Speeding-up Enzyme-Catalyzed Synthesis of Polyamides using ω-Amino-α-Alkoxy-Acetate as Monomer

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ABSTRACT:The design of ω-amino-α-alkoxy-acetate as monomer enablestodramaticallyspeeduptheformationofpolyamidescatalyzedbythesupportedlipase B of*Candida antarctica*. Only 30 min areneededtoreach 93% conversionwhereas 240 h arenecessarytoobservethe same levelofconversionwith a monomer devoidedof an oxygenatom in position β relative totheelectrophiliccarbonylgroup. Yields, *Mn*, *Mw*, DPnand PDI reachverygoodvaluesfor an enzymaticsynthesisofpolyamides.

KEYWORDS: polyamides; enzymes; monomers .

1. Introduction

For many years, users and developers from a broad range of industries particularly value the properties of polyamides. These include high mechanical and thermal stress resistance, excellent low-friction properties and abrasion resistance, good chemical stability, low stress crack resistance and good electrical insulating behavior [1]. These interesting properties result

mainly from hydrogen bonds induced by the amide function. These multiple interactions among adjacent strands are energetically strong due to the high dipolar moment of the amide group. The symmetrical and regular character of the backbone of the polyamide and the strength of interchain interactions lead often to highly crystalline polymers. However, the low solubility associated to high melting and glass transition temperatures caused by theircrystallinityinherent to their backbone lead to difficulties in synthesis, characterization and processing of polyamides [2-4].

The bio-inspired synthesis of polymers is recognized as a powerful tool, mostly because enzymes react under mild conditions and provide sustainable chemical processes [5-10]. Polyesters are readily available from bio-catalyzed lactones ring-opening, condensation of ω-hydroxyesters, or copolymerization of diesters with diols [5-12]. Comparatively, very few examples of enzymatic syntheses of polyamides have been reported, even though it is an attractive alternative to usual methodologies [13-20].

Regarding lipase-promoted lactam ring-opening strategy, only the unsubstituted β-lactam ring enables the formation of poly(β-alanine) [13]. Polycondensations leading to Nylon 6,12 and Nylon 6,6 have been successfully achieved by Gross [14]. The influence of the carbon chain-length on the polymerization degree was emphasized in this study. The faster propagation of Nylon 6,12 chains as compared to Nylon 6,6 ones is in fair agreement with the chemical properties of lipases designed by nature for the hydrolysis of long chain fatty esters. Cheng and coworkers have also reported the lipase-catalyzed synthesis of polyamides, through an AA/BB strategy [15]. They coupled saturated diamines, heteroatom-containing diamines and even polyamines with various diesters according to an enzymatic process. This study clearly demonstrated that the use of various lipases as polymerization catalysts led to the obtention of polymers with molecular properties similar to those obtained by a chemical approach at 180°C, but with better polydispersity indexes. Moreover, the cationic resins

obtained from these products showed physical properties close to those of polymers derived from chemical processes. Bio-catalyzed bulk polymerizations were expanded to the formation of polyamides and polyester/amides based on polydimethylsiloxane blocks [16-17], sometimes coupled with an aliphatic fluorinated backbone [18].

Our group has been involved during the last years in the improvement of the formation of amide bond catalyzed by lipases and proteases [21-23], with the goal to make it compatible with thiyl radical-promoted racemization of aliphatic amines in a dynamic kinetic resolution process (DKR) [24-27]. The obvious extension of this DKR was to synthesize optically active polyamides by coupling the free-radical racemization with the efficient enzymatic polymerization we have recently developed. This strategy was designed and successfully applied by Meijer and coworkers to the synthesis of chiral polyesters [28-30].

However, despite the efficiency of the enzymatic synthesis of polyamides, the reaction times needed to reach good monomer conversion levels were far too high compared to the time necessary to complete the radical-promoted racemization [19-20]. In this publication, we present an enzymatic polymerization involving the supported variant of the lipase B of *Candida antarctica,* Novozym 435 (N435), which leads to high degrees of conversion within only a few minutes of reaction. This improvement was made possible by the design and synthesis of "methoxyacetate-like" monomers.

