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Mono-thioesters and di-thioesters by lipase-catalyzed reactions of α,ω-alkanedithiols with palmitic acid or its methyl ester

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Abstract 1-*S*-Mono-palmitoyl-hexanedithiol and 1-*S*-mono-palmitoyl-octanedithiol were prepared in high yield (80–90%) by solvent-free lipase-catalyzed thioesterification of palmitic acid with the corresponding α,ω-alkanedithiols in vacuo. Similarly, 1,6-di-*S*-palmitoyl-hexanedithiol and 1,8-di-*S*-palmitoyl-octanedithiol were prepared in moderate yield (50–60%) by solvent-free lipase-catalyzed thioesterification of palmitic acid with 1-*S*-Mono-palmitoyl-hexanedithiol and 1-*S*-mono-palmitoyl-octanedithiol, respectively. An immobilized lipase preparation from *Rhizomucor miehei* (Lipozyme RM IM) was more effective than a lipase B preparation from *Candida antarctica* (Novozym 435) or a lipase preparation from *Thermomyces lanuginosus* (Lipozyme TL IM). Lipase-catalyzed transthioesterifications of methyl palmitate with α,ω-alkanedithiols using the same enzymes were less effective than thioesterification for the preparation of the corresponding 1-*S*-mono-palmitoyl thioesters.

Introduction

Thioesters are activated esters which are utilized as versatile intermediates in organic chemistry for the preparation of various compounds, e.g., peptides, macrolide antibiotics and other pharmaceuticals (Bianchi and Cesti 1990; Gauthier et al. 1986; Kunieda et al. 1981; Li et al. 1998; Masamune et al. 1975; Reißig and Scherer 1980). Acyl thioesters are active acylation intermediates in biochemical and bioorganic nucleophilic reactions, having higher reactivity and selectivity than the corresponding oxygen analogues (Lambert et al. 1997; Nakajima et al. 1975; Taylor and Weber 1993). Methods for the preparation of acyl thioesters mostly use expensive or highly toxic reagents (Ahmad and Iqbal 1986; Imamoto et al. 1982; Kunieda et al. 1981; Masamune et al. 1975). This disadvantage led to the development of lipase-catalyzed thioesterification and transthioesterification processes for the preparation of acyl thioesters. For example, short-chain flavor thioesters (Caussette et al. 1997; Cavaille-Lefebvre and Combes 1997; Zaks and Klibanov 1985) and long-chain thio-wax esters (Weber et al. 1998, 1999a, 2000) were formed by lipase-catalyzed thioesterification and transthioesterification, respectively, between alkanethiols and carboxylic acids or carboxylic acid esters—usually in organic media with a molecular sieve as an acceptor for water and short-chain alcohols. Such reactions were also efficiently carried out without molecular sieve in vacuo (Weber et al. 1999b).

In this paper, we describe a solvent-free enzymatic method for the preparation of long-chain mono-thioesters and di-thioesters by the thioesterification and transthioesterification of palmitic acid and palmitic acid methyl ester, respectively, with α,ω-alkanedithiols, using immobilized lipases as catalysts and evacuation for the removal of water or methanol.

Dedicated to Professor Dr Helmut K. Mangold, former Executive Director, Federal Centre for Lipid Research, Institute for Biochemistry and Technology, Münster, Germany, on the occasion of his 80th birthday on 19 June 2004

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Materials and methods

Materials

1,6-Hexanedithiol, 1,8-octadanedithiol, palmitic acid, methyl palmitate and Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid)] were obtained from Sigma–Aldrich (Deisenhofen, Germany). An immobilized lipase preparation from *Rhizomucor miehei* [Lipozyme RM IM; containing 10% water, with 23 batch interesterification units/g, defined as the amount of enzyme required to incorporate 1 μmol palmitic acid/min into trioleoylglycerol from an equimolar mixture at 40°C], a lipase B preparation from *Candida antarctica* (Novozym 435; containing 10,500 propyl laurate units/g, 2% water) and a lipase preparation from *Thermomyces lanuginosus* [Lipozyme

TL IM; containing 170 interesterification units Novo/g] were kindly provided by Novozymes, Bagsvaerd, Denmark.

