# Solvent-Free Lipase-Catalyzed Thioesterification and Transthioesterification of Fatty Acids and Fatty Acid Esters with Alkanethiols *in Vacuo*

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**ABSTRACT:** Palmitic acid hexadecylthioester and other long-chain acyl thioesters have been prepared in high yield (80–85%, purity >98%) by solvent-free lipase-catalyzed thioesterification of fatty acids with alkanethiols *in vacuo*. A lipase B preparation from *Candida antarctica* was more effective than a lipase preparation from *Rhizomucor miehei* and, particularly, those from papaya latex and porcine pancreas. Lipase-catalyzed transthioesterification of fatty acid methyl esters with alkanethiols was less effective than thioesterification for the preparation of acyl thioesters. However, in transthioesterification, a lipase preparation from *R. miehei* was more effective than a lipase B preparation from *C. antarctica*. Lipases from papaya latex and porcine pancreas led to moderate conversions to acyl thioesters in both thioesterification and transthioesterification reactions, whereas only small proportions of thioesters were formed using lipase from *Rhizopus arrhizus*. Lipases from *Chromobacterium viscosum* and *Candida rugosa* were not effective at all.

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**KEY WORDS:** Fatty acid thioesters, lipases, thioesterification, transthioesterification.

Thioesters are activated esters that are utilized as versatile intermediates in organic chemistry for the preparation of various compounds, e.g., peptides, macrolide antibiotics, and other pharmaceuticals (1–6). Acyl thioesters are active acylation intermediates in biochemical and bioorganic nucleophilic reactions, having higher reactivity and selectivity than the corresponding oxygen analogs (7–9). Most methods for the preparation of acyl thioesters use expensive or highly toxic reagents (3,4,10,11). These disadvantages led to the development of lipase-catalyzed thioesterification and transthioesterification processes for the preparation of acyl thioesters. For example, short-chain flavor thioesters and long-chain thio wax esters 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 (12–16). In this paper we describe a solvent-free enzymatic method for the preparation of long-chain thioesters by thioesterification and transthioesterification of fatty acids (FA) and fatty acid methyl esters (FAME), respectively, with long-chain thiols using immobilized lipases as catalysts and evacuation for the removal of water and methanol.

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## EXPERIMENTAL PROCEDURES

*Materials.* 1-Dodecanethiol, 1-tetradecanethiol, 1-octadecanethiol, lauric acid, palmitic acid, methyl palmitate, *Carica papaya* latex, and *Candida rugosa* Type VII lipase (850,000 units/g) were obtained from Sigma-Aldrich-Fluka (Deisenhofen, Germany). 1-Hexadecanethiol was a product of TCI (Tokyo, Japan). The granular papaya latex preparation was ground in a mortar with pestle to a fine powder to pass through a 0.8-mm mesh width sieve and was used as a source of lipase. The immobilized lipase preparation from *Rhizomucor miehei* (Lipozyme IM 20®; 23 Batch Interesterification units/g; 10% w/w water) and lipase B preparation from *C. antarctica* (Novozym 435®; 10,500 propyl laurate units/g; 2% w/w water) were kindly provided by Novo Nordisk (Bagsvaerd, Denmark). Lipases from *Chromobacterium viscosum* (144,000 units/g) and porcine pancreas (4,500 units/g) were products of Biocatalysts (Pontypridd, Mid Glamorgan, United Kingdom). Lipase from *Rhizopus arrhizus* (50,000 units/mL) was obtained from Boehringer-Mannheim (Mannheim, Germany).

*Lipase-catalyzed reactions.* As a typical example, palmitic acid (16:0-FA, 51.5 mg, 0.2 mmol), was esterified with 1-octadecanethiol (18:0-thiol, 172 mg, 0.6 mmol) in the presence of 50 mg of one of the lipase preparations (50 μL suspension in the case of lipase from *R. arrhizus*) by magnetic stirring in a screw-capped tube *in vacuo* (20–50 mbar) at 60°C for various periods with water-trapping in the gas-phase using KOH pellets. Samples of the reaction products were withdrawn at various intervals, taken up in isohexane and centrifuged to separate the biocatalyst. An aliquot of the supernatant was analyzed as described below.

Methyl palmitate (16:0-FAME, 54.1 mg, 0.2 mmol) was interthioesterified with 1-octadecanethiol (172 mg, 0.6 mmol)

under identical conditions as described above for the thioesterification reaction.

Enzyme units were calculated from the initial rates (4 h) of thioesterification or transthioesterification of FA and FAME, respectively, with 1-alkanethiols. One unit of enzyme activity was defined as the amount of enzyme (in g) that produced 1 µmol of fatty acid alkyl thioester/min.