2. Experimental

2.1.Materials

All chemicals were analytical grade and were used as received. Glyme was distilled before use from sodium/benzophenoneketyl. *Candida antarctica* Lipase B (CAL-B) immobilized on macroporous acrylic resin (Novozym 435 (N435)) was a gift from Novozymes (Denmark).

2.2.General Procedure

2.2.1. A/B type polymerization under reduced pressure

In a typical experiment, ester **1** (219 mg, 1 mmol) in Ph_2O (1 g) was introduced in a 10 mL flask. After stirring for 15 min, dried Novozym 435 (100 mg) was added. The mixture was then heated at 80 °C under 10 mbars for 240 h. After completion, the mixture was cooled at 60 °C, 10 mL of chloroform were added. The hot solution was filtered to remove the enzyme, the latter was washed with hot chloroform and the solution was concentrated to give the product as a sticky oilwhich was then triturated in methanol and dried under vacuum at 40 °C for 48 h (155 mg). ^{1}H NMR (300 MHz, D_2O): 7.86 (bs, NH), 4.14 (q, *J*=7.2, CH_3*CH_2*O, terminations), 4.09 (s, O*CH_2*COOEt, terminations), 3.92 (s, O*CH_2*CONH), 3.59 (m, O*CH_2CH_2*O), 3.46 (m, *CH_2O*CH_2CH_2O*CH_2*), 3.23 (m, *CH_2*NHCO), 2.99 (m, *CH_2*NH_2), 1.58 (bs, *CH_2*CH_2NHCO et OCH$_2$*CH_2*CH_2NH), 1.21 (t, *J*=7.2, *CH_3*CH_2OCO, terminations). ^{13}C NMR (75 MHz, D_2O): 173.0 (CONH), 172.1 (COOEt), 72.4 (CH_2), 72.3 (CH_2), 72.2 (CH_2), 71.7 (CH_2), 71.6 (CH_2), 71.5 (CH_2), 69.8 (CH_2), 62,3 (CH_2), 40.0 (CH_2), 28.4 (CH_2), 27.8 (CH_2), 15.0 (CH_3).

2.2.2. AA/BB type copolymerization under reduced pressure

In a typical experiment, diester**5** (58.5 mg, 0.25 mmol) and diamine**4** (111 mg, 0.25 mmol) in Ph_2O (340 mg) were added in a 10 mL flask. After stirring for 15 min, dried Novozym 435 (60 mg) was added. The mixture was then heated at 80 °C under 10 mbars for 240 h. After completion, the mixture was cooled at 60 °C, 10 mL of chloroform were added. The hot solution was filtered to remove the enzyme, the latter was washed with hot chloroform and the solution was concentrated up to 2 mL. The precipitate formed after addition of methanol was filtered, and then washed with the same solvent. The polymer was recovered after drying at 40 °C under vacuum for 48 h (130 mg). ^{1}H NMR (400 MHz, $CDCl_3$): 6.70 (bs, NH), 4.16

(m, O*CH*$_2$CH$_3$, terminations), 4.13 (s, O*CH*$_2$COOEt, terminations), 4.01 (s, O*CH*$_2$CONH), 3.72 (s, O*CH*$_2$*CH*$_2$O), 3.67-3.56 (m, O*CH*$_2$*CH*$_2$O*CH*$_2$*CH*$_2$O), 3.44 (t, *J*=6.8, *CH*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$O*CH*$_2$*)*, 3.29 (dt, *J*=7.0, 6.8, *CH*$_2$NHCO), 1.61-1.47 (m, *CH*$_2$CH$_2$O et *CH*$_2$CH$_2$NHCO), 1.36-1.26 (m, (*CH*$_2$)$_7$), 0.88 (t, *J*=6.8, *CH*$_3$CH$_2$O, terminations).^{13}C NMR (100 MHz, $CDCl_3$): 170.4 (CONH), 71.6 (CH_2), 70.7 (CH_2), 70.6 (CH_2), 70.1 (CH_2), 39.0 (CH_2), 31.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 26.9 (CH_2), 26.1 (CH_2).

