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Section: Microbial and Enzyme Technology

**Thiol-functionalized copolymeric polyesters by lipase-catalyzed esterification and transesterification of 1,12-dodecanedioic acid and its diethyl ester, respectively, with 1-thioglycerol**

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**Keywords** Esterification; 1,12-Dodecanedioic acid; Immobilized lipase B from *Candida antarctica*; Poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol); Poly(1,12-dodecanedioic acid-*co*-1-thioglycerol); 1-Thioglycerol (3-mercaptopropane-1,2-diol)

**Abstract** Copolymeric polyoxoesters containing branched-chain methylenethiol functions, i.e. poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol), were formed by lipase-catalyzed polyesterification and polytransesterification of 1,12-dodecanedioic acid and diethyl 1,12-dodecanedioate, respectively, with 1-thioglycerol (3-mercaptopropane-1,2-diol) using immobilized lipase B from *Candida antarctica* (Novozym 435) in vacuo without drying agent in the reaction mixture. After 360 to 480 h, both polyoxoesters were purified by extraction from the reaction mixtures followed by solvent fractionation. The precipitate of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) demonstrated a  $M_w$  of  $\sim 170,000$  Da, whereas a  $M_w$  of  $\sim 7,100$  Da only was found for poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol). Both polycondensates were analyzed by GPC/SEC, alkali-catalyzed transmethylation, NMR- and FTIR-spectrometry.

## Introduction

Various compounds containing thiol groups, such as mercaptoacetic and mercaptopropionic acids as well as mercaptoethanol and 1-thioglycerol (monothioglycerol; 3-mercaptopropane-1,2-diol), are used as antioxidants. Polyfunctional thioalkanols, e.g., 1-thioglycerol, dithiothreitol and others, may also serve as starting materials for the preparation of polyesters (polyoxoesters, PE) containing free thiol functions or polythioesters. There is increasing interest in such sulfur-

containing functional polymers for various applications. For example, sulfur-containing polyesters may be suitable as antioxidative ingredients for polymers, coatings, paints and lubricants and as polymers with antibacterial properties (Kúdelka et al. 1985; Uhrich 2003). PE having high molecular weights are formed by biotransformation of hydroxy alkanolic acids in microorganisms (Kim and Lenz 2001; Steinbüchel and Hein 2001) or by lipase-catalyzed esterification and transesterification of  $\alpha,\omega$ -alkanedioic acids and their methyl esters, respectively, with  $\alpha,\omega$ -alkanediols in vitro (Kazlauskas and Bornscheuer 1998; Kobayashi and Uyama 2001; Rehm 2006). In contrast to polythioesters, polyoxoesters are more easily decomposed by microbial hydrolases such as lipases or depolymerases (Jendrossek 2001; Tokiwa and Calabia 2004). This is also expected for PE containing thiol or thioether functions, as compared to polythioesters, which may be of advantage from an environmental point of view. Recently, we have described the preparation of such copolymeric poly(thioalkanedioates) containing thioether groups by lipase-catalyzed esterification and transesterification of 3,3'-thiodipropionic acid and its dimethyl ester with  $\alpha,\omega$ -alkanediols (Fehling et al. 2008). The aim of the present work was to develop an enzymatic process for the preparation of thiol-functionalized polycondensates by esterification and transesterification of 1,12-dodecanedioic acid and its diethyl ester, respectively, with 1-thioglycerol using a commercial immobilized microbial lipase as biocatalyst. The final polycondensates containing methylenethiol groups are expected to have antioxidative properties and may be suitable as, e.g., non-migrating stabilizers of polymers for both food use and technical applications.

## **Materials and methods**

### Lipase-catalyzed preparation of copolymeric PE containing methylenethiol groups

As a typical example, 1,12-dodecanedioic acid (460 mg, 2 mmol) was esterified with 1-

thioglycerol (216 mg, 2 mmol) in the presence of 100 mg Novozym 435 [immobilized lipase preparation from *Candida antarctica* (lipase B; 10,500 propyl laurate units/g; 2% w/w water); kindly provided by Novozymes] by magnetic stirring in a screw-capped tube (placed in a Schlenk vessel) in vacuo at 80°C for periods up to 480 h with water-trapping in the gas-phase using potassium hydroxide pellets. Diethyl 1,12-dodecanedioate (516 mg, 2 mmol) was transesterified with 1-thioglycerol using similar reaction conditions as given above.

#### Gas chromatography (GC)

Aliquots of the reaction mixtures of esterification assays were methylated using an ethereal solution of diazomethane followed by silylation with *N*-methyl-*N*-trimethylsilylheptafluorobutyramide (MSHFBA). The resulting mixtures of dimethyl 1,12-dodecanedioate, silylated 1-thioglycerol as well as various other derivatized reaction products were separated by gas chromatography as described previously (Fehling et al. 2008).

