Lipase-Catalyzed Polyester Synthesis in Organic Medium. Study of Ring−Chain Equilibrium

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ABSTRACT: Enzyme-catalyzed synthesis of aliphatic, unsaturated, and semiaromatic polyesters from diesters and diols was carried out in toluene at different temperatures in the presence of supported lipases from *Candida antarctica*. Whatever the monomer structure, rings were formed concurrently with linear chains except when dimethyl fumarate was used. The yield of cyclics depends on several parameters such as the monomer structure and the initial concentration of the reactive functions and strongly influences the average molar masses of unfractionated samples. Qualitatively, this is a general feature of the ring−chain equilibrium. Once the existence of this equilibrium was shown, the molar cyclization equilibrium constants (K) for aliphatic, unsaturated, and semiaromatic polyesters were determined, and it would appear that the molar distribution of the ringed species obeys the Jacobson−Stockmayer equation ($\ln K = \ln B - {}^5/_2 \ln n$, where B and n represent a constant and the number of monomer units of each type in the ring, respectively) except when n is equal to 1. The so-called *cyclic dimer exception* is discussed in relation to the catalyst's enzymic nature.

Introduction

The use of enzyme catalysis for polyester synthesis in an organic medium has experienced important developments over the past few years, and many polymer characteristics, such as average molar mass and polydispersity index, are now largely available.[1] Concerning the average molar masses, the literature gives scattered results due to the wide variety of the experimental reaction conditions, *i.e.*, monomer structure, enzyme nature, initial concentration of the reactive functions, solvent hydrophobicity, temperature, etc. In spite of this reservation, two clear trends emerge from all of those works, depending on the recovery procedure used for the enzymatically prepared polyester samples: (*i*) Polyester samples recovered from the reaction medium by precipitation (often by pouring into a large excess of methanol) present relatively high molecular weights, which is consistent with the fractionation connected to the recovery by precipitation. (*ii*) Those recovered by solvent evaporation, the so-called unfractionated samples, have rather low molecular weights and often contain cyclic species when the monomer structure and the flexibility of the resulting chain allow cyclization.

For instance, Morrow and Wallace[2,3] obtained cycle-free polyester samples by pouring the reaction medium into anhydrous methanol ($\bar{M}_n = 8200$, $\bar{M}_w/\bar{M}_n = 1.44$, yield = 89%). Linko *et al.*[4] prepared several poly(tetramethylene decanedioate) samples by polycondensation of bis(2,2,2-trifluoroethyl) decanedioate with 1,4-butanediol; the polyester with the highest $\overline{DP}_n$ (184) was prepared in diphenyl ether using *Mucor miehei* powder as catalyst and was obtained after precipitation by adding methanol to the reaction mixture.

Recently, we showed that the number-average molecular weights of poly(hexamethylene maleate)[5] and poly(hexamethylene isophthalate)[6] unfractionated samples are strongly influenced by the yield of cyclics so that cyclization was finally postulated as the main molecular weight limiting factor. Furthermore, Gutman *et al.*[7] studied the enzymic polymerization *vs* lactonization of ω-hydroxymethyl ester and, referring to Flory's works,[8] concluded in favor of the influence of the methylene group number in the monomer structure on the yield of cyclics.

The object of this article is to define the general features of linear polymer formation *vs* ring formation in lipase-catalyzed polyester synthesis carried out in organic solution. We extensively studied the synthesis of polyesters from dimethyl esters and diols in toluene in the presence of supported lipases from *Candida antarctica*. A qualitative approach to the ring−chain equilibrium has already been reported[9] and is briefly described in the first part of this article; in the second part, the experimental data obtained in the different studies (diol length, diester structure and temperature effect) are tested in the framework of the Jacobson−Stockmayer theory on intramolecular reaction in polycondensation.[10] Assumptions on the enzymic mechanism of cyclization are also proposed as the basis of further kinetic studies.

Experimental Section

^{1}H NMR Analysis. Proton (^{1}H) NMR spectra were recorded at 250 MHz on a Brucker spectrometer. Polymer samples were analyzed at 2% (w/v) in $CDCl_3$. ^{1}H NMR chemical shifts (δ) in parts per million (ppm) are referenced to $CDCl_3$ as the internal reference at 7.26 ppm. Experimental parameters were as follows: relaxation delay 2 s, 16K data points, acquisition time 2 s, and 16 transients. The extent of reaction p was defined as the ratio of the number of ester function formed (N) to the number of initial methyl ester functions (N_0). N is proportional to the integral values of signals relative to $-CO_2CH_2-$ in linear chains and rings (I_1). N_0 is proportional to those of signals relative to $-CH_2CO_2-$ (aliphatic polyester, I_2), $-CH{=}CH-CO_2-$ (unsaturated polyester, I_3) and aromatic protons (semiaromatic polyester, I_4). p was calculated according to $p = I_1/I_2$, $p = I_1/2I_3$, and $p = I_1/I_4$ for aliphatic, unsaturated, and semiaromatic polyester samples, respectively.