2.3. Characterization methods

2.3.1. Gel Permeation Chromatography Analyses

The analyses and the monitoring of polymerization reactions were achieved by gel permeation chromatography (GPC) using Macherey-Nagel Nucleogel GPC 500, 100 and 50 (10 µm porosity; 300 x 7.7 mm) analytical columns fitted in series after a GPC (50 x 7.7 mm) pre-column. The system was piloted by Azur (Jasco©) software, and equipped with a Waters 600 pump, a Varian oven (model 510) and a Waters differential diffractometer (model 410). Analyses used a 0.1 M solution of LiBr in *N*,*N*-dimethylacetamide (HPLC grade, degassed and filtered on a Millipore membrane before use) at 80 °C (0.6 mL/min rate of flow). Sample concentrations of 1-3 mg/mL and injection volumes of 200 µL were used. Toluene (500 µL/100 mL) was introduced in the mobile phase as elution marker.

System calibration data and relative molar mass calculations were acquired and processed using PSS WinGPC (Polymer laboratories) software. Narrow distribution polyethylene glycol standards (Varian) with number-average molecular weight *(Mn)* values of 106, 194, 430, 615, 1010, 1970, 3390, 7980, 12140 and 21030 Da were used to establish the calibration curves. Weight-average molecular weight (*Mw*), (*Mn*) and polydispersity index (PDI= *Mw*/*Mn*) were calculated from the chromatograms.

2.3.2. Instrumentals

DSC analyses were effected with a Q200 DSC TA Instrument, the calorimeter under nitrogen (30 mL/min) being connected to a cryostat from the same manufacturer. DSC scans were run at temperatures ranging from -150 to 150 °C with a heating rate of 5 °C/min. A controlled cooling rate of -10 °C/min was applied between heating runs.

TGA analyses were recorded with TGA Q500 TA Instruments apparatus, under an air flux (60 mL/min). The measurements were carried out with a rate of 10 °C/min.

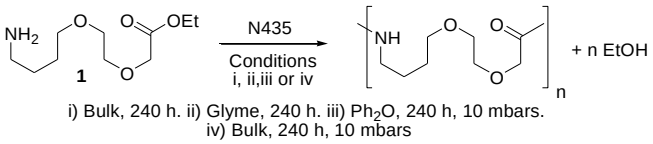
3. Results and discussion

The acceleration of lipase-catalyzed kinetic resolution using ethyl methoxyacetate as acyl donor has been known for several years [31]. It allows the completion of the enzymatic reaction with excellent yields in short reaction times, without loss of enantioselectivity. Park and coworkers have suggested [32] that the rate would be increased by the electronic effect of the α-oxygen atom enhanced by hydrogen bonding with the NH group in the transition state of the aminolysis. The introduction of such an oxygenated acylating moiety into monomers might lead to macromolecules with good average degrees of polymerization in reduced reaction times.

3.1.A/B Polymerization

To reach this goal, amino-ester **1** was synthesized. It presents an ethyl alkoxyacetate moiety suitable for the kinetic study of A/B polymerizations. The presence of the ethylene glycol moiety ensures the solubility of the resulting polymer in organic solvents.

Monomer **1** was obtained in eight steps from allylethoxyethanol (see supporting information). A/B polymerizations were carried out at 80 °C [33],either in bulk or in solvents (Scheme 1). All the reactions were run during 240 hours; this time was determinedas optimal to reach good DPn values in our previous work [19].



Scheme 1. A/B polymerization involving amino-ester.