*Isolation and purification of long-chain acyl thioesters.* As a typical example, the products resulting from the reaction of palmitic acid (51.3 mg, 0.2 mmol) with 1-hexadecanethiol (155.1 mg, 0.6 mmol) using Novozym 435® (50 mg) at 60°C and 50 mbar for 48 h were dissolved in 5 mL isohexane, centrifuged, and the supernatant was applied to a silica gel 60 (E. Merck, Darmstadt, Germany) column (20 × 0.5 cm, i.d.). The column was eluted first with 20 mL isohexane/diethyl ether (99:1, vol/vol) to remove 1-hexadecanethiol, then with 20 mL isohexane/diethyl ether (95:5, vol/vol) to yield 80–85 mg palmitic acid hexadecyl thioester, m.p. 56–57°C (from isohexane), mass spectrometry [*m/z* (rel.%)]: 271 (13.4, $[M - C_{16}H_{33}]^+$); 257 (3.8, $[C_{16}H_{33}S]^+$); 239 (59.9, $[M - C_{16}H_{33}S]^+$); 57 (100).

*Analytical methods.* In thioesterification reactions, aliquots of products were treated with a solution of diazomethane in diethyl ether to convert the unreacted FA to methyl esters; the resulting mixture of methyl esters, unreacted alkanethiols, and acyl thioesters was analyzed by gas chromatography. In transthioesterification reactions aliquots of products consisting of methyl esters, unreacted alkanethiols, and acyl thioesters were analyzed directly by gas chromatography. A Hewlett-Packard (Böblingen, Germany) HP-5890 Series II gas chromatograph equipped with a flame-ionization detector was used. Separations were carried out on a 0.1-µm Quadrex 400-5HT (Quadrex Corp., New Haven, CT) fused-silica capillary column, 25 m × 0.25 mm i.d., using hydrogen as the carrier gas (column pressure 50 kPa), initially at 120°C for 2 min, followed by linear programming from 120 to 200°C at 5°C · $min^{-1}$ and from 200 to 350°C at 20°C · $min^{-1}$, finally at 350°C for 6 min. The split ratio was 1:10, the injector as well as the flame-ionization detector temperature was 350°C. Peaks in gas chromatograms were assigned by comparison of their retention times with those of known standards. Peak areas and percentages were calculated using Hewlett-Packard 3365 Series GC ChemStation software.

## RESULTS

The formation of long-chain acyl thioester by lipase-catalyzed thioesterification of palmitic acid with 1-octadecanethiol over a period of 72 h is shown in Figure 1. In the thioesterification reaction, a lipase B preparation from *C. antarctica* (Novozym 435®) with an activity of 9.3 thioesterification units/g led to >98% conversion of palmitic acid. The data presented in Figure 1 also show that a lipase B preparation from *C. antarctica* was superior to a lipase preparation from *R. miehei* (8.8 thioesterification units/g) and, particularly, to lipase preparations from papaya latex (0.7 thioesterification units/g) and porcine pancreas (0.3 thioesterification units/g). Moreover, only small proportions, if any, of acyl thioesters were formed using lipases from *C. viscosum*, *R. arrhizus,* and *C. rugosa* (data not shown).

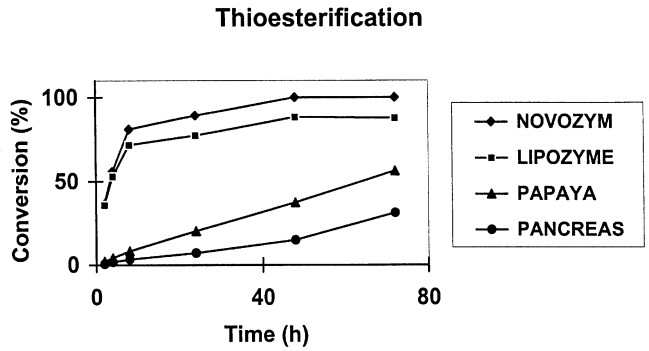


**FIG. 1.** Time course of the formation of palmitic acid octadecyl thioester by lipase-catalyzed thioesterification of palmitic acid with 1-octadecanethiol in the presence of various lipases at 60°C *in vacuo.* Novozym and Lipozyme (Bagsvaerd, Denmark); papaya latex as a source of lipase (Sigma-Aldrich-Fluka, Deisenhofen, Germany); porcine pancreas lipase (Biocatalysts, Pontypridd, Mid Glamorgan, United Kingdom).

In a typical reaction of palmitic acid with 1-hexadecanethiol, catalyzed by Novozym 435®, the reaction products were fractionated by column chromatography on silica gel, as described in the Experimental Procedures section, to yield palmitic acid hexadecyl thioester in a purity of 98% and an isolated yield of 80–85% with respect to the amount of palmitic acid used.