Qualitative and Quantitative SEC Analyses. Average molecular weights were measured by gel permeation chromatography (SEC) using a Waters Model 510 pump and Model 410 refractive index detector with 500, 10^3, 5×10^3, and 10^4 Å Ultrastyragel columns in series. Tetrahydrofuran (THF) was

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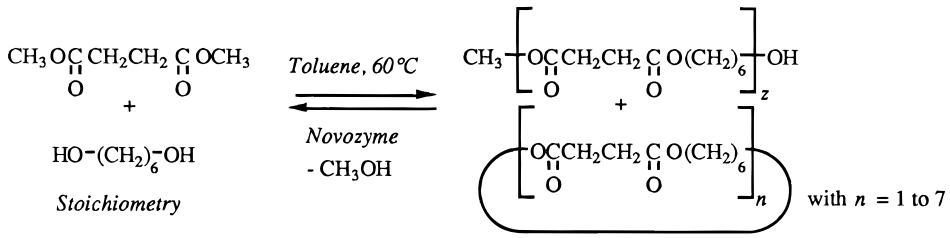
Scheme 1



used as an eluent at a flow rate of 1.0 mL/min. Polystyrene standards with low polydispersity were used to calibrate the studies when internal calibration was not possible. The yields of cyclics were calculated from SEC chromatograms of unfractionated samples recorded using a Waters Model 510 pump and LDC Analytical Model IV refractive index (RI) detector with 50, 100, and 500 Å Plgel columns in series and THF as an eluent at a flow rate of 0.5 mL/min. The sample concentration and injection volume were 1−1.5% (w/v) and 80 μL, respectively. The molecular weight and yield calculations were performed on a NEC Model Powermate SX Plus station using a Maxima 820 SEC data program at a recording rate and slice width of 1.0 point/s and 30 s, respectively. The RI responses of cyclics and linear chains were assumed to be the same and were assumed to be independent of the chain length.

Materials. Dimethyl succinate, dimethyl maleate, and dimethyl isophthalate (Aldrich) were used without further purification. 1,6-Hexanediol, 1,8-octanediol, 1,10-decanediol, and 1,12-dodecandiol (Aldrich) were dried on P_2O_5 over several hours. Toluene (SDS) was dried and stored on activated 5Å molecular sieves. Novozyme was purchased from Novobioindustry and is a two enzyme-containing preparation: both lipases from *C. antarctica* (E.C. 3.1.1.3) are immobilized on a macroporous acrylic resin.

Enzymatic Polyester Synthesis and Sample Recovering. A typical procedure consisted of introducing the diol and the diester in a stoichiometric ratio, toluene (150 mL), and the enzymic preparation (5 g for 1 mol·L^{-1} initial concentration of the reactive functions) into a thermostated double jacket reactor. The reactor was surmounted by a thermostated tube. Methanol elimination was favored by nitrogen bubbling (0.3 L·min^{-1}). The removed methanol and solvent were collected in a flask at the outlet of the tube. The volume of the solution was held constant by adding fresh toluene periodically. After the reaction, the supported catalyst was removed by filtration, and the solvent was distilled off at 40 °C under reduced pressure. The residue was dried under reduced pressure at 40−50 °C until its weight was constant. The polyester sample obtained in these conditions is called the unfractionated sample.

Error of Measurement. The molar cyclization equilibrium constant K was determined from the concentration of rings at equilibrium [R_{nn}] and the extent of reaction p (see further Jacobson−Stockmayer relationships). The error of measurement was estimated to be mainly due to that of the surface area relative to each cyclic species (SEC analysis) from which [R_{nn}] arises and that of the extent of reaction p (^{1}H NMR analysis). For K values, it was calculated according to $\Delta K/K = \Delta[R_{nn}]/[R_{nn}] + 2n\Delta p/p$ with $\Delta[R_{nn}]/[R_{nn}] = 0.05$ and $\Delta p/p = 0.02$. For subsequent calculations (leading to ΔH^0 and ΔS^0 in particular), $\Delta K/K$ and $\Delta \ln K = 0.1$, 0.15, 0.15, 0.2, 0.25, and 0.3 for $n = 1-6$, respectively. Slope values were calculated from the lowest and the highest slopes of straight lines fitting within the error field.

Abbreviations Used for Polymers: Poly(hexamethylene succinate), PHS; poly(octamethylene succinate), POS; poly(decamethylene succinate), PDeS; poly(dodecamethylene succinate), PDoS; poly(hexamethylene fumarate), PHF; poly(hexamethylene maleate), PHM; poly(hexamethylene isophthalate), PHiP.

Results and Discussion

Empirical Approach to the Ring−Chain Equilibrium. Poly(1,6-hexanediyl succinate) (PHS) was synthesized from dimethyl succinate and 1,6-hexanediol at different initial concentrations of the reactive functions, C_0, in a stoichiometric ratio at 60 °C in toluene in the presence of supported *C. antarctica* lipases (Novozyme) (Scheme 1).

Linear chains and rings were characterized by SEC and MALDI−TOF spectrometry[11] and we recently reported that the content of cyclic species increases on dilution,[9] which is a trivial feature of the ring−chain equilibrium.