The low number of methylene groups included in the backbone of **1** did not allow the purification of the corresponding polymer by precipitation. After filtration of the catalyst, work-up was realized by washing the polymer. Organic extracts obtained after washing were analyzed by proton NMR, confirming that they did include only a few residual monomer and no trace of polymer,no signal corresponding to an amide function was observed.The high yield observed for the isolated products are consistent with this observation.

The resulting sticky oil was analyzed by SEC to evaluate the molecular properties of the polymer.The average molecular masses in number were confirmed by ^{1}H NMR [34],by comparing the integration of the signals relative to the methylene groupsattached to the NH of the amide function (*CH*$_2$NHCO), with those relative to the methylene groups in αposition of ester terminations (O*CH*$_2$COOEt).The results fell in the same range, confirming the viability of the SEC sequence despite the lack of SEC references for polyamides.

The main results are compiled in Table 1.

Table 1
Molecular properties of synthesized A/B-type polymers with monomer **1**.

	Conditions	M_n [b]	M_n [c]	M_w [b]	PDI [b]	Yield	DPn[b]
a	Bulk	2020	2530	3050	1.50	71%	11.7
b	Glyme	1750	2320	3270	1.90	79%	10.1
c	Ph_2O, 10 mbars	2540	3250	4160	1.60	90%	14.7
d	Bulk, 10 mbars	3160	3880	5140	1.60	89%	18.3
e[a]	Bulk, 10 mbars	450	-	1260	2.80	-	2.6

[a]blank experiment performed without catalyst.[b] determination by SEC.[c] determination by ^{1}H NMR.

The SEC analyses clearly highlighted the fact that monomer **1** was a good candidate for enzymatic polymerization. The bulk reaction, at 80 °C under atmospheric pressure (entry a), enabled the isolation of a polymer showing good properties, with a DPn equal to 11.7. This good reactivity was retained in solvent, as illustrated by the experiment performed in glyme (entry b) (DPnclose to 10).

The use of reduced pressure, either in a high-boiling solvent (entry c) or in bulk (entry d) confirmed the suitability of **1** for enzymatic reaction. In both cases, the isolated polymers presented very good average degrees of polymerization, higher than those already reported in diphenyl oxide for equivalent reaction times [19-20]. Reaction in bulk under 10 mbars led to a DPn higher than 18 units (entry d). This value, which ranges among the best DPn reported for related enzymatic synthesis of polyamides, has to be linked with the impressive yield in isolated polymer close to 90%. The 1.60 value of the polydispersity index is rather good for an enzymatic polymerization. All these results clearly underline the compatibility of **1** with the used enzymatic polymerization conditions. Futhermore, polymerization of **1** without catalyst failed to give a polymer with a DPn higher than 2.6 units (entry e).

3.2.Rate of reaction.

The polymerizations involving monomer **1** were monitored by ^{1}H NMR. The evolution of the conversion versus time was evaluated by the ratio of the integration of the signals of

OCH_2CO_2Et terminations to that of the methylene protons in α -position relative to the amide nitrogen atom.

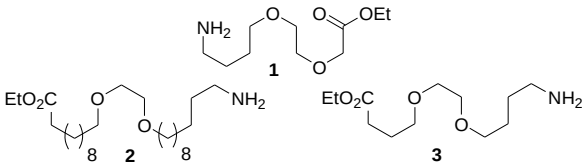
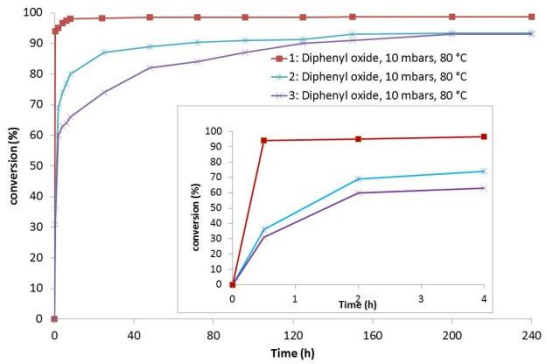


Fig 1. Amino-esters structures.