Lipase-catalyzed reactions

As a typical example, palmitic acid (51.5 mg, 0.2 mmol) was thioesterified with 1,8-octanedithiol (178.4 mg, 5×0.2 mmol, each, added at 0, 6, 24, 48 and 72 h) in the presence of 50 mg of one of the three immobilized lipase preparations, with magnetic stirring in a screw-capped tube in vacuo at 60°C for various periods and water-trapping in the gas phase, using potassium hydroxide pellets. A moderate vacuum (80 kPa) was used to prevent substantial loss of α,ω-alkanedithiols. Samples of the reaction products were withdrawn at various intervals, taken up in methyl-*t*-butyl ether at 50°C and filtered through a 1-μm syringe filter to separate the biocatalyst. An aliquot of the filtrate was analyzed as given below. Similarly, palmitic acid (0.2 mmol) was thioesterified with 1,6-octanedithiol (150.3 mg, 5×0.2 mmol, each, added at 0, 6, 24, 48 and 72 h).

Methyl palmitate (54.1 mg, 0.2 mmol) was transthioesterified with 1,8-octanedithiol (178.4 mg, 1 mmol) or 1,6-hexanedithiol (150.3 mg, 1 mmol) under identical conditions as described above for thioesterification reaction, using the above three lipases.

To prepare 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8), respectively, 58.3 mg 1-*S*-monopalmitoyl-hexanedithiol-(1,6) and 62.5 mg 1-*S*-monopalmitoyl-octanedithiol-(1,8), 150 μmol each, synthesized as described above and isolated as given below, were esterified with palmitic acid (115.4 mg, 450 μmol; molar ratio 1:3) in the presence of 75 mg Lipozyme RM IM, with magnetic stirring in a screw-capped tube in vacuo (2–4 kPa) at 80°C for various periods and water-trapping in the gas phase, using KOH pellets. Samples of the reaction products were withdrawn at various intervals and isolated as described above.

Enzyme units were calculated from the initial rates (24 h) of thioesterification or transthioesterification of palmitic acid and methyl palmitate, respectively, with α,ω-alkanedithiols or 1-*S*-mono-palmitoyl-α,ω-alkanedithiols. One unit of enzyme activity was defined as the amount of enzyme (g) that produced 1 μmol mono-palmitoyl or di-palmitoyl α,ω-alkanedithiol/min.

Isolation and purification of 1-*S*-monopalmitoyl-hexanedithiol-(1,6) and 1-*S*-monopalmitoyl-octanedithiol-(1,8)

As a typical example, the products resulting from the reaction of palmitic acid (51.3 mg, 0.2 mmol) with 1,8-octanedithiol (178.4 mg, 1 mmol) using Lipozyme RM (50 mg) at 60°C and 80 kPa for 96 h were dissolved in 3 ml diethyl ether-*i*-hexane (2:1, v/v) and applied to a Silica gel 60 (E. Merck, Darmstadt) column (20 cm long, 0.5 cm i.d.). The column was eluted first with 20 ml *i*-hexane, then with 20 ml *i*-hexane(diethyl ether (95:5, v/v) and 20 ml *i*-hexane-diethyl ether (9:1, v/v), to yield 35 mg 1-*S*-monopalmitoyl-octanedithiol-(1,8), m.p. 51–52°C (after repeated crystallization from diethyl ether-*i*-hexane, 4:1, v/v, followed by diethyl ether). Similarly, 1-*S*-monopalmitoyl-hexanedithiol-(1,6), m.p. 43–44°C (from diethyl ether-*i*-hexane, 4:1, v/v), was prepared by the reaction of palmitic acid with 1,6-hexanedithiol.

Isolation and purification of 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8)

As a typical example, the products resulting from the reaction of 1-*S*-monopalmitoyl-octanedithiol-(1,8) (62.6 mg, 0.15 mmol) with palmitic acid (115.4 mg, 0.45 mmol), using Lipozyme RM IM (75 mg) at 80°C and 2–4 kPa for 144 h, were dissolved in 2 ml dichloromethane and applied to a Silica gel 60 (Merck, Darmstadt) column (20 cm long, 0.5 cm i.d.). The column was eluted first with 20 ml *i*-hexane, then with 20 ml *i*-hexane-diethyl ether (95:5, v/v) and 20 ml *i*-hexane-diethyl ether (9:1, v/v) to yield, after crystallization from dichloromethane, 38 mg 1,8-di-*S*-palmitoyl-octanedithiol-(1,8), m.p. 76–77°C. Similarly, 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6), m.p. 75–76°C (from dichloromethane), was prepared starting from palmitic acid and 1-*S*-monopalmitoyl-hexanedithiol-(1,6).