Another evidence for the ring−chain equilibrium is provided as linear chains are liable to give rings and shorter chains. As previously reported,[9] cycle-free PHS was prepared by enzyme catalysis and obtained after methanol fractionation to eliminate the cyclic fraction. This sample made of linear chains only was dissolved in toluene at 60 °C and the reaction mixture was stirred for 1 day in the presence of Novozyme as catalyst. After reaction, the sample was recovered by solvent evaporation and analyzed by SEC. The presence of rings was easily detected, and their formation was accompanied by a broadening distribution and a shift toward low molecular weight. We also verified that the shortest chains did not result from hydrolysis since a small water content is needed into supported enzyme to be active in organic solution. This was possible by comparing the content of hydroxy end groups before and after the experiment. Thus linear chains lead to both rings and shortest linear chains which confirms that a ring−chain equilibrium takes place during the enzymic polycondensation.

A last experiment consisted in concentrating a toluene solution containing a mixture of rings and chains in which rings are largely predominant. Thus, 0.302 g of the mixture obtained from a 0.05 M toluene solution (Figure 1a) was dissolved in 7.5 mL of toluene. The corresponding mixture concentration (40 g·L^{-1}) is equivalent to that obtained from a 0.4 M solution. After 1 day in the presence of the enzymatic preparation (100 mg), the solution was filtrated and toluene was distilled off. The residual mixture was analyzed by SEC.

Figure 1b clearly shows that a new ring−chain distribution is obtained where long chains are now predominant. In Figure 1, chromatogram b is qualitatively compared with that of a polyester sample obtained from a 0.4 M toluene solution. The ring content and distribution are approximatively similar. This confirms the possibility that rings, in the presence of a few end groups, lead to long chains and the existence of a ring−chain equilibrium. Furthermore, we verified by ^{1}H-NMR that the end group content or the extent of reaction is practically the same before and after the experiment ($p = 0.95$ and 0.94, respectively). The slight difference is probably due to ring opening by some free water molecules, which increases the end-group content and decreases the extent of reaction.

Jacobson−Stockmayer Relationships. In the 1950s, Jacobson and Stockmayer developped an elegant

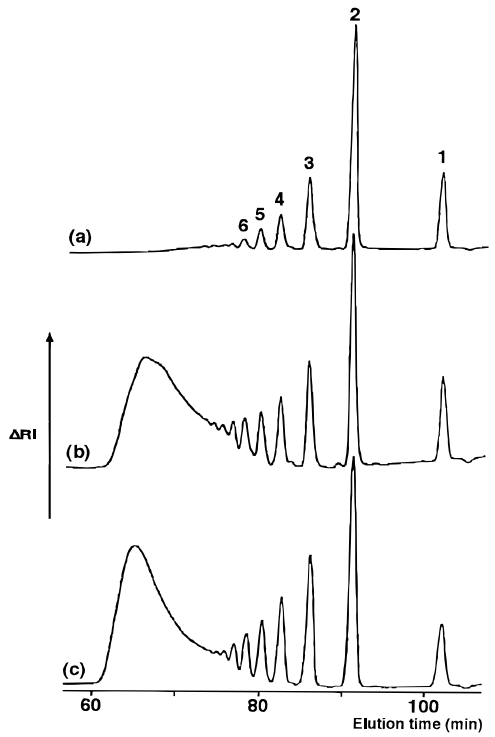


Figure 1. SEC profiles: (a) the ring-chain mixture obtained from dimethyl succinate and 1,6-hexanediol ($C_0 = 0.05$ mol·L^{-1}); (b) the same sample after concentration in toluene solution (40 g·L^{-1}) and after 24 h reaction in the presence of enzyme at 333 K; (c) the ring−chain mixture directly obtained from dimethyl succinate and 1,6-hexanediol ($C_0 = 0.4$ mol·L^{-1}). Plgel columns were used (500, 100, and 50 Å; 60 cm long); flow rate (THF) = 0.5 mL·min^{-1}; refractometry was used. The unimolecular peaks were assigned to rings with n values ranging from 1 to 6 at least where, according to Jacobson−Stockmayer lettering,[10] n represents the unit number of each monomer type in the rings (cyclic n,n-mer, DP = 2n).

theory on intramolecular reaction in polycondensation.[10] They distinguished three different cases of polymer structure. All initial monomers are identical and their end groups are of the same nature (case I); in case II, all the monomers are identical but each of them bears different functional groups. Case III concerns monomers of two different types, but each molecule bears two end groups of the same kind.

The polymers on study belong to case III (adipic acid−decamethylene glycol case); the corresponding ring−chain equilibrium is described by eq 1 (see Appendix) and the molar distribution of rings is given by eq 2 (see Appendix). In principle, eq 3 (see Appendix) should be used for analyzing ring−chain equilibria, but unfortunately it is very difficult to determine the fractions of reacted end groups in the chain fraction (x and y) which are essential to calculate the molar cyclization equilibrium constant. The mixture of rings and linear chains indeed do not allow a direct determination of x and y. We used a simplified form of eq 3 where x and y were assumed to be very close to the extent of reaction p (eq 4; see Appendix). This approximation is valid as long as linear chains predominate in the ring−chain mixture so that the fraction of reacted end-groups leading to ring formation can be neglected. Thus, experimental K values were available once the concentration of rings at equilibrium and the extent of reaction were known.