#### Alkali-catalyzed transmethylation

Carboxy end groups of purified poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) were converted to the methyl esters by the addition of an ethereal diazomethane solution. Under standard conditions, 2 ml of 0.5 M methanolic sodium methylate solution were added to ~15 mg of the thiol-functionalized PE and the transmethylation mixture was heated to 50°C for ~15 min. After cooling, the derivatization mixture was neutralized by the addition of 75  $\mu$ l acetic acid followed by concentrated aqueous sodium hydrogen carbonate solution, dried in a stream of nitrogen and finally silylated using MSHFBA reagent. Silylated samples, 1-2  $\mu$ l, were directly injected into gas chromatograph and separated on a J&W DB-5HT column as described previously (Fehling et

al. 2008). This way, methyl esters were formed from carboxy ester groups of PE, whereas hydroxy and thiol groups were converted to the silyl derivatives.

#### GC-MS analyses

Samples withdrawn from the reaction mixtures at various times or low-molecular compounds formed by alkali-catalyzed transesterification of purified thiol-functionalized PE were methylated and silylated as described above. GC-MS analyses of these derivatives were carried out on a Hewlett-Packard model 5890 series II GC/5889A apparatus as described previously (Fehling et al. 2008).

#### Gel Permeation Chromatography / Size Exclusion Chromatography (GPC/SEC)

The reaction products from esterification and transesterification of 1,12-dodecanedioic acid and diethyl 1,12-dodecanedioate, respectively, with 1-thioglycerol were repeatedly extracted for GPC/SEC using tetrahydrofuran (THF; stabilized with 25 mg BHT/l) at 50°C and filtered through a 1  $\mu\text{m}$  PTFE syringe filter. The molecular masses of copolymeric PE containing methylenethiol groups were determined by GPC/SEC using polystyrene standards for calibration as described previously (Fehling et al. 2008).

#### Solvent fractionation

As an example, the combined reaction products (100 mg) from various incubations of 1,12-dodecanedioic acids with 1-thioglycerol were repeatedly extracted with THF at 50°C and filtered through a 1  $\mu\text{m}$  PTFE syringe filter. THF extract was evaporated in a stream of nitrogen at 45°C to a concentration of around 20 mg/ml, *iso*-hexane was added to a final ratio of 2:3 (v/v) and

cooled to  $-20^{\circ}\text{C}$ . Low-molecular constituents were extracted this way from the crude reaction products. The white polymer (60 mg) formed, i.e. poly(1,12-dodecanedioic acid-*co*-1-thioglycerol), was separated by centrifugation. The *iso*-hexane–THF supernatant was isolated to give 40 mg of white precipitate after evaporation of the solvents. The proportions of low- ( $M_{\text{W}} < 600$  Da), medium- ( $M_{\text{W}} > 600 - < 1,500$  Da), and high-molecular ( $M_{\text{W}} > 1,500$  Da) constituents of both fractions were determined by GPC/SEC analysis as described above. Precipitation of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) was also studied using other solvent mixtures such as THF–*iso*-hexane–dichloromethane or THF–*iso*-hexane–*iso*-propanol. Similarly, the combined reaction products of polytransesterification of diethyl 1,12-dodecanedioate with 1-thioglycerol were repeatedly extracted with methyl-*tert*.-butyl ether (MTBE) at  $50^{\circ}\text{C}$  and filtered as described above. Solvent fractionation of poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) reaction mixtures (200 mg) with MTBE–*iso*hexane (1:4) led to 170 mg of PE precipitate and 30 mg of supernatant.

#### FTIR- and NMR-Spectrometry

Fourier Transform Infrared (FTIR) spectra of PE precipitates were obtained using a Bruker IFS 28 spectrometer. Absorbance was measured at a resolution of  $1\text{ cm}^{-1}$  and a total of 30 scans were collected. Solid-state polymer spectra were measured using pressed potassium bromide discs containing  $\sim 1.5\%$  polymer.

$^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker AV400 spectrometer ( $^1\text{H}$ , 400.13 MHz;  $^{13}\text{C}$ , 100.62 MHz) as well as a Varian unity plus 600 instrument ( $^1\text{H}$ , 599.6 MHz;  $^{13}\text{C}$ , 150.8 MHz). The measurements were carried out at 298 K with samples of  $\sim 50$  mg of the PE precipitates isolated from the reaction mixtures by solvent fractionation as described above and dissolved in  $\sim 1$  ml  $d_8$ -THF. In addition, DEPT, gCOSY, gHMBC and gHSQC experiments were performed with the above PE precipitates.