Thin-layer chromatography

Aliquots were withdrawn from the reaction mixtures and the conversion was checked by thin-layer chromatography (TLC) on 0.3-mm layers of Silica gel H (E. Merck, Darmstadt, Germany), using *i*-hexane-dichloromethane (65:35, v/v) or *i*-hexane-methyl-*t*-butyl ether (98:2, v/v). Compounds containing thiol groups were detected by spraying the plate with Ellman's reagent (2 g/l) in ethanol–water (2:1, v/v); and other spots were located by iodine staining. The R_f values of various compounds in both solvent systems were as follows: 0.57 for α,ω-alkanethiols, 0.43 for 1-*S*-monopalmitoyl-hexanedithiol-(1,6) and 1-*S*-monopalmitoyl-octanedithiol-(1,8), 0.27 for 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8), 0.33 for methyl palmitate and <0.1 for palmitic acid. Similarly, 0.5-mm layers of Silica gel H were used for preparative TLC.

Gas chromatography

In both thioesterification and transthioesterification reactions, aliquots of products were treated with a solution of diazomethane in diethyl ether to convert the unreacted or hydrolyzed fatty acids into methyl esters; and the resulting mixture of methyl palmitate, unreacted α,ω-alkanedithiols and mono-palmitoyl and di-palmitoyl α,ω-alkanedithiols were analyzed by gas chromatography, using a HP-5890 series II gas chromatograph (Hewlett-Packard, Böblingen, Germany) equipped with a flame ionization detector. Separations were carried out on a 0.1-μm fused silica capillary column (Quadrex 400-1HT, 15 m long, 0.25 mm i.d.; Quadrex Corp., New Haven, USA), using hydrogen as the carrier gas (column pressure 45 kPa), initially at 80°C for 6 min, followed by linear programming from 80°C to 400°C at 20°C/min and finally kept at 400°C for 2 min. Injector and detector temperatures were maintained at 380°C. Peaks in gas chromatograms were assigned by comparison of their retention times with those of known standards and purified reaction products. Peak areas and percentages were calculated using Hewlett-Packard GC ChemStation software.

High-performance liquid chromatography

Aliquots of extracts were analyzed for their composition by HPLC as follows. The HPLC system consisted of a Merck–Hitachi pump L-6200 (E. Merck) equipped with a column oven (VDS Optilab, Berlin, Germany) set at 40°C, a Kontron UV/Vis HPLC 332 detector (Kontron Instruments, Milan, Italy) set to a wavelength of 210 nm and an Alltech ELSD detector model 500 (thermostated to 60°C; Alltech, Unterhaching, Germany) which were used in series. UV and mass traces were monitored and evaluated using a KromaSystem 2000 data acquisition unit (Kontron Instruments). Mono-*S*-palmitoyl and di-*S*-palmitoyl esters of α,ω-alkanedithiols and palmitic acid or methyl palmitate were separated at 40°C with a 85:15 (v/v) mixture of acetonitrile–tetrahydrofurane (THF), isocratically until 12 min, then for 8 min using a gradient (from 85:15 to 60:40, v/v), followed by 5 min isocratically (60:40, v/v), then for 5 min using a gradient (from 60:40, v/v, to 100% THF) and finally isocratically (100% THF) until 35 min on a Phenomenex 5-μm LiChrospher RP-18 end-capped column (250 mm long, 4 mm i.d.; Phenomenex, Aschaffenburg, Germany) and a precolumn. The flow rate was set at 0.8 ml/min. Injections (around 20–60 μg) of the reaction mixture in acetonitrile–THF (1:1, v/v) were carried out with a Rheodyne 7161 sample injector (Cotati, Calif., USA) equipped with a 20-μl sample

loop. Peak areas and percentages were calculated using KromaSystem 2000 software.

Standards isolated from the reaction mixtures by column chromatography followed by crystallization from diethyl ether-*i*-hexane (4:1, v/v; mono-thioesters) or dichloromethane (di-thioesters) were used for comparison. HPLC standards dissolved in acetonitrile or THF were not stable and were, therefore, prepared freshly every day. Response factors of mass detector were determined, e.g., for 1-*S*-monopalmitoyl-octanedithiol-(1,8) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8) and for methyl palmitate, using purified thioester compounds and a methyl palmitate standard.