Figure 2 shows the formation of long-chain acyl thioester by lipase-catalyzed transthioesterification of methyl palmitate with 1-octadecanethiol. The data demonstrate that with both Novozym 435® (6.5 transthioesterification units/g) and Lipozyme IM 20® (7.0 transthioesterification units/g) biocatalysts distinctly lower amounts of acyl thioesters were formed by transthioesterification than thioesterification (Figs. 1 and 2). Lipase preparations from papaya latex (1.3 transthioesterification units/g) and porcine pancreas lipase (1.3 transthioesterification units/g) catalyzed transthioesterification reactions at similar rates, and both enzymes showed higher conversion

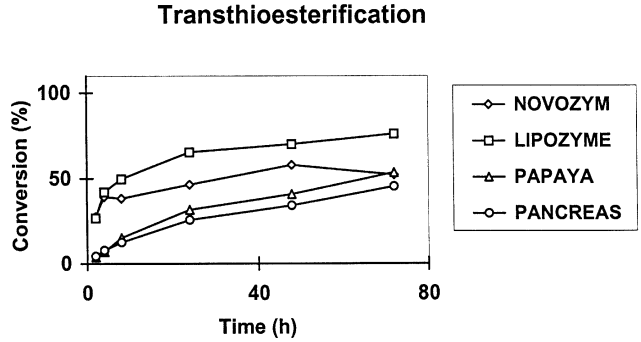


**FIG. 2.** Time course of the formation of palmitic acid octadecyl thioester by lipase-catalyzed transthioesterification of methyl palmitate with 1-octadecanethiol in the presence of various lipases at 60°C *in vacuo.* For lipase sources see Figure 1.

**TABLE 1**
**Solvent-Free Lipase-Catalyzed Thioesterification of Fatty Acids with 1-Alkanethiols *in vacuo* Catalyzed by Novozym 435®**

| Reaction products | Conversion (%)[a] | Enzyme activity[b] (units/g) |
|---|---|---|
| Lauric acid dodecyl thioester | 93 | 9.3 |
| Lauric acid tetradecyl thioester | 96 | 9.9 |
| Lauric acid hexadecyl thioester | 93 | 9.8 |
| Lauric acid octadecyl thioester | 92 | 10.4 |
| Palmitic acid dodecyl thioester | 92 | 13.0 |
| Palmitic acid tetradecyl thioester | 92 | 12.3 |
| Palmitic acid hexadecyl thioester | 95 | 9.8 |
| Palmitic acid octadecyl thioester | 98 | 9.3 |

[a]Conversion of products was determined by gas chromatography. For reaction conditions see the Experimental Procedures section; reaction time, 48 h.
[b]Enzyme units were calculated from the initial rates (4 h) of thioesterification as described in the Experimental Procedures section. Novozym 435® from *Candida antarctica* was provided by Novo Nordisk (Bagsvaerd, Denmark).

rates in transthioesterification than in thioesterification (Figs. 1 and 2).

Table 1 shows that conversions (determined by gas chromatography) of well over 90% of long-chain acyl thioesters are obtained by thioesterification of lauric and palmitic acids with various 1-alkanethiols, catalyzed by Novozym 435® (enzyme activity: 9–13 thioesterification units/g enzyme) *in vacuo*.

## DISCUSSION

As an extension of previous studies (15,16), the present work reveals that long-chain acyl thioesters can be obtained almost quantitatively by thioesterification of a FA with a 1-alkanethiol or in high amounts by transthioesterification of a FAME with an 1-alkanethiol, catalyzed by lipases, *in vacuo* in the absence of an organic solvent. Our recent studies reported lipase-catalyzed thioesterification and transthioesterification using molecular sieve as drying agent with or without organic solvents (15,16). The molecular sieve (4Å) used in these experiments ensured the removal of water in thioesterification and of methanol in transthioesterification reactions (15,16).

By comparing the reactions *in vacuo* (Table 1, Figs. 1 and 2) with those in the presence of molecular sieve (16) or solvents plus molecular sieve (15) one can see that conversions of thioesters were doubled when the reactions were performed without solvents. Similar conversions (>90%) were observed when starting materials were reacted in the presence of molecular sieve at normal pressure (16) or under vacuum without molecular sieve (Table 1, Figs. 1 and 2). In reactions *in vacuo,* no further chemicals are needed, which may be of advantage particularly for industrial applications. To our knowledge the vacuum conditions reported here were being used for the first time in thioesterification and transthioesterification reactions with lipases. However, other lipase-catalyzed esterifications and transesterifications *in vacuo* are well known (e.g., 17–19).

To summarize, long-chain acyl thioesters (thio wax esters) have been prepared in high conversion (up to >98%, isolated yield 80–85%) by solvent-free esterification of FA with long-chain 1-alkanethiols *in vacuo,* catalyzed by lipase preparations from *C. antarctica* B (Novozym 435®), *R. miehei* (Lipozyme IM 20®), *C. papaya* latex, and porcine pancreas. In thioesterification reaction Novozym 435® as a biocatalyst was superior to lipase preparations from *R. miehei* (Lipozyme IM 20®), papaya latex, and porcine pancreas. Lipase-catalyzed solvent-free transthioesterification *in vacuo* of FAME with 1-alkanethiols was less effective for the preparation of acyl thioesters than thioesterification of fatty acids with 1-alkanethiols. In transthioesterification Lipozyme IM 20® was slightly more effective as biocatalyst than Novozym 435® as well as papaya latex and porcine pancreas. Similar results have been obtained for both the thioesterification of lauric and palmitic acids with various 1-alkanethiols (Table 1) and the transthioesterification of methyl laurate with tetradecanethiol (data not shown).

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