## Results and discussion

Functional polyesters with antioxidative and/or antimicrobial properties are of great current interest as components of synthetic polymers for, e.g., food packaging as well as technical, medical and cosmetic applications. Such properties can be expected for sulfur-containing polycondensates such as polythioesters, polythioethers or polymers containing thiol groups (Kúdelka et al. 1985; Nathan 2007; Shaglaeva et al. 2005; Uhrich 2003). Here we present the Novozym 435-catalyzed preparation of copolymeric polyesters (PE) containing methylenethiol functions which are formed by polyesterification and polytransesterification of bifunctional 1,12-dodecanedioic acid and its diethyl ester, respectively, with trifunctional 1-thioglycerol.

Fig. 1 shows the time-course of Novozym 435-catalyzed polycondensation of 1,12-dodecanedioic acid (Fig. 1a) or diethyl 1,12-dodecanedioate (Fig. 1b) with 1-thioglycerol to the corresponding low- ( $M_w > 600 - < 1,500$  Da) and high-molecular weight ( $M_w > 1,500$  Da) esterification product, i.e. poly(1,12-dodecanedioic acid-*co*-1-thioglycerol), and transesterification product, i.e. poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol). The formation of both copolymers was studied over a period of up to 480 h using GPC/SEC analyses for the determination of the degree of conversion and polymerization (calibration using polystyrene standards). Fig. 1c shows the time course of the increase of  $M_w$  of the above methylenethiol functions-containing PE. The chromatograms were analyzed using a GPC software; the intervals chosen for the separation of the reaction products are given in Fig. 2. Fig. 1d demonstrates the polydispersities ( $M_w/M_n$ ) of the polymeric reaction products at different times.

(Fig. 1)

(Fig. 2)

It is obvious from these results that the proportions of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) ( $M_w > 1,500$  Da) in the reaction mixture increase with time. After an initial increase (24 h), the proportion of oligomers ( $M_w > 600 - < 1,500$  Da) does not rise as is typical of reaction intermediates (Figs. 1 and 2). In addition, the rapid disappearance of both starting materials and oligomeric intermediates ( $M_w < 1500$  Da) indicates that Novozym 435 is highly specific for the esterification of these two groups of reactants. Generally, copolymeric thiol-functionalized PE are formed in high to moderate yield (oligo- plus polyesters  $M_w > 600$  Da, conversion: ~89% for polyesterification and 51% for polytransesterification after 24 h;  $M_w > 1,500$  Da, conversion: ~88% for polyesterification and 31% for polytransesterification after 96 h) as is shown in Figs. 1 and 2. Moreover,  $M_w$  of both copolymeric PE ( $M_w > 600$  Da) steadily increase with time up to ~100,000 Da for poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and up to ~4,400 Da for poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol). However, from Fig. 1b and c, the increase of the polytransesterification product is rather low. Fig. 1c also shows that  $M_w$  of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) decreases after 360 h. This is attributed to insoluble polymers most probably generated by the formation of small proportions of crosslinking disulfide bridges which were detected by NMR analyses (see below).

Table 1 demonstrates that similar enzyme activities were found for the polyesterification and polytransesterification of 1,12-dodecanedioic acid and its diethyl ester, respectively, with 1-thioglycerol using Novozym 435 in the absence of a solvent. The data of 24 h incubations were used to calculate the mean polyesterification and polytransesterification activities of Novozym 435. Substantially higher activity was determined, however, when polyesterification was started after dissolving the reactants in a small volume of MTBE (193.1 vs. 31.6 units/g, after MTBE treatment) – the solvent was removed in vacuo before adding the enzyme – demonstrating that higher solubility of 1,12-dodecanedioic acid is responsible for the increase in esterification activity. Under similar reaction conditions, the addition of MTBE only led to a slight increase of the polytransesterification activity of Novozym 435 using diethyl 1,12-dodecanedioate and 1-

thioglycerol as substrates (Table 1). It is worth noting that the conversion of substrates in the absence of lipase was negligibly low (data not shown).

(Table 1)

Fig. 2 shows the GPC/SEC separation of the reaction products of Novozym 435-catalyzed polyesterification of 1,12-dodecanedioic acid (Fig. 2a) and polytransesterification of diethyl 1,12-dodecanedioate (Fig. 2b) both with 1-thioglycerol after 24, 96 and 360 h and 24, 192 and 360 h, respectively. The dotted lines show the above limits set for oligomeric and polymeric fractions. Generally, copolymeric PE having maximum molecular weights of approximately  $10^7$  Da were formed by polyesterification of 1,12-dodecanedioic acid with 1-thioglycerol and those with maximum molecular weights of up to  $8 \times 10^4$  Da by polytransesterification of diethyl 1,12-dodecanedioate with 1-thioglycerol (Fig. 2a and b).