Structural analysis by derivatization for GC using trimethylsulfonium hydroxide

Under standard conditions, 30–60 µl trimethylsulfonium hydroxide (TMSH) were added to 0.4 mg of a compound containing thiol and/or thioester groups (molar ratio TMSH:thiol and/or carboxy equivalents around 4:1). The derivatization mixture was heated to 120°C for 3 min and 1–2 µl were directly injected into the GC. Analysis of the reaction mixtures containing derivatives of thiol and/or thioester compounds formed by the pyrolytic reaction with TMSH was carried out as described above. Under the conditions described, 1-*S*-monopalmitoyl-α,ω-alkanedithiols and 1,8-di-*S*-palmitoyl-α,ω-alkanedithiols were completely converted to the corresponding α,ω-di-*S*-methyl alkanedithiols and methyl palmitate (Weber and Klein 2000).

GC-MS analyses

GC-MS analyses of monopalmitoyl and dipalmitoyl thioesters of α,ω-alkanedithiols were performed in chemical ionization (CI; methane as reagent gas) and electron ionization (EI; 70 eV) modes on a Hewlett-Packard model 5890 series II/5989 A equipped with a 0.10-µm HAT-5 fused silica capillary column (SGE, Germany), 12 m long, 0.22 mm i.d. The carrier gas was helium at a flow rate of 1.0 ml/min. The column temperature was initially kept at 80°C for 6 min and then programmed from 80°C to 400°C at 20°C/min; and the final temperature was held for 6 min. Other operating conditions were split/splitless injector in splitless mode (temperature 400°C), interface temperature 400°C and ion source temperature 200°C.

Results

The chemical structures of 1-*S*-monopalmitoyl-hexanedithiol-(1,6) and 1-*S*-monopalmitoyl-octanedithiol-(1,8) which were prepared by lipase-catalyzed reaction of palmitic acid or methyl palmitate with α,ω-alkanedithiols at 60°C in vacuo are shown as structure 1 in Fig. 1. In addition, the structures of 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8) which were formed similarly by the reaction of palmitic acid with the corresponding 1-*S*-monopalmitoyl α,ω-alkanedithiols at 80°C in vacuo are shown as structure 2 in Fig. 1.

The reaction products were purified by preparative TLC or column chromatography as described in the Materials and methods. The reaction of the various thioesters formed, such as 1-*S*-monopalmitoyl-α,ω-alkanedithiols and α,ω-di-*S*-palmitoyl-α,ω-alkanedithiols with TMSH led to typical methylated derivatives, i.e., α,ω-di-*S*-methyl alkanedithiols and methyl palmitate (Weber and Klein 2000).

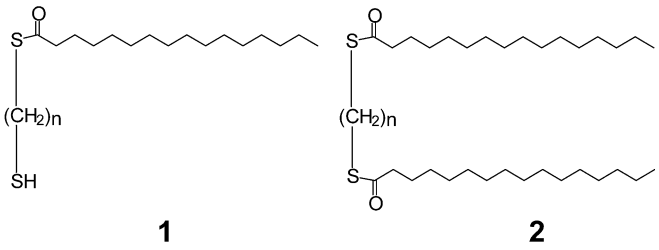

Fig. 1 Chemical structures of **1** 1-*S*-monopalmitoyl-hexanedithiol-(1,6) and 1-*S*-monopalmitoyl-octanedithiol-(1,8) and **2** 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8). Number of carbon atoms n=6 or n=8