The polymerization in diphenyl oxide under reduced pressure was compared with the polymerization of monomers **2** and **3** (Fig 1), already described in our previous study as highly efficient [19]. The results are gathered in graph 1.



Graph 1. Evolution of the conversion of amino-esters **1**, **2** and **3** all along polymerizations under reduced pressure in Ph_2O.

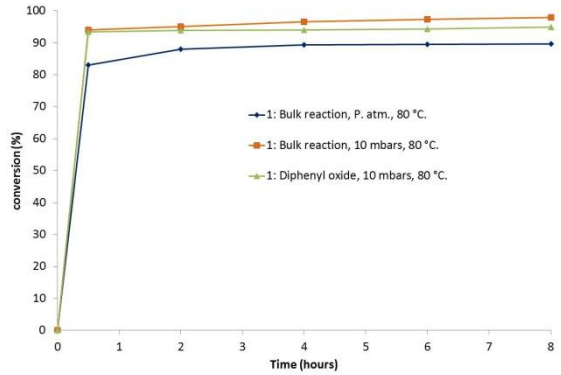
This monitoring clearly highlighted the positive impact of the alkoxyacetate moiety in **1** on the rate of polymerization. The conversion of **1** reached 93% after only 30 min. The direct comparison with amino-esters **2** and **3** is even more representative of this dramatic effect, as conversion after 30 min reached only 36% for **2** and 31% for **3** that is three times less than for **1**.

If the gap was reduced as the polymerization evolved, a major difference still remained. Between 2 and 8 hours, the conversion of **1** varied from 95 to 98%, and remained near this

level after 240 h. The rate of conversion of **2**only reached 80% after 8 h, to gain 10% more at the end of the reaction. The evolution of the polymerization of **3** was slower, going from 66% conversion after 8 h to reach 93% at the end. The slight difference in reactivity between **2** and **3** could be correlated to the high methylene content of **2**, depicted by Gross and coworkers as responsible of an important enhancement of the rate of polymerization in the case of oxygenated polyesters [35].

In light of these results, it became obvious that the use of **1** in an enzymatic polymerization procedure did not require long reaction times to be fully efficient and to lead to a well-featured polymer, in opposition to monomers **2** and **3**.

This enhanced rate is not linked to the experimental conditions, as demonstrated in graph 2, which shows the evolution of the conversion of **1** during the first 8 h of the polymerization in bulk under atmospheric pressure, under reduced pressure, and in diphenyl oxide under reduced pressure.



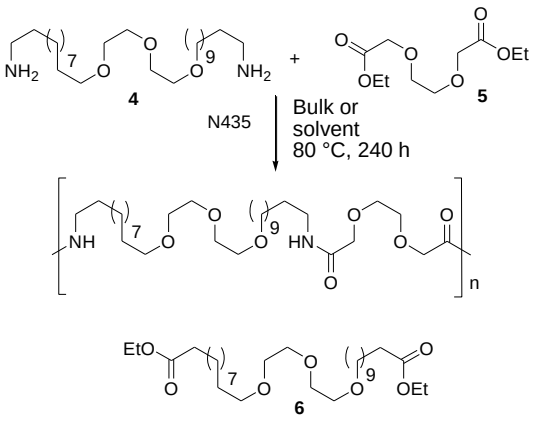
Graph 2. Evolution of the conversion of amino-ester **1** during the first 8 h of polymerization under different conditions.

Reactions at 10 mbars, either in bulk or in Ph_2O present similar profiles. The conversion of **1** reached a value close to 93% after only 30 min. The reaction in bulk under atmospheric pressure did not reach such a high level, but the observed conversion was 83% after 30 min, to end at 90% in 8 h. It seems so that the difference in term of conversion is only related to the

benefit of working under 10 mbars pressure, and that the ensuing kinetic improvement could be observed whatever the experimental conditions.

3.3.AA/BB Polymerization

In parallel with this study, the possibility to transpose the rate enhancement observed in A/B reactions to a AA/BB sequence has been investigated using thediester**5**which presents two ethyl alkoxyacetate moieties.