Copolymeric PE containing methylenethiol groups were purified by solvent fractionation in order to remove the remaining reactants as well as low molecular reaction products. The PE fractions of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) were extracted from the reaction mixtures with THF and filtered. The mainly high-molecular components were precipitated by addition of *iso*-hexane and cooling at  $-20^\circ\text{C}$ . Similarly, crude fractions of poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) were dissolved in MTBE and purified by solvent fractionation from MTBE-*iso*-hexane (1:4, v/v) at  $-20^\circ\text{C}$  (Table 2). Using GPC/SEC analyses, a distinct increase in  $M_w$  of PE precipitates (1,12-dodecanedioic acid derived polyesters:  $8 \times 10^4$  vs.  $1.7 \times 10^5$  Da; diethyl 1,12-dodecanedioate derived polyesters:  $5.4 \times 10^3$  vs.  $7.1 \times 10^3$  Da) is observed when low-molecular compounds are removed by solvent fractionation from the crude polyester fractions (Table 2). The precipitates contained largely uniform copolymeric poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) of  $M_w \sim 170,000$  Da or poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) of  $M_w \sim 7,100$  Da (Table 2). It is obvious from these results that both starting materials and low-molecular esters were largely removed from the PE precipitates, whereas substantial proportions of low-molecular material were concentrated in the supernatants.

Experiments performed to increase  $M_w$  by precipitation of the polymeric fractions using other solvent mixtures did not lead to PE with higher  $M_w$  (Table 2).

(Table 2)

Precipitates of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) having highest  $M_w$  (Table 2) were chosen for FTIR as well as  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR analyses. FTIR spectra of the thiol-functionalized PE showed the carbonyl band of the carboxy ester groups at  $1740\text{ cm}^{-1}$  ( $\nu$ ,  $\text{C}=\text{O}$ , strong) as well as typical thiol absorption at  $2571\text{ cm}^{-1}$  ( $\nu$ ,  $\text{S}-\text{H}$ , weak), as is characteristic for thiol-containing compounds including polymers (Yu et al. 1996). Typical  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR resonances were observed for the two methylenethiol-functionalized PE formed by polyesterification or polytransesterification. The spectra of the two PE precipitates, i.e. poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol), demonstrate very close sets of chemical shifts and indicate only some variations with regard to the ratio of integration. For example, the PE precipitate from the esterification of 1,12-dodecanedioic acid with 1-thioglycerol shows characteristic resonances at:  $\delta_{\text{H}}$  5.01 (m,  $[\text{CH}_2]_2-\text{CH}-\text{O}$ ); 4.32 (dd,  $^2\text{J}=11.9\text{ Hz}$ ,  $^3\text{J}=3.7\text{ Hz}$ ,  $\text{O}-\text{CH}_2-\text{CH}$ ); 4.15 (dd,  $^2\text{J}=11.9\text{ Hz}$ ,  $^3\text{J}=5.5\text{ Hz}$ ,  $\text{O}-\text{CH}_2-\text{CH}$ ); 2.7 (m,  $\text{CH}_2-\text{SH}$ ); 2.28 (m,  $\text{CO}-\text{CH}_2$ ); 1.89 (tr,  $^3\text{J}=9.0\text{ Hz}$ ,  $\text{CH}_2-\text{SH}$ ); 1.58 (m,  $\text{CO}-\text{CH}_2-\text{CH}_2$ ); 1.30 (m,  $-\text{[CH}_2\text{-]}_n$ );  $\delta_{\text{C}}$  197.7 ( $-\text{CO}-\text{S}-$ ); 173.5-172.6 ( $-\text{CO}-\text{O}-$ ); 73.24 ( $[\text{CH}_2]_2-\text{CH}-\text{O}$ ); 63.78 ( $\text{O}-\text{CH}_2-\text{CH}$ ); 34.5 ( $\text{CO}-\text{CH}_2$ ); 30 ( $-\text{[CH}_2\text{-]}_n$ ); 25.78 ( $\text{CO}-\text{CH}_2-\text{CH}_2$ ); 25.22 ( $\text{CH}_2-\text{SH}$ ). NMR data also reveal that the polyester prepared from diethyl 1,12-dodecanedioate and 1-thioglycerol additionally contains chains with terminal carboxyethyl groups [ $\delta_{\text{H}}$  4.04 ( $\text{O}-\text{CH}_2-\text{CH}_3$ ) and 1.19 ( $\text{O}-\text{CH}_2-\text{CH}_3$ );  $\delta_{\text{C}}$  60.4 ( $\text{O}-\text{CH}_2-\text{CH}_3$ ) and 14.58 ( $\text{O}-\text{CH}_2-\text{CH}_3$ )].