In addition, the reaction products were analyzed by CI and EI GC-MS and the results of the GC-MS analyses of monopalmitoyl and dipalmitoyl thioesters of α,ω-alkanedithiols are given in Table 1. The CI mass spectra (methane as reagent gas) of 1-*S*-monopalmitoyl-α,ω-alkanedithiols and α,ω-di-*S*-palmitoyl-α,ω-alkanedithiols demonstrated typical $[M+1]^+$ ions, whereas $[M+29]^+$ ions were observed for 1-*S*-monopalmitoyl-α,ω-alkanedithiols only. The EI mass spectra of all compounds showed typical fragments, e.g., thiopalmitoyl (*m/z* 271, $[C_{16}H_{31}OS]^+$) and palmitoyl (*m/z* 239, base peak, $[C_{16}H_{31}O]^+$) ions, which were formed by the loss of 1-mercaptoalkyl and α,ω-mercaptoalkanethio groups, respectively (Table 1). In addition, sulfur-containing mass fragments were observed, such as 1,6-hexanedithiol and 1,8-octanedithiol ions (*m/z* 150 $[C_6H_{14}S_2]^+$, *m/z* 178 $[C_8H_{18}S_2]^+$), respectively) and 1-hexanethio and 1-octanethio ions (*m/z* 116 $[C_6H_{14}S]^+$, *m/z* 143 $[C_8H_{17}S]^+$, respectively). These data agree well with those published recently for acyl thioesters (Weber et al. 1998, 2000) and with data given in earlier literature (Spiteller 1966). Molecular ions and typical mass fragments of monothioesters and di-thioesters of 1,6-hexanedithiol and 1,8-octadanedithiol with palmitic acid obtained by CI and EI GC-MS confirm the chemical structures of the reaction products, as given in Fig. 2.

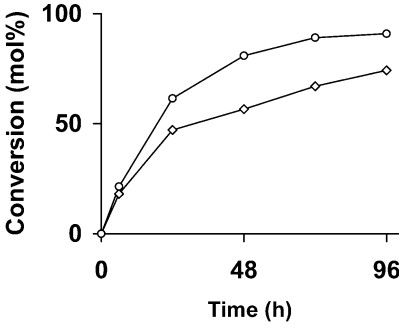

Fig. 2 Time-course of the formation of 1-*S*-monopalmitoyl-octanedithiol-(1,8) by thioesterification of palmitic acid (*circles*) or transthioesterification of methyl palmitate (*diamonds*) with 1,8-octanedithiol catalyzed by immobilized lipase from *R. miehei* (Lipozyme RM IM) at 60°C in vacuo, as described in the Materials and methods

Table 1 Mass ions of monopalmitoyl and dipalmitoyl thioesters of α,ω-dithiols obtained by CI and EI GC-MS. CI mass spectra of all thioesters demonstrated typical $[M+1]^+$ ions, whereas $[M+29]^+$ ions were found for the mono-thioesters only. All other fragment ions were obtained by EI GC-MS

1-*S*-Monopalmitoyl-hexanedithiol-(1,6) (*m/z*, rel.%)	1,6-*S*-Dipalmitoyl-hexanedithiol-(1,6) (*m/z*, rel.%)	1-*S*-Monopalmitoyl-octanedithiol-(1,8) (*m/z*, rel.%)	1,8-*S*-Dipalmitoyl-octanedithiol-(1,8) (*m/z*, rel.%)
417 $[M+29]^+$	–	445 $[M+29]^+$	–
389 $[M+1]^+$	627 $[M+1]^+$	417 $[M+1]^+$	655 $[M+1]^+$
388 (<1) $[M]^+$	–	416 (<1) $[M]^+$	–
–	387 (3) $[M\text{-}C_{16}H_{31}O]^+$	–	415 (4) $[M\text{-}C_{16}H_{31}O]^+$
–	355 (6) $[M\text{-}C_{16}H_{31}OS]^+$	–	383 (1) $[M\text{-}C_{16}H_{31}OS]^+$
271 (7) $[C_{16}H_{31}OS]^+$	271 (6) $[C_{16}H_{31}OS]^+$	271 (8) $[C_{16}H_{31}OS]^+$	271 (5) $[C_{16}H_{31}OS]^+$
239 (100) $[C_{16}H_{31}O]^+$	239 (100) $[C_{16}H_{31}O]^+$	239 (100) $[C_{16}H_{31}O]^+$	239 (100) $[C_{16}H_{31}O]^+$
150 (17) $[C_6H_{14}S_2]^+$	150 (6) $[C_6H_{14}S_2]^+$	178 (14) $[C_8H_{18}S_2]^+$	178 (6) $[C_8H_{18}S_2]^+$
116 (27) $[C_6H_{12}S]^+$	116 (15) $[C_6H_{12}S]^+$	143 (9) $[C_8H_{17}S]^+$	143 (14) $[C_8H_{17}S]^+$