Dilution Effect. K values were calculated from the yield of each cyclic species determined by computing the SEC profiles (Table 1). Equation 3 was tested for rings present in the unfractionated PHS synthesized at initial

Table 1. Ring−Chain Equilibrium Data for Poly(1,6-hexanediyl succinate) Made by Enzyme-Catalyzed Polytransesterification from 1,6-Hexanediol and Dimethyl Succinate in Toluene, Showing the Influence of the Initial Concentration of the Reactive Functions on the Molar Distribution of Rings

		10^3K (mol·L^{-1})[a]					
C_0[b] (mol·L^{-1})	p[c]	$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$
0.05	0.99	4.4	5.8	1.4	0.5	0.25	0.11
0.10	0.99	6.2	9.4	2.3	1.1	0.6	0.5
0.20	0.99	6.5	9.6	4.2	2.1	1.3	0.8
0.30	0.99	6.7	12.2	4.8	2.7	1.6	1.1
0.40	0.98	7.7	13.2	5.1	2.8	1.9	nd
0.50	0.99	7.7	13.7	4.9	2.5	1.6	1.3
0.75	0.98	6.9	12.1	4.9	2.6	nd	nd
1.00	0.98	8.3	14.5	5.7	3.2	1.8	nd

[a] Determined from SEC analysis; Plgel columns (500, 100, 50 Å, 60 cm long); flow rate (THF) = 0.5 mL·min^{-1} at room temperature. Error of measurement: $\Delta K/K = 0.1$, 0.15, 0.15, 0.2, 0.25, and 0.3 for $n = 1$ to 6, respectively (for more details, see Experimental Section). [b] Enzyme-catalyzed synthesis of poly(hexanediyl succinate) in toluene at 333 K; C_0 is the initial concentration of the reactive functions (stoichiometry); enzyme concentration (see Experimental Section for explanation); nitrogen flow = 0.3 L·min^{-1}. [c] Determined by ^{1}H NMR from $-CH_2OH$ and $-CO_2CH_2-$ signals (see Experimental Section for calculation and for error of measurement). Error of measurement: $\Delta p/p = 0.02$ (for more details, see Experimental Section).

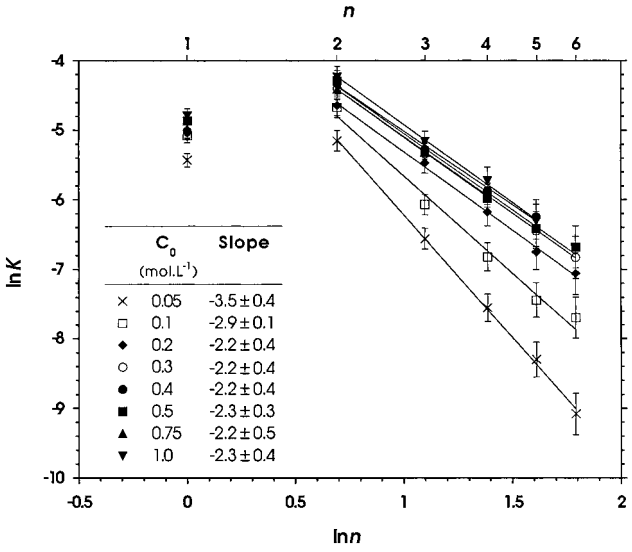


Figure 2. Experimental molar cyclization equilibrium constants K (mol·L^{-1}) for cyclics in the PHS toluene solution at 333 K as a function of the repeating unit number in the ring n. Key (ln K *vs* ln n for different initial concentrations of the reactive functions): $C_0 = 1.0$ (▼), 0.75 (▲), 0.5 (■), 0.4 (●), 0.3 (○), 0.2 (◆), 0.1 (□), and 0.05 mol·L^{-1} (×). Error bars are given according to $\Delta \ln K = 0.1$, 0.15, 0.15, 0.2, 0.25, and 0.3 for $n =$ 1 to 6, respectively (see Experimental Section for more details).

concentrations of the reactive functions ranging from 1 to 0.05 mol·L^{-1}. In Figure 2, ln K *vs* ln n plots clearly show that the cyclic species obey a linear relation when n is above one (according to Jacobson−Stockmayer lettering,[10] n represents the unit number of each monomer type in the rings [cyclic n,n-mer, DP = 2n]). When C_0 ranges from 0.2 to 1 M, the experimental slope values are -2.2 ± 0.4, -2.3 ± 0.3, -2.2 ± 0.5, and -2.3 ± 0.4 for $C_0 = 0.2$ to 0.4, 0.5, 0.75, and 1.0 mol·L^{-1}, respectively, which is very close to the theoretical value (-2.5) and confirms that linear chains and rings equilibrate in the reaction system as predicted by the Jacobson−Stockmayer equation (eq 3). Unlike the other rings, the cyclic dimer ($n = 1$) does not obey eq 3 as expected for a 12-membered ring. This exception will