The intensity of the carbon resonances at  $\delta_{\text{C}}$  197.7 ppm (*thioester*) is generally low as compared to the resonances at  $\delta_{\text{C}}$  173 ppm (*oxoester*) demonstrating rather small proportions of thioester linkages in comparison with both primary and secondary oxoester groups. These results are in good agreement with the chemical structures of poly(1,12-dodecanedioic acid-*co*-1-

thioglycerol) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol). The NMR resonances at  $\delta_{\text{H}}$  2.69 as well as  $\delta_{\text{C}}$  44.5 point at small proportions of disulfide bridges formed by partial interconversion of the polymeric thiol functions into disulfides. This was verified by comparing  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR resonances of the above thiol-functionalized PE with resonances of diisobutyl-disulfid at  $\delta_{\text{H}}$  2.60 and  $\delta_{\text{C}}$  48.6. In addition, the chemical structures of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) were confirmed by DEPT, gCOSY, gHSQC and gHMBC experiments (data not shown). The chemical structure of purified PE precipitates – isolated by solvent fractionation as described above – was also analyzed by alkaline methanolysis followed by silylation. The results of methanolyses demonstrated the formation of dimethyl 1,12-dodecanedioate and silylated 1-thioglycerol as confirmed by GC and GC-MS analyses. Finally, the results of FTIR, NMR and alkaline methanolysis reveal that both polymers predominantly consist of polyoxoesters containing branched-chain methylenethiol groups.

Interestingly, preferential *O*-acylation was also observed for the lipase-catalyzed transesterification of various ethyl alkanooates with 1-thioglycerol, dithiothreitol and other hydroxyalkanethiols (Baldessari et al. 1993; Iglesias et al. 1998). Moreover, copolymeric polyoxoesters are commonly obtained by enzymatic polyesterification or polytransesterification of various alkanedioic acids or alkanedioates with alkanediols using immobilized lipases such as lipase B from *C. antarctica* (Gross et al. 2001; Kazlauskas and Bornscheuer 1998), whereas  $\alpha,\omega$ -end thiol-functionalized PE are formed by lipase-catalyzed ring-opening polymerization of  $\omega$ -alkanelactones with various mercaptoalkanols (Takwa et al. 2008).

Recently, we described various environmentally friendly, solvent-free lipase-catalyzed preparations of sulfur-containing antioxidants and polycondensates such as dialkyl 3,3'-thiodipropionates as well as copolymeric polythioesters and polyesters containing thioether groups using immobilized lipases from *C. antarctica* (lipase B; Novozym 435) and *Rh. miehei* (Lipozyme RM IM) as biocatalysts (Fehling et al. 2007; 2008; Weber et al. 2006a; b). Similarly,

enzymatic preparation of thiol-functionalized polycondensates may be of advantage over chemical synthesis because expensive and toxic reagents are mostly applied to the latter one (Maignan 2002; Moad et al. 2007; Watanabe et al. 2000; Wilczewska et al. 2002) whereas no chemical activators are used in the polyesterification and polytransesterification reactions described here. Moreover, chemical side-reactions are limited to a minimum and only small proportions of organic solvents may be necessary in some cases to disperse and/or dissolve the reactants at the beginning of the enzymatic conversion. Polyoxoesters are widely degraded by microbial hydrolases (Jendrossek 2001; Tokiwa and Calabia 2004). This is expected for polyoxoesters containing methylenethiol groups as well, whereas polythioesters are hardly perceptible to microbial degradation (Steinbüchel 2005).

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## Figure legends

**Fig. 1** Time course of the Novozym 435-catalyzed polyesterification and polytransesterification of 1,12-dodecanedioic acid and diethyl 1,12-dodecanedioate, respectively, with 1-thioglycerol to the corresponding polycondensates containing methylenethiol functions as calculated from GPC/SEC chromatography data. **(a)** Formation of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol), weight average molecular mass ( $M_W$ ) >1,500 Da ( $\circ$ ), and of the corresponding oligomeric esters,  $M_W$  >600 – <1,500 Da ( $\bullet$ ). **(b)** Formation of poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol),  $M_W$  >1,500 Da ( $\square$ ) and of the corresponding oligomeric esters,  $M_W$  >600 – <1,500 Da ( $\blacksquare$ ). **(c)** Time course of the increase of  $M_W$  of thiol-functionalized polycondensates formed by Novozym 435-catalyzed ( $\bullet$ ) polyesterification and ( $\circ$ ) polytransesterification of 1,12-dodecanedioic acid and diethyl 1,12-dodecanedioate, respectively, with 1-thioglycerol as determined by GPC/SEC. **(d)** Polydispersity ( $M_W/M_n$ ) of ( $\bullet$ ) poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and ( $\circ$ ) poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) at different times. The chromatographic conditions for GPC/SEC separation are given in Materials and methods. Values are given as mean  $\pm$  SEM ( $n = 4$ )