Fig. 3 Time-course of the formation of 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) (*circles*) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8) (*diamonds*) by thioesterification of palmitic acid with 1,8-octanedithiol catalyzed by immobilized lipase from *R. miehei* (Lipozyme RM IM) at 80°C in vacuo, as described in the Materials and methods

Table 2 shows the enzyme activities of various immobilized lipases in thioesterification and transthioesterification reactions of palmitic acid or methyl palmitate with α,ω-alkanedithiols in vacuo. It is obvious from these results that, in thioesterification reaction, immobilized lipase preparation from *R. miehei* led to up to >90% conversion of palmitic acid. The data presented in Table 2 also show that the immobilized lipase preparation from *R. miehei* (0.82 thioesterification units/g) was superior to that from *C. antarctica* lipase B (0.72 thioesterification units/g) and *T. lanuginosus* (0.64 thioesterification units/g).

Moreover, Table 2 shows that thioesterification of palmitic acid with 1,6-hexanedithiol or 1,8-octanedithiol, catalyzed by immobilized lipase preparations from *R. miehei*, *C. antarctica* and *T. lanuginosus* yield the corresponding 1-*S*-monopalmitoyl thioesters (maximum conversions around 80–90 mol%; mean enzyme activities respectively 0.7–0.8, 0.6–0.7 and 0.5–0.6 thioesterification units/g enzyme). Mean enzyme activities are generally lower for the corresponding transthioesterification reactions with methyl palmitate catalyzed by the lipases described above (mean enzyme activities respectively 0.4–0.6, 0.4–0.5 and 0.3–0.4 transthioesterification units/g enzyme). Maximum conversions were around 61–74 mol % in transthioesterification reactions yielding 1-*S*-monopalmitoyl-hexanedithiol-(1,6) or 1-*S*-monopalmitoyl-octanedithiol-(1,8).

Mean enzyme activities are by far lower for the corresponding thioesterification reactions of 1-*S*-monopalmitoyl-hexanedithiol-(1,6) or 1-*S*-monopalmitoyl-octanedithiol-(1,8) with palmitic acid (Table 2), catalyzed by an immobilized lipase preparation from *R. miehei* (mean enzyme activity 0.24–0.45 thioesterification units/g enzyme), leading to 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) or 1,8-di-*S*-palmitoyl-octanedithiol-(1,8).

The time-course of the formation of long-chain acyl monothioesters by lipase-catalyzed thioesterification or transthioesterification of palmitic acid and methyl palmitate, respectively, with α,ω-alkanedithiols over a period of 96 h is shown in Fig. 2; and it exhibits a distinctly higher rate of thioesterification than transthioesterification.

Figure 3 demonstrates the time-course of thioesterification reactions of 1-*S*-monopalmitoyl-hexanedithiol-(1,6)

Table 2 Enzyme activities of various immobilized lipases in thioesterification and transthioesterification reactions of palmitic acid or methyl palmitate, respectively, with α,ω-alkanedithiols and thioesterification of palmitic acid with 1-*S*-monopalmitoyl-hexanedithiol-(1,6) or 1-*S*-monopalmitoyl-octanedithiol-(1,8). Experimental conditions for mono-thioester synthesis were as follows: 200 μmol palmitic acid or methyl palmitate (molar ratio of palmitic acid or methyl palmitate:α,ω-alkanedithiol 1:5), amount of various immobilized lipases 50 mg each, 80 kPa. Experimental conditions for di-thioester synthesis were: 150 μmol 1-*S*-monopalmitoyl-hexanedithiol-(1,6) or 1-*S*-monopalmitoyl-octanedithiol-(1,8) with 450 μmol palmitic acid (molar ratio 1:3), amount of immobilized Lipozyme RM IM 75 mg, 2–4 kPa. Conversions were determined by GC; and mono-thioester fractions additionally contained small proportions (2–4%) of di-thioesters. Enzyme units were calculated as 1 μmol/min mono-thioester or di-thioester formed using 1 g lipase from the initial rates (24 h) of thioesterification or transthioesterification as described in the Materials and methods. Number of experiments (*n*)=*x*, *SD* standard deviation