Table 2. Ring−Chain Equilibrium Data for Polyesters Made by Enzyme-Catalyzed Polytransesterification from Diols and Dimethyl Succinate in Toluene,[a] Showing the Influence of the Diol Length on the Molar Distribution of Rings

	10^3K (mol·L^{-1})[b]				
polyester[c]	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$
PSH	7.7	13.3	5.1	2.8	1.9
POS	13.6	11.4	3.8	2.3	nd
PDeS	6.4	9.4	3.9	1.8	nd
PDoS	9.7	7.9	3.2	nd	nd

[a] Enzyme-catalyzed synthesis of poly(alkylene succinate) in toluene at 333 K; $C_0 = 0.4$ mol·L^{-1} (stoichiometry). [b] See footnote *a* Table 1. [c] Key: PHS, poly(hexamethylene succinate); POS, poly(octamethylene succinate); PDeS, poly(decamethylene succinate); PDoS, poly(dodecamethylene succinate).

be further discussed. When $C_0 = 0.1$ and 0.05 M, the corresponding plots are straight lines with the same exception relative to the cyclic dimer; the slope values however decrease on dilution. In fact, in this range of concentrations, the rings predominate so that the extent of reaction is no longer a satisfactory approximation to x and y (eq 4). K values are therefore underevaluated, and the more the rings predominate and the larger they are, the stronger the variation.

Cyclic Dimer RI Response. We verified that the behavior of the cyclic dimer does not result from an artifact due to a different RI response during SEC analysis. The yield of the cyclic dimer was therefore calculated by ^{1}H NMR from the intensities of the $-CH_2-CO_2-$ signals at 4.2 and 4.1 ppm characteristic of the cyclic dimer and to the other rings and linear chains, respectively (submitted as Supporting Information). For $C_0 = 0.05$ mol·L^{-1}, the cyclic species are largely predominant so that the content of the cyclic dimer (compared to all cyclic species) can be directly determined from ^{1}H NMR analysis. It is equal to 17.3 and 17 wt % according to ^{1}H NMR and SEC analyses, respectively. The good agreement between these values confirms that the cyclic dimer has the same RI response as the other rings. The corresponding cyclization equilibrium constant is therefore lower than the value expected from eq 3, which is definitely consistent with the presence of short-range steric effect for a 12-membered ring, and this will be confirmed in the study of the temperature effect. On the other hand, we showed that eq 4 could not be applied for the $C_0 = 0.05$ and 0.1 M concentration ranges.

Influence of Diol Length. The influence of the diol length on the cyclization was studied considering poly(octamethylene succinate), poly(decamethylene succinate), and poly(dodecamethylene succinate) (POS, PDeS, and PDoS, respectively). The corresponding cyclization equilibrium constants were determined as before (Table 2), and eq 3 was tested, taking care to work in a concentration range where eq 4 is valid. Whatever the length of the alcohol moities, straight lines were obtained with similar slope values (-2.2 ± 0.4, -2.4 ± 0.4, -2.4 ± 0.5, and -2.3 ± 0.7 for PHS, POS, PDeS, and PDoS, respectively) very close to the theoretical value (-2.5). The molar distribution of the rings with n more than one is not influenced by the diol length and is well-described by the Jacobson−Stockmayer equation. On the other hand, for a given cyclic n,n-mer with $n > 1$, the cyclization equilibrium constant decreases with increasing length of the alcohol moities. This confirms that the probability of ring closure decreases when the number of skeletal atoms in the ring structure increases either by increasing the number of monomer units or by increasing the number of methylene groups in the alcohol moities.

Table 3. Ring−Chain Equilibrium Data for Polyesters Made by Enzyme-Catalyzed Polytransesterification from 1,6-Hexanediol and Dimethyl Esters in Toluene,[a] Showing the Influence of the Temperature on the Molar Distribution of Rings

		10^3K (mol·L^{-1})[b]				
polyester[c]	T (K)	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$
PHS	313	6.7	13.5	5.0	nd	nd
	323	6.1	9.8	4.6	nd	nd
	333	7.7	13.3	5.1	2.8	1.9
	343	8.8	12.2	4.7	2.8	1.9
	353	8.6	13.4	5.1	2.7	1.6
POS	313	11.2	10.2	4.8	nd	nd
	323	10.9	9.6	3.9	nd	nd
	333	13.6	11.4	3.8	2.2	nd
	343	16.1	11.0	5.7	nd	nd
	353	14.8	9.9	4.3	nd	nd

[a] Enzyme-catalyzed synthesis of poly(alkylene succinate) in toluene; $C_0 = 0.4$ mol·L^{-1} (stoichiometry). [b] See footnote *a* Table 1. [c] Key: PHS, poly(hexamethylene succinate); POS, poly(octamethylene succinate).