Scheme 2.AA/BB polymerization involving diester**5** and **6**.

Diamine**4** was selected as reactant. It presents an oxygenated moiety to insure the solubility of the resulting polymers, and a high methylene contents.Experimental conditions involving reduced pressure in presence of Ph_2O were selectedto insure a good diffusion of the monomers all along the reaction and to prevent an inconvenient elevation of the viscosity [19]. The molecular properties of the products are depicted in Table 2.

Table 2
Molecular properties of synthesized AA/BB type polymers with monomers **4** and **5**.

	Conditions	Yield	M_n	M_w	PDI	DPn
a	Ph_2O, 240 h, 10 mbars	89%	7350	11770	1,60	12.5
b	Ph_2O, 240 h, 10 mbars[a]	-	-	-	-	2.6

[a] Blank experiment in the absence of enzyme.

As in the previous examples, the use of reduced pressure dramatically increased the efficiency of the reaction [19].This time, a polymer with a DPn equal to 12.5, and a polydispersity index

of 1.60 was isolated in very good yield. Despite the fact that these results did not reach the highest values of DPnand PDIthat had already been reported, the compatibility of diester**5** with the enzymatic procedure makes no doubt. The polymer produced from **4** and **5** could be precipitated at the end of the reaction, due to the high hydrophobic character of diamine**4**.

The AA/BB reactions were also monitored. However, the NMR methodology could not be applied to the reactions of diamine**4** with diesters**5** and **6** due to the impossibility to determine the exact nature of the end-groups of the polymeric chains during the polymerization. The reaction was therefore followed by the injection in size-exclusion chromatography of small aliquots withdrawn from the flask to determine the average degree of polymerization of the sample. The plot of DPn versus time during the first hours of reaction is shown in Table 3.

Table 3
Evolution of the DPn of polymers resulting from the reaction between diamine**4** and diester**5** and **6** under reduced pressure in Ph_2O.

Monomers	DPn (1 h)	DPn (3 h)	DPn (8 h)	DPn (20h)	DPn[a](240 h)
4+5	2.8	3.1	3.3	6.3	12.5
4+6	2.3	3.1	3.7	3.9	16.7

[a]Isolated product.

The major improvement observed in the case the A/B reaction was no more observed in the AA/BB experiments involving diamine**4** and diester**5**as compared to **6.** Indeed during the first hours, a slight difference could be detected as the copolymerization with **5** led to a polymer with a DPn equal to 2.8, whereas in the case of **6,**the DPn was only 2.3. The small gap no longer remained after 8 h**.**The curves crossed again 12 hours later with the polymer issued from **5** showing a slightly better DPn value. The higherDPn observed in the case of diester**6**at the end of the polymerization could be attributed to the influence of the carbon chain-length on the polymerization degree, which is in agreement with Gross and coworkers [14].

It is difficult to rationalize this absence of acceleration. An inhibition of the “key and lock” interaction predicted by Park and co-workers, caused by the structure of one of the monomers might be suspected.

The polymer obtained from **4** and **5**was purified by precipitation to give a solid residue,thelatterwas thermally characterized through an ATG analysis.

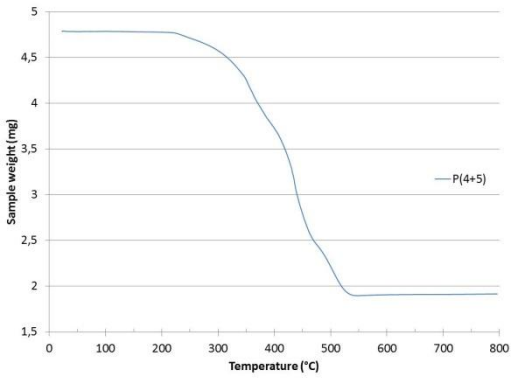


Fig2. Thermogravimetric degradation profile of polymer **P(4+5)**.

