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Journal of Molecular Catalysis B: Enzymatic 27 (2004) 51–53
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Enzymatic synthesis of surfactants based on polyethylene glycol and stearic or 12-hydroxystearic acid

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