Biotechnology Letters, Vol 19, No 4, April 1997, pp. 307–309

Enzymatic polymerization catalyzed by surfactant-coated lipases in organic media

Sadafumi Noda, Noriho Kamiya, Masahiro Goto* and Fumiyuki Nakashio

Department of Chemical Science & Technology, Faculty of Engineering, Kyushu University, Fukuoka 812–81, Japan

Structural ring-opening of lactones driven by enzymatic polymerization has been performed using low concentration dosages of surfactant-coated lipases in organic media. By comparison, enzymatic polymerization rate with coated lipase proceeded at a rate 100-fold better than native powder. Similarly a higher polymeric molecular weight (21,300), narrow dispersity (Mw/Mn = 1.9) and better conversion (100%) were obtained following polyesterification tests with surfactant-coated lipase.

Introduction

Recently, research on enzymatic polymerization in organic media has attracted much attention as a new procedure for synthesizing polymers (Uyama *et al.*, 1995). Generally enzymes possess high selectivity for specific substrates and their reactions proceed under mild conditions. In organic media, such properties of enzymes become more important because the usage of lipase in nonaqueous media facilitates synthesis reactions such as esterification which is the reverse reaction in aqueous media (Klibanov, 1986). Furthermore, polyesterification using lipases is another interesting application. However, in the most of research work on enzymatic polymerizations the obtained polymers have had low molecular weights (MacDonald *et al.*, 1995; Chaudhary *et al.*, 1995). Lately, Uyama and co-workers (1995) have reported that polymers with a high molecular weight are simply produced in enzymatic ring-opening polymerization. However, in these documented reactions, a considerably higher dosage of lipase is required to obtain a satisfactory reaction rate and conversion. Further, Mw/Mn values of polymers were over 2.0 for the most part due to the heterogeneous reactions concomitant with powder lipases. Surfactant-coated lipases are easily soluble in hydrophobic organic solvents and show better catalytic activity compared with native lipase during interesterification and enantioselective esterification (Goto *et al.*, 1995; 1996).

Enzymatic ring-opening polymerization of lactones in organic media is one of the interesting routes to polymer-forming reaction. In the present study, we have performed ring-opening polymerization of three lactones which have different ring size by using surfactant-coated lipases. We focused on the effects of the ring size of lactones and the reaction temperature on product molecular weight and conversion in the polymerization using the surfactant-coated lipases. Furthermore, the polymerizaton reaction rate with surfactant-coated lipases has been studied with a view to draw a comparison with results obtainable in native powder lipases.

Experimental methods

Materials and method

Lipase from *Pseudomonas cepacia* (PS) was employed in the present study. The lipase was kindly supplied by Amano Pharmaceutical Co., Ltd. All other chemicals used in this work were obtained commercially and were of analytical grade. All solvents were of the highest purity commercially available and were dried with 3Å molecular sieves prior to use. The nonionic surfactant, glutamic acid dioleyl ester ribitol amid, required for coating the lipase was synthesized as described in the previous paper (Goto *et al.*, 1994). The preparation of surfactant-coated lipase was conducted according to the previous paper (Kamiya *et al.*, 1995). Native lipase was used after lyophilizing in a phosphate buffer solution (pH 6.9). The lyophilized lipase was previously equilibrated under a saturated LiCl solution at 4°C for 24 hours. A typical run was as follows: 1 mmol of a lactone, 1 ml of cyclohexane and 0.005 g of lipase were placed in a test tube and sealed. The polymerization was performed in a water bath which was maintained for 72 hours.

Analytical measurements

After the reaction, the volatile solvent was removed by a rotary evaporator. Then the residue was dissolved in

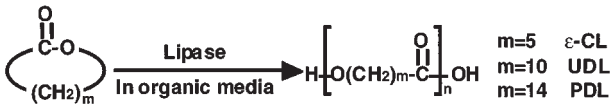

Figure 1 Reaction scheme of ring-opening polymerization.

chloroform and filtered off. The filtrate was analyzed by GPC with a refractive index (RI) detector and an intelligent integrator (Hitachi D-2520) under the following conditions: Shodex GPC K-series column and chloroform eluent at a flow rate of 1.0 ml/min. The calibration curves for GPC analyses were obtained using polystyrene standards (molecular weight: 794, 2360, 3700, 12000, 18700, 44000).

Results and discussion

The effects of monomer structure and temperature on the ring-opening polymerization by the surfactant-coated lipase and the comparison between surfactant-coated and native lipases are summarized in Table 1.