The degradation of the polyetheramide**P(4+5)** took place in one step comprising between 290 and 510 °C (Fig 2). These data are in good agreement with already reported values [19-20] and confirmed the good thermal behavior of such a class of polymers, which can face those obtained from Nylons synthesized using more conventional pathways [36-37].

The potential crystalline behavior of **P(4+5)** was then investigated by differential scanning calorimetry (Fig 3).

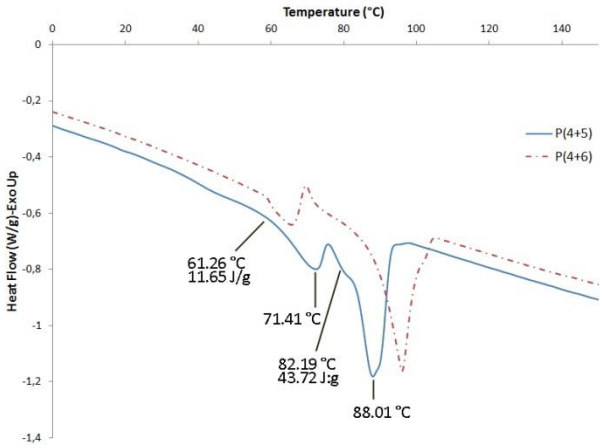


Fig3.DSC curves ofpolymers**P(4+5)**and **P(4+6)**.

The presence of two melting temperatures (71.41 °C and 88.01 °C) is in agreement with a semi-crystalline character for **P(4+5)**. This observation might also argue in favor of the co-existence of two types of supramolecular organizations, resulting from the presence of different intermolecular interactions between polymeric chains [38]. Such behavior has been already observed in the case of **P(4+6)**[19], which DSC curves is also showed in the figure 3.

4. Conclusion

Inserting an ethyl alkoxyacetate moieties in the backbone of A/B type monomer dramatically accelerates the formation of polyamides catalyzed by the lipase B of *Candida antarctica*. The "activated" amino-ester monomer allows to reach in 30 min conversion rates three times higher than those observed with conventional monomers, whatever the experimental conditions. The efficient polymerization procedures give rise to soluble polymers whose molecular, thermic and crystalline properties reach the best standard reported in this field.With this improvement, enzymatic polymerization becomes kinetically compatible with free radical-mediated racemization. This is a step forward towards the development of an efficient ITC procedure for the synthesis of optically active polyamides starting from racemic monomer.

Acknowledgment

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, atdoi:

References

[1] Joachimi D. Kunstoffe 2005;95:86.

[2] Kricheldorf HR, Boehme S, Schwarz G. Macromolecules2002;34:8879.

[3] Onciu M. J. Appl. Polym. Sci.2006;103:2013.

[4] Hsiao H, Lin KH. Polymer 2004;45:7877.

[5] Kobayashi S, Uyama H, Kimura S. Chem. Rev. 2001;101:3793.

[6] Gross RA, Kumar A, Kalra B. Chem. Rev. 2001;101:2097.

[7] Kobayashi S, Makino A. Chem. Rev. 2009;109:5288.

[8] Cheng HN, Gross RA. Polymer biocatalysis and Biomaterials, *ACS Symposium series*, **900**, in: Cheng HN, Gross RA. *Polymer Biocatalysis and Biomaterials*, American chemical society, Washington DC (2005), p. 1.

[9] Cheng HN, Gross RA. Polymer biocatalysis and Biomaterials, *ACS Symposium series*, **999**, in: Cheng HN, Gross RA. *Polymer Biocatalysis and Biomaterials II*, American chemical society, Washington DC (2008), p. 1.

[10] Baum I,Elsässer B, Schwab LW, Loos K,Fels G. ACS Catal. 2011;1:323.

[11] Varma IK, Albertsson AC, Rajkhowaa R, Srivastava RK. Prog. Polym. Sci. 2005;30:949.