Table 4. ΔS^0 and ΔH^0 Values for PHS and POS Cyclization in Toluene,[a] Showing the Influence of the Monomer Unit Number, n

	ΔH^0 [b] (kJ·mol^{-1})		ΔS^0 [b] (J·mol^{-1}·K^{-1})	
n	PHS[c]	POS[d]	PHS	POS
1	9 ± 1	8 ± 2	-15 ± 2	-11 ± 6
2	0	0	-36 ± 1	-37 ± 1
3	0	0	-44 ± 1	-44 ± 1
4	0		-49 ± 2	
5	0		-53 ± 2	

[a] See footnote *a* Table 3. [b] See Experimental Section for indications of the error of the measurements. [c] PHS: poly(hexamethylene succinate). [d] POS: poly(octamethylene succinate).

However, for 12-, 14-, 16- and 18-membered rings (PHS, POS, PDeS, and PDoS cyclic dimers, respectively), we did not find any relation between K values and the number of skeletal atoms. In this case the probability of ring closure cannot be expressed as a well-defined function of ring size. The frame of the Jacobson−Stockmayer theory is based indeed on the probability of ring closure and does not take into account enthalpic contributions. This means obviously that enthalpic and entropic effects contribute to the formation of PHS, POS, PDeS, and PDoS cyclic dimers. This is clearly confirmed by the study of temperature effect.

Temperature Effect. The PHS and POS cyclization equilibrium constants were determined at different temperatures ranging from 40 to 80 °C (Table 3). The corresponding Arrhenius plot shows that temperature has no effect on the cyclization equilibrium constants when n is above 1. On the other hand, ln K slightly decreases with increasing temperature when $n = 1$, which must be correlated with short-range steric effects. The same observations hold for POS equilibrium constants with again the same exception relative to the cyclic dimer. All these observations are summarized in Table 4 where ΔH^0 and ΔS^0 are reported. A significant enthalpic contribution is observed only when n is equal to 1. In the case of PHS and POS, it appears for the 12- and 14-membered rings, respectively. When the number of skeletal atoms is 24 (PHS) or 28 (POS) and up, the cyclization equilibrium constant only depends on ΔS^0. Thus, when $n > 1$, K is an exponential function of ΔS^0, which decreases with the increasing number of

Table 5. Ring−Chain Equilibrium Data for Polyesters Made by Enzyme-Catalyzed Polytransesterification from 1,6-Hexanediol and Dimethyl Esters in Toluene,[a] Showing the Influence of the Diester Structure on the Molar Distribution of Rings

	10^3K (mol·L^{-1})[b]				
polyester[c]	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$
PHS	8.3	14.5	5.7	3.2	1.8
PHM	27	23	7.0	3.6	2.1
PHiP	0	3.9	1.2	0.7	0.4

[a] Enzyme-catalyzed synthesis in toluene at 333 K; $C_0 = 1.0$ mol·L^{-1} (stoichiometry). [b] See footnote *a* Table 1. [c] Key: PHS, poly(hexamethylene succinate); PHM, poly(hexamethylene maleate); PHiP, poly(hexamethylene isophthalate).

repeating units. When $n = 1$, an enthalpic contribution is observed. As demonstrated in Jacobson−Stockmayer works, the ΔS^0 variation is correlated to the probability of the ring closure according to $\Delta S^0 = k \ln(P/n)$, where k, P, and n are the Boltzmann constant, the probability of ring closure, and the number of repeating units. This corroborates the assumptions made in the previous part.

The influences of diol length and temperature showed that the cyclic dimers behave atypically according to Jacobson−Stockmayer theory. They do not obey eq 3, and this implies that an enthalpic contribution must be taken into account as demonstrated for PHS and POS cyclic dimers. On the other hand, the presence of short-range steric effects in PDeS and PDoS cyclic dimers must be also considered since we observed the same exception. If for 12- and 14-membered rings these results were expected (it corresponds to the limit given by Jacobson and Stockmayer), they are surprising in the case of 16- and 18-membered rings.

On the other hand, Semlyen *et al.*[12] showed that the characteristic slope of *ca.* −2.5 is observed from $n = 2$ for the solution ring−chain equilibria of poly(decamethylene adipate) (PDA) and that the experimental K_x values for the cyclic dimer and tetramer (18 and 36 skeletal atoms, respectively) are *ca.* 25 and 7.9 mmol·L^{-1}. In the present study, the experimental K_x values for the cyclic dimer and tetramer of PDoS (same number of skeletal atoms) are 9.7 and 7.9 mmol·L^{-1} (Table 2). Although the PDA and PDoS chemical structures slightly differ, the K_x values for the two tetramers are very close. In the case of the cyclic dimers, three parameters can be retained in order to explain the difference between the K_x values: (*i*) temperature (130 °C[12] *vs* 60 °C in this study); (*ii*) solvent (chlorobenzene[12] and toluene) and catalyst (tetraisopropyl orthotitanate[12] and lipases from *C. antarctica*).