Although a high molecular weight of polymer was expected for the substrate ϵ-CL due to the high ring strain, the molecular weight and conversion were smaller than those of UDL and PDL. This may be due to the inherent low specificity of lipase; hydrophobic substrates were preferred by lipase. Therefore, a hydrophobic substrate was found to be a better monomer for enzymatic polymerization catalyzed by lipases. In fact, results show that the polymerization of PDL was the most favored among the lactones tested from the view point of the product molecular weight. In order to improve the molecular weight of polymer, a relatively high temperature was found to be necessary. The main reason is that high solubility and molecular mobility of the substrates are attainable under slightly higher temperature conditions. These results compared well with those of native powder lipase reported by Uyama and co-workers (1995).

The comparison in the polymerization of PDL between surfactant-coated and native lipases is presented by run

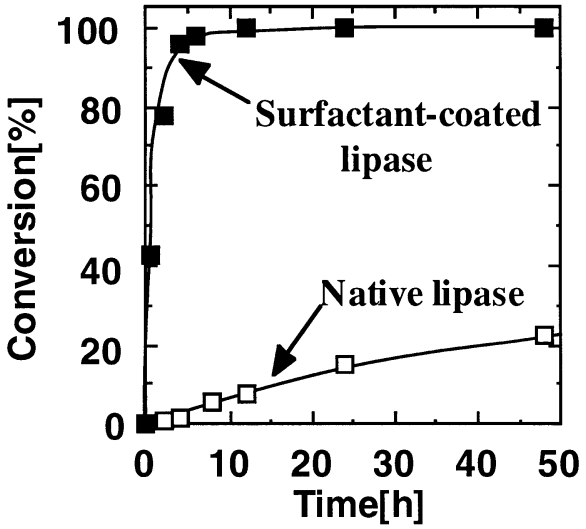

Figure 2 Conversion profile of monomer during the polyesterification of PDL at 60°C.

3 and 7 in Table 1 and Figure 2. These results show that the molecular weight using surfactant-coated lipase was larger than that obtained by using native lipase. Furthermore, the Mw/Mn values using surfactant-coated lipase was lower than the native lipase. Moreover, it is obvious from Figure 2 that the polymerization rate of surfactant-coated lipase was a 100-fold better than that of native lipase. This phenomenon was considered to be arise from the chemical characteristic differences between homogenous and heterogeneous reactions.

The surfactant-coated lipase showed a better catalytic activity for the ring-opening polymerization owing to the higher solubility in the organic solvent. It must be noted that the contained lipase in the reaction medium was less than one tenth of that in an ordinary powder enzymatic system. Although a high conversion could not be attained at a low temperature range (30° or 45°C) for the powder system (MacDonald *et al.*, 1995), it was found that the polymerization of PDL proceeded well even under such mild conditions by using the surfactant-coated lipase.

Table 1 Enzymatic polyesterification by surfactant-coated lipase PS for a 72-hour period.

Run	Lipase	Lactone	Temp.[°C]	Mw ($\times 10^{-3}$)	Mw/Mn	Conv. [%]
1	Surfactant-coated lipase	ϵ-CL	60	3.3	1.4	56
2	Surfactant-coated lipase	UDL	60	12.6	1.9	91
3	Surfactant-coated lipase	PDL	60	21.3	1.9	100
4	Surfactant-coated lipase	PDL	30	3.2	1.6	99
5	Surfactant-coated lipase	PDL	45	8.2	1.8	97
6	Surfactant-coated lipase	PDL	75	5.5	1.6	96
7	Native lipase	PDL	60	9.8	2.1	41

In order to elucidate the effect of surfactant coating on catalytic activity, we conducted a subsequent experiment; the surfactant and native lipase were placed in the reaction mixture separately. The concentrations of the surfactant and lipase were adjusted to the same content in the coated lipase. The results on a molecular weight, dispersity and conversion were comparable to that of native lipase. It is worth mentioning that the coexisting of surfactant molecules in the reaction does not affect enzymatic polymerization in organic media. Thus the coating of lipase with surfactant molecules in the preparation was found to have a significant effect on resultant enzymatic activity.

Conclusions

In ring-opening polymerization, hydrophobic lactones, PDL and UDL, were effectively polymerized by surfactant-coated lipase PS. The polymerization rate was strongly enhanced by coating lipases with surfactant molecules. The coated lipase has been authenticated to be among leading biocatalysts currently available for enzymatic polymerization. Further research is on hand to extend our findings to other polymerization systems.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research (No. 07555245 and 08543267) from the Ministry of Education, Science, Sport and Culture of Japan.

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Received 21 January 1997