**Fig. 2** GPC/SEC chromatogram of typical reaction products of the Novozym 435-catalyzed polyesterification and polytransesterification of **(a)** 1,12-dodecanedioic acid and **(b)** diethyl 1,12-dodecanedioate, respectively, with 1-thioglycerol after 24, 96 and 480 h or 24, 192 and 480 h: polymeric fractions ( $M_W$  >1,500 Da), <23.7 ml elution volume; oligomeric fractions ( $M_W$  >600 – <1,500 Da), >23.7 – 25.4 ml elution volume; starting materials ( $M_W$  <600 Da), >25.4 ml elution volume. The chromatographic conditions for GPC/SEC separations are given in Materials and Methods. The dotted lines show the limits of oligomeric and polymeric fractions as compared with polystyrene standards

**Table 1** Lipase activities of immobilized lipase B from *Candida antarctica* (Novozym 435) used for the polyesterification and polytransesterification of 1,12-dodecanedioic acid and diethyl 1,12-dodecanedioate, respectively, with 1-thioglycerol

Substrates	Solvent MTBE	Lipase activity <sup>a</sup> [units/g $\pm$ SEM] (n = x)
1,12-Dodecanedioic acid		
1-thioglycerol	without	30.0 <sup>b</sup> $\pm$ 5.4 (n=4)
1-thioglycerol	with	193.1 <sup>c</sup> $\pm$ 13.2 (n=8)
Diethyl 1,12-dodecanedioate		
1-thioglycerol	without	18.3 <sup>b</sup> $\pm$ 1.8 (n=2)
1-thioglycerol	with	31.6 <sup>c</sup> $\pm$ 3.6 (n=2)

<sup>a</sup> Esterification units were calculated from the initial rates (24 h) of polyesterification or polytransesterification reactions, as determined by GPC/SEC (see below). One esterification unit of enzyme activity was defined as the amount of enzyme (g) that produced 1  $\mu$ mol of monoester/min, irrespective of the position of the esterified hydroxy group at the glycerol backbone. Small proportions of thioesters formed were also calculated as “esterification units”. n=x, number of assays

<sup>b</sup> Assay conditions: as given in Materials and methods. Samples of the reaction products were withdrawn at various intervals, extracted with tetrahydrofurane at 50°C and filtered through a 1  $\mu$ m PTFE syringe filter to separate the biocatalyst. Aliquots of the filtrate were analyzed by GPC/SEC

<sup>c</sup> Same assay conditions, but before starting the reaction, ~0.5 mL methyl *tert*-butyl ether (MTBE) was added to dissolve diethyl 1,12-dodecanedioate or partially dissolve 1,12-dodecanedioic acid. After 10 min of incubation at 40°C, MTBE was evaporated in vacuo followed by drying at 1 kPa (40°C; 10 min). The reaction was started by adding the immobilized lipase and a moderate vacuum (80 kPa) was used to remove reaction water or ethanol. Incubations without Novozym 435 were used as blanks

**Table 2** Determination of molecular weight by GPC/SEC in various fractions of poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) obtained by solvent fractionation

Fractions	Poly(1,12-dodecanedioic acid- <i>co</i> -1-thioglycerol)						Poly(diethyl 1,12-dodecanedioate- <i>co</i> -1-thioglycerol)					
	M <sub>w</sub> range (%)			M <sub>w</sub>	M <sub>n</sub>	M <sub>w</sub> /M <sub>n</sub>	M <sub>w</sub> range (%)			M <sub>w</sub>	M <sub>n</sub>	M <sub>w</sub> /M <sub>n</sub>
	<600 Da	>600 – <1.500 Da	>1.500 Da				<600 Da	>600 – <1.500 Da	>1.500 Da			
Crude polycondensate <sup>a</sup>	6.3	7.1	86.6	80,700	3,890	20.8	9.8	19.0	71.2	5,380	1,880	2.9
Supernatant 1 <sup>b</sup>	24.2	29.0	46.8	2,060	1,150	1.8	38.1	43.8	18.1	1,140	790	1.4
Supernatant 2 <sup>c</sup>	16.4	20.8	62.8	3,720	1,490	2.5	–	–	–	–	–	–
Supernatant 3 <sup>d</sup>	17.6	20.2	62.2	3,750	1,490	2.5	–	–	–	–	–	–
Precipitate 1 <sup>b</sup>	2.3	1.8	95.9 <sup>e</sup>	168,000	11,200	15.0	4.6	13.3	82.1 <sup>f</sup>	7,100	2,830	2.5
Precipitate 2 <sup>c</sup>	2.4	1.8	95.8 <sup>e</sup>	93,400	9,130	10.2	–	–	–	–	–	–
Precipitate 3 <sup>d</sup>	2.4	1.7	95.9 <sup>e</sup>	111,000	9,150	12.1	–	–	–	–	–	–

