculated from polycondensation (1.93). The crude poly(1,6-hexanediyl isophthalate) **2** is characterized by a very broad molecular weight distribution. As it is shown in Fig. 3, the sample contains macrolactones observed on the high elution time side and linear chains on the low one.

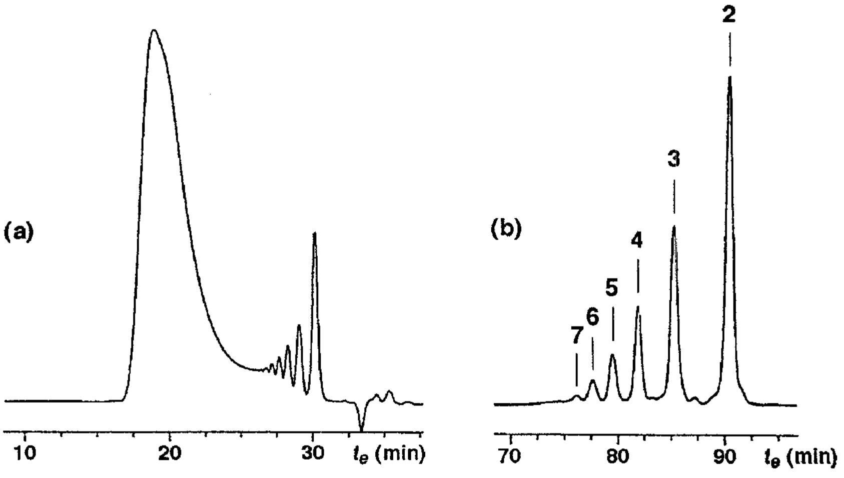


Fig. 3. *Steric exclusion chromatograms of* **2** *(a) and* **2b** *(b). y values are assigned above each peak. See experimental part for analysis conditions.*

Tab. 1. $\overline{M}_n$, $\overline{M}_w$ and I_p of **1**, **2**, **2a** and **2b** Determined by SEC [a]

Sample No.	Yield (%)	$\overline{M}_n$ (eq.PS)	$\overline{M}_w$ (eq.PS)	I_p
1	100	1860	2790	1.5
2	100	4100	29900	7.3
2a	91	6100	31700	5.2
2b	9	430 [640 [b]]	600	1.4 [1.2 [b]]

[a] Determined by SEC in THF using standard poly(styrene) for calibration.

[b] $\overline{M}_n$ (g/mol) and I_p were determined using an internal calibration based on the assumption that only cycles are present (see also Fig. 3).

The number-average molar mass of the linear chains **2a** is relatively high which obviously results from the fractionation of the crude sample **2** as the macrocycles with low molecular weights remained in the methanol solution. The absolute number-average molar mass of the macrocycles **2b** was obtained from an internal calibration (see exp. part) and is relatively low. This confirmed that the binodal distribution of **2** is due to the presence of linear chains with high molecular weights and of macrocycles with low molecular weights.

Thermal analyses

The thermal properties of **1**, **2**, **2a** and **2b** were compared by DSC (Tab. 2).

The melting temperature (T_m) of the enzymatically prepared poly(1,6-hexanediyl terephthalate) **1** was observed at 138°C. Taking account its relatively high melting enthalpy (ΔH_m) and its number-average molar mass, it is crystalline. Moreover, glass temperature (T_g) was not observed.

The thermal properties of **2** can be discussed taking into account that it corresponds to a mixture of linear chains **2a** and macrocycles **2b** (91 and 9 % weight, respectively). In fact, **2** can be regarded as linear chains containing about 9 % of macrocyclic impurities, thus, T_m of **2** is lower than that of **2a**. On the other hand, T_g and ΔH_m values of **2a** are very close to those of **2**.

DSC analysis of macrocycles **2b** exhibits three endotherms. The endothermic variation at 68 °C is attributed to a crystal-crystal transition as it is often observed in cyclic polyester thermograms while the endotherms at 175 and 193 °C correspond to melting ; T_g was not observed. According to these data, **2b** is in fact a mixture of macrocycles whose thermal properties depend on their size.

Tab. 2. Thermal Properties of **1**, **2**, **2a** and **2b** Determined by DSC [a]

Sample No.	T_g (°C) [b]	T_m (°C) [c]	ΔH_m (J.g^{-1})	T_k (°C)
1	not observed	138	65	-
2	13	88	36	-
2a	17	104	41	-
2b	not observed	175 and 193	not determined	68

[a] Glass, melting and crystal-crystal transition temperatures (T_g, T_m, T_k) and melting enthalpy (ΔH_m) were measured with a differential scanning calorimeter at heating and cooling rates of 20°C/min and 5°C/min respectively.

[b] First heating (20°C/min).

[c] Second heating (20°C/min) after cooling at 5°C/min.

Conclusion

The enzyme-catalyzed preparations of poly(1,6-hexanediyl terephthalate) and poly(1,6-hexanediyl isophthalate) were successfully achieved in toluene at 60°C using an enzymatic system called Novozyme. However, these semi-aromatic polyesters have relatively low molar masses ; this is due to precipitation during the poly(1,6-hexanediyl terephthalate) preparation and to macrolactones formation during the poly(1,6-hexanediyl isophthalate) synthesis. For the first time, it was possible to synthesize poly(alkylene phthalates) from their monomers and by using an enzymatic process ; however, this enzyme-catalyzed synthesis does not work when the monomer is *o*-phthalate. This raises the problem of the enzyme specifity since it recognizes only the methyl ester functions in meta and para positions.

On the other hand, even though that the relatively low molar masses we observed, the polyesters exhibit crystallinity and in particular, the macrocyclic poly(isophthalate)s with DP ranging from 4 to 14 exhibit very high melting temperatures. We can assume that each macrocycle with a well determined size has specific thermal properties. It would be interesting to separate these different species.

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