Apparently, the chain conformation required by the enzyme binding and catalytic sites leads to those steric effects even in the case of rings larger than 14−15 skeletal atoms; during the cyclization process, several skeletal atoms are engaged in these sites (in the acylenzyme intermediate for instance) resulting in a partial freedom loss of the chain. Thus a 18-membered chain behaves as a shorter one, and the chain stress generated by these conformational requirements progressively disappears for a larger number of skeletal atoms as is observed in the case of the PHS or POS cyclic tetramers.

Influence of Diester Structure. We also investigated the influence of the diester structure on the enzymatic cyclization (Table 5). For poly(hexamethylene maleate) (PHM, slope value −2.6 ± 0.4) and poly(hexamethylene isophthalate) (PHiP, slope value −2.5 ± 0.3), the molar distribution of rings was found to fit eq 3 with the same exception relative to the cyclic dimers. In the case of the phthalic structure,[6] the cyclic dimer was not detected by SEC, and on the other hand, the highest value was obtained with the maleic cyclic dimer.[5] It must be added that poly(hexamethylene fumarate) does not contain any rings.[5] The configuration of the double bond and the presence of rigid moities such as phthalic structure strongly influence the possibility of the enzyme-catalyzed ring closure but not the relative distribution of the cyclic species larger than the dimer.

Assumptions on the Enzymatic Cyclization Mechanism. The reaction scheme involving the active site of a lipase (or of a serine-hydrolase) can be written in a simplified form (Scheme 2). In the acylation step (eq 5), the acylenzyme intermediate results from the reaction of the hydroxy function of the catalytic serine residue on the ester (or acid) function of the substrate. The deacylation step (eq 6) consists in the nucleophilic attack of the second substrate (an alcohol) on the activated ester function of the acylenzyme intermediate.

The formation of rings from a linear chain should obey the same scheme with acylation-deacylation steps (Scheme 3). Thus, at any time of the polycondensation, the linear chains present three different structures according to the nature of the end-groups. There are the α,ω-dihydroxy **1**, the α,ω-dimethyl ester **5** and the α-hydroxy, ω-methyl ester chains **8**. Reacting on the free enzyme **2**, these chains do not lead to the same acylenzyme intermediate. An α,ω-dihydroxy chain **1** leads to the ω-hydroxyacylenzyme (**3**, eq 7); an α,ω-dimethylester chain **5** leads to the ω-methyl ester acylenzyme (**6**, eq 8); an α-hydroxy, ω-methylester chain **8** lead to **3** (eq 9) or **6** (eq 10) according to the orientation of the reactive ester function in the chain with respect to the nature of the end-groups.

Rings cannot be formed in the acylation step but in the deacylation one. Only the ω-hydroxy acylenzyme is liable to form rings (eq 11). In this case, the ring formation obeys a back-biting reaction involving the hydroxy end group and the activated ester function of **3**. On the other hand, **6** cannot lead to ring formation.

Nevertheless, the formation of rings from α,ω-dimethyl ester chain **5** can be explained by the following path: Equation 8 leads to **6** and **7** species; the latter is an α-hydroxy, ω-methyl ester chain shorter than that of **5** and is able to cyclize according to eqs 9 and 11.

These assumptions are consistent with the overall behavior of the polyester chains in solution since no difference was observed whatever the nature of the end groups. They can be a basis for further enzymatic kinetics. On the other hand, they show that cyclization intervenes in the deacylation step, and from this point of view, the short-range steric effects pointed out for the PSH and POS cyclic dimers can appear in this step.

Conclusion

The enzymic preparation (supported lipases from *C. antarctica*) used in toluene for the syntheses of aliphatic, unsaturated, and semiaromatic polyesters catalyzes the formation of linear chains as well as the ring closure. The ring−chain equilibrium was demonstrated as a general feature of these reaction systems and was successfully examined in the framework of Jacobson−Stockmayer theory on intramolecular reaction in polycondensation.

However, a peculiar behavior was observed for all cyclic dimers and particularly for the largest ones. If one easily understands that the formation of 12- and 14-membered rings is accompanied by a slight enthalpic contribution as mentioned by these authors themselves, it is rather difficult to explain the atypical behavior of cyclic dimers having 16 and *a fortiori* 18 skeletal atoms.

In order to explain the *cyclic dimer exception*, we propose an interpretation based on the possibility that the conformational requirements imposed by the enzyme binding and catalytic sites artificially diminish the effective length of the chain that is going to cyclize. Thus, these requirements do not change anything for the longest chains but lead to a decrease of the cyclization equilibrium constant in the case of shorter chains. This interpretation must be supported by complementary studies and we are now focusing on the study of cyclic dimer formation only. Interestingly, more flexible diols such as α,ω-dihydroxy ethers will be studied in the near future. Besides, rings with 20, 22, and odd numbers of skeletal atoms and the influence of the diester length will be considered.

Supporting Information Available: Figure showing ^{1}H-NMR spectrum (250 MHz, $CDCl_3$ solution) of enzymatically synthesized PHS at 333 K in toluene ($C_0 = 0.05$ mol·L^{-1}) with extension from 3.4 to 4.4 ppm (1 page). Ordering information is given on any current masthead page.