[12] van Buijtenen J, van As BAC, Verbruggen M, Roumen L, Vekemans JAJM, Pieterse K, Hilbers PAJ, Hulshof L, Palmans ARA, Meijer EW. J. Am. Chem. Soc. 2007;129:7393.

[13] Schwab LW, Kroon R, Schouten AJ, Loos K. Macromol. Rapid. Commun. 2008;29: 794.

[14] Azim A, Azim H, Sahoo. B, Gross RA. Polym. Mater. Sci. Eng. 2005;93:743.

[15] Cheng HN, Maslanka WW, Gu QM. US 6677427;2004.

[16] Sharma S, Azim A, Azim H, Gross RA, Zini E, Focarete ML, Scandola M. Macromolecules 2007;40:7919.

[17] Poojari Y, Clarson SJ. Macromolecules 2010;43:4616.

[18] Palsule AS, Poojari Y. Polymer 2010;51:6161.

[19] Poulhès F, Mouysset D, Bertrand MP, Gil G, Gastaldi S. Polymer2012;53:1172.

[20] Poulhès F, Mouysset D, Bertrand MP, Gil G, Gastaldi S. Tetrahedron Asymmetry.2012;23,867.

[21] Nechab M, Azzi N, Vanthuyne N, Bertrand M, Gastaldi S, Gil G. J. Org. Chem. 2007;72:6918.

[22] Nechab M, El Blidi L, Vanthuyne N, Gastaldi S, Bertrand MP, Gil G. Org. Biomol. Chem. 2008;6:3917.

[23] Bottalla AL, Queroy S, Azzi-Schue N, Vanthuyne N, Gastaldi S, Bertrand MP, Gil G. Tetrahedron: Asymmetry 2009;20:2823.

[24] Gastaldi S, Escoubet S, Vanthuyne N, Gil G, Bertrand MP. Org. Lett. 2007;9:837.

[25] El Blidi L, Nechab M, Vanthuyne N, Gastaldi S, Bertrand MP, Gil G. J. Org. Chem. 2009;74:2901.

[26] El Blidi L, Vanthuyne N, Siri D, Gastaldi S, Bertrand MP, Gil G. Org. Biomol. Chem. 2010;8:4165.

[27] Poulhès F, Vanthuyne N, Bertrand MP, Gastaldi S, Gil G. J. Org. Chem. 2011;76:7281.

[28] van Buijtenen J, van As BAC, Meuldijk J, Palmans ARA, Vekemans JAJM, Hulshof LA, Meijer EW. Chem. Commun. 2006;3161.

[29] van As BAC, van Buijtenen J, Mes T, Palmans ARA, Meijer EW. Chem. Eur. J. 2007;13:8325.

[30] Kanca U, van Buijtenen J, van As BAC, Korevaar PA, Vekemans JAJM, Palmans ARA, Meijer EW. J. Polym. Sci., Part A: Pol. Chem. 2008;46:2721.

[31] Balkenhohl F, Ditrich K, Hauer B, Ladner W. J. Prakt. Chem. 1997;339:381.

[32] Cammemberg M, Hult K, Park S. ChemBioChem 2006;7:1745.

[33]] Kumar A, Gross RA. Biomacromolecules 2000;1:133.

[34] Izunobi JU, Higginbotham CL. J.Chem. Educ. 2011;88:1098.

[35] Gross RA, Sahoo B, Bhattacharya A, Fu H, Gao W. Polymeric Materials: Science & Engineering 2005;93:745

[36] Cui X, Yan D, Xiao D. e-Polymers 2004;068.

[37] Preston J. in *Encyclopedia of Polymer Science and Technology*, Vol. Sup. 2, ed. Bikales NM, Mark HF, Gaylord NG. Eds, Interscience, New York, 1977, p. 84.

[38] For similar observations on *n*-alkylthiols monolayers, see: Porter MD, Bright TB, Allara DL, Chidsey CED. J. Am. Chem. Soc. 1987;109:3559.