<sup>a</sup> After 480 h reaction time; mixed reaction product from 4 assays

<sup>b</sup> Poly(1,12-dodecanedioic acid-*co*-1-thioglycerol) was precipitated from THF-*iso*-hexane (40:60) and poly(diethyl 1,12-dodecanedioate-*co*-1-thioglycerol) from MTBE- *iso*-hexane (20:80), both at -20°C

<sup>c</sup> Precipitated from THF-*iso*-hexane-dichloromethane (40:57:3) at -20°C

<sup>d</sup> Precipitated from THF-*iso*-hexane-*iso*-propanol (40:57:3) at -20°C

<sup>e</sup> Maximum molecular weight calculated from GPC/SEC data:  $1 \times 10^7$  Da

<sup>f</sup> Maximum molecular weight calculated from GPC/SEC data:  $8 \times 10^4$  Da

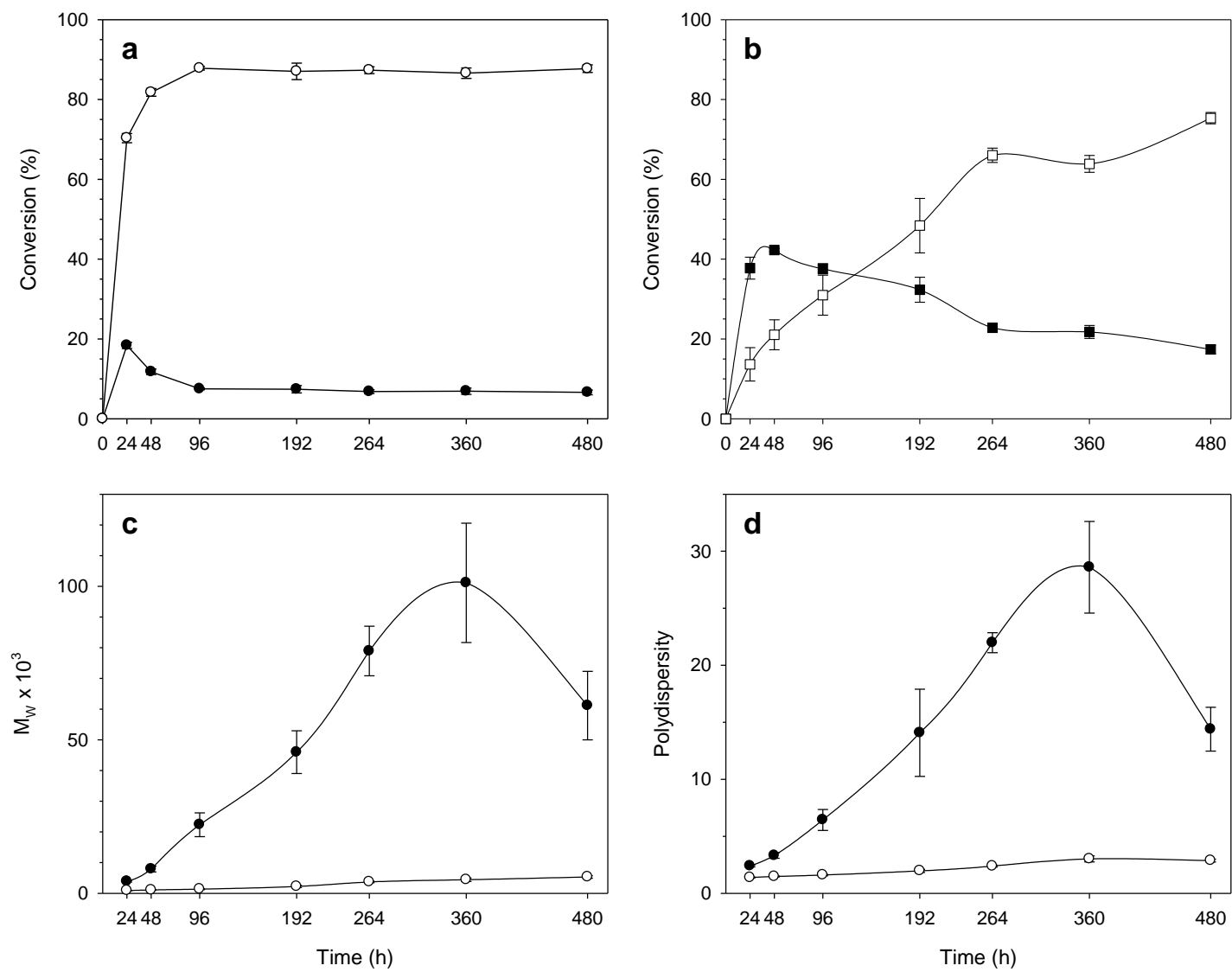


Fig. 1

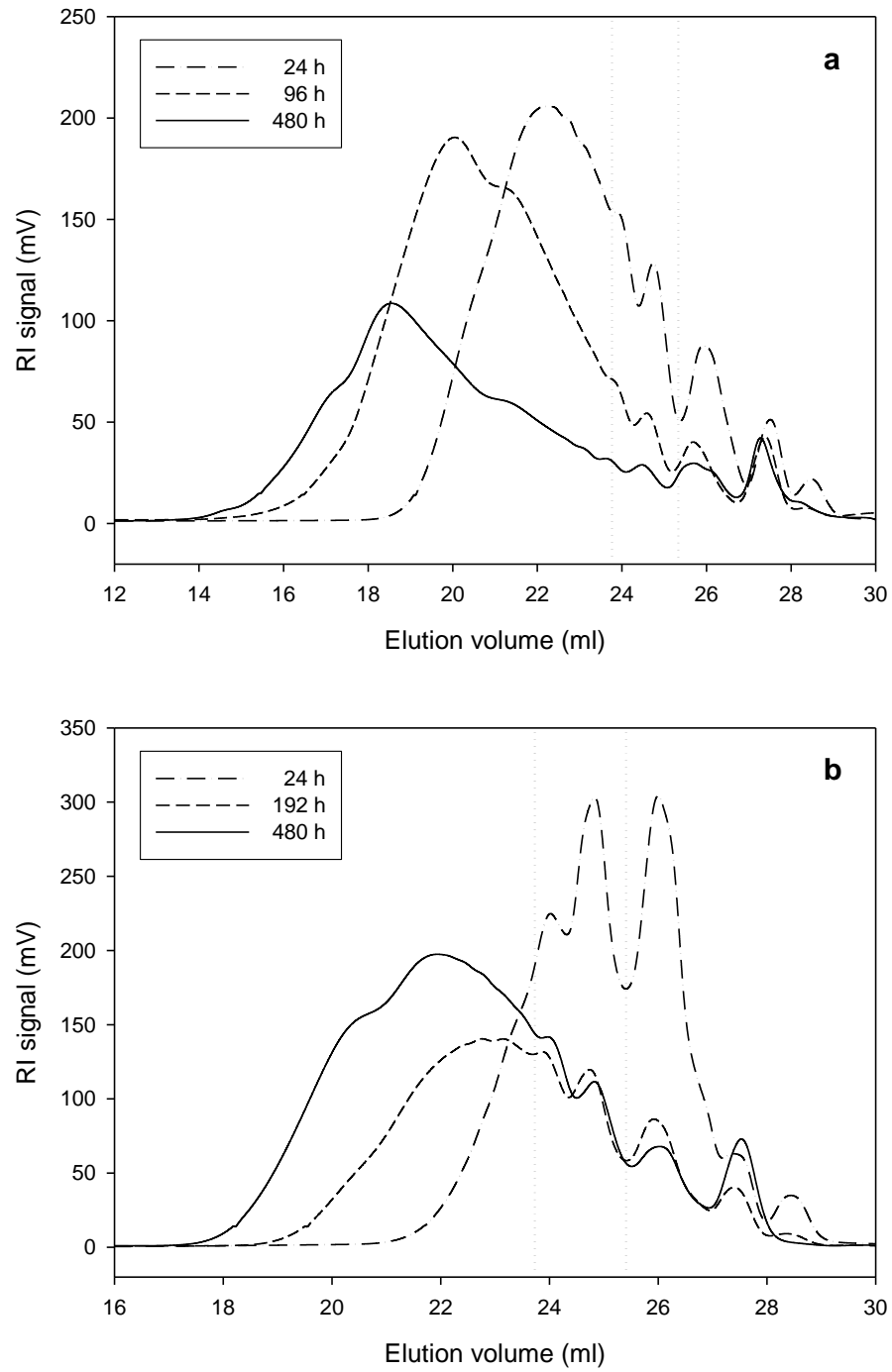


Fig. 2