Appendix: Equations

Equations 1−4 referred to in the text are listed below.

$$C_{n+m,n+s} \overset{K}{\rightleftharpoons} C_{ms} + R_{nn} \quad (1)$$

$C_{n+m,n+s}$ represent $n + m,n + s$-mer linear chains; C_{ms} represents m,s-mer linear chains; R_{nn} represents n,n-mer rings (the two indices referring to the number of monomers of the first and second kinds, respectively); K is the molar cyclization equilibrium constant.

$$B_{nn} = BVn^{-5/2}x^n y^n \quad \text{with} \quad B = [3/(2\pi\nu)]^{3/2}/2b^3 \quad (2)$$

B_{nn} is the mole number of n,n-mer rings; B is a constant depending on the effective link length b of the polymer chain and on the number of chain atoms per monomer unit ν; V is the reaction volume; x and y are the fractions of reacted end groups in the chain fraction.

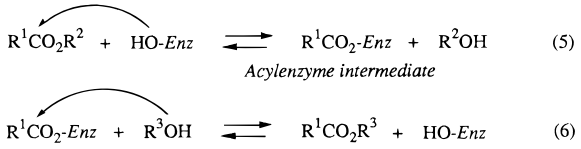
$$\ln K = \ln B - {}^5/_2 \ln n \quad \text{with}$$
$$K = B_{nn}/Vx^n y^n = [R_{nn}]/x^n y^n \quad (3)$$

$[R_{nn}]$ is the concentration of rings at equilibrium.

$$K = [R_{nn}]/x^n y^n \approx [R_{nn}]/p^{2n} \quad (4)$$

p is the extent of the reaction.

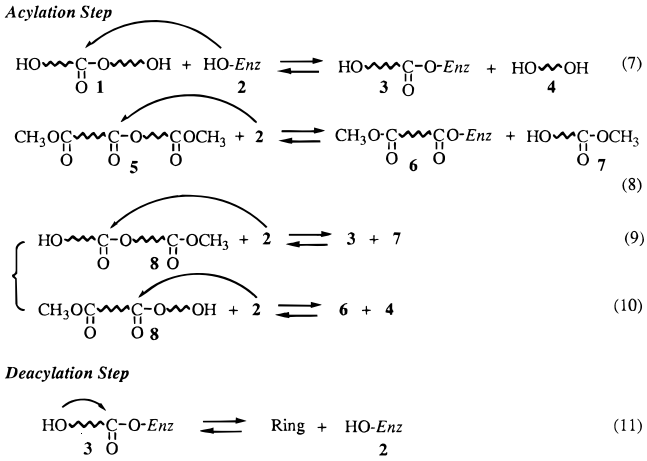
Scheme 2



(5)

(6)

Scheme 3



References and Notes

(1) For reviews, see: (a) Morrow, C. J.; Wallace J. S. In *Biocatalysis*; Abramowicz, D. A., Ed.; Van Nostrand Reinhold: New York, 1990; p 25. (b) Morrow, C. J. *MRS Bull.* **1992**, *17*, 43. (c) Lalot, T.; Brigodiot, M.; Maréchal, E. In *Comprehensive Polymer Science*; Aggarwal, S. L., Russo, S., Eds.; Pergamon: Oxford, England, New York, Tokyo, 1996; 2nd Suppl., p 29.
(2) Wallace, J. S.; Morrow, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **1989**, *27*, 2553 and 3271.
(3) Morrow, C. J.; Wallace, J. S.; Brazwell, E. M.; Bybee, G. M.; Reda, K. B.; Williams, M. E. *Mater. Res. Soc. Symp. Proc.* **1990**, *174*, 197.
(4) Linko Y.-Y.; Wang, Z.-L.; Seppälä, J. *Enzym. Microb. Technol.* **1995**, *17*, 506.
(5) Mezoul, G.; Lalot, T.; Brigodiot, M.; Maréchal, E. *Macromol. Rapid Commun.* **1995**, *16*, 613.
(6) Mezoul, G.; Lalot, T.; Brigodiot, M.; Maréchal, E. *Polym. Bull.* **1996**, *36*, 541.
(7) Gutman, A. L.; Oren, D.; Boltanski, A.; Bravdo, T. *Tetrahedron Lett.* **1987**, *28*, 5367.
(8) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
(9) Berkane, C.; Mezoul, G.; Lalot, T.; Brigodiot, M.; Maréchal, E. *Macromol. Chem. Phys., Macromol. Symp.*, in press.
(10) Jacobson, H.; Stockmayer, W. H. *J. Chem. Phys.* **1950**, *18*, 1600.
(11) Mezoul, G.; Lalot, T.; Brigodiot, M.; Maréchal, E. *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33*, 2691.
(12) Wood, B. R.; Semlyen, J. A.; Hodge, P. *Polymer* **1997**, *38*, 191.
(13) Lalot, T.; Brigodiot, M.; Maréchal, E. To be published.

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