α,ω-Alkanedithiols or *S*-monopalmitoyl derivatives of α,ω-alkanedithiols	Enzymes	Fatty acid or fatty acid ester	Time (h)	Maximum conversion (mol%)	Mean enzyme activity (units/g)	Enzyme activity range (SD; *n*=*x*)
1,6-Hexanedithiol	Lipozyme TL IM	Palmitic acid	120	89.2	0.49	0.37–0.60 (0.09; *n*=8)
		Methyl palmitate	120	63.7	0.40	0.39–0.42 (0.02; *n*=4)
	Novozym 435	Palmitic acid	120	76.9	0.59	0.48–0.70 (0.10; *n*=4)
		Methyl palmitate	120	65.2	0.36	0.28–0.41 (0.06; *n*=4)
	Lipozyme RM IM	Palmitic acid	120	87.1	0.75	0.49–0.93 (0.19; *n*=4)
		Methyl palmitate	120	61.1	0.39	0.33–0.44 (0.06; *n*=3)
1,8-Octanedithiol	Lipozyme TL IM	Palmitic acid	96	86.8	0.64	0.56–0.81 (0.02; *n*=5)
		Methyl palmitate	96	68.9	0.49	0.39–0.59 (0.10; *n*=3)
	Novozym 435	Palmitic acid	96	84.7	0.74	0.68–0.78 (0.05; *n*=4)
		Methyl palmitate	96	69.2	0.43	0.36–0.62 (0.12; *n*=5)
	Lipozyme RM IM	Palmitic acid	96	90.9	0.82	0.73–0.95 (0.10; *n*=5)
		Methyl palmitate	96	74.3	0.59	0.52–0.65 (0.07; *n*=3)
1-*S*-Monopalmitoyl-hexanedithiol-(1,6)	Lipozyme RM IM	Palmitic acid	144	68.8	0.45	0.42–0.48 (0.03; *n*=3)
1-*S*-Monopalmitoyl-octanedithiol-(1,8)	Lipozyme RM IM	Palmitic acid	144	51.5	0.24	0.20–0.30 (0.04; *n*=4)

and 1-*S*-monopalmitoyl-octanedithiol-(1,8) with palmitic acid catalyzed by an immobilized lipase preparation from *R. miehei*, leading to 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8), respectively, in moderate yield (around 40–60%). From these data, it is obvious that distinctly fewer α,ω-dipalmitoylalkanedithiol thioesters were formed when using Lipozyme RM IM (0.24–0.45 thioesterification units/g enzyme), as compared with mono-thioester formation (0.75–0.82 thioesterification units/g), demonstrating that enzyme activity is by far lower for the introduction of the second palmitoyl group (Table 2, Fig. 2).

Discussion

As an extension of previous studies (Weber et al. 1998, 1999b), the present work reveals that long-chain monoacyl thioesters can be obtained in high yield by thioesterification of palmitic acid with an α,ω-alkanedithiol or in moderate conversion by transthioesterification of a methyl palmitate with an α,ω-alkanedithiol, catalyzed by lipases in vacuo in the absence of an organic solvent. Similarly, 1,6-di-*S*-palmitoyl-hexanedithiol-(1,6) and 1,8-di-*S*-palmitoyl-octanedithiol-(1,8) are formed in moderate yield by lipase-catalyzed reaction of 1-*S*-monopalmitoyl-hexanedithiol-(1,6) and 1-*S*-monopalmitoyl-octanedithiol-(1,8), respectively, with palmitic acid. In reactions in vacuo no further chemicals are needed, which should be of advantage, particularly for industrial applications.

To summarize, long-chain monopalmitoyl thioesters are prepared in high conversion (up to >90%) by solvent-free esterification of palmitic acid with α,ω-alkanedithiols in vacuo catalyzed by lipase preparations from *R. miehei* (Lipozyme RM IM), *C. antarctica* (lipase B, Novozym 435), and *T. lanuginosus* (Lipozyme TL IM). Lipase-catalyzed solvent-free transthioesterification in vacuo of methyl palmitate with α,ω-alkanedithiols is less effective for the preparation of acyl thioesters than thioesterification of palmitic acid with α,ω-alkanedithiols. Both in thioesterification and transthioesterification, Lipozyme RM IM is slightly more effective as biocatalyst than Novozym 435 and Lipozyme TL IM. Similar results have been obtained for both the thioesterification and transthioesterification of long-chain fatty acids and long-chain fatty acid methyl esters, respectively, with long-chain 1-alkanethiols (Weber et al. 1998, 1999a, 1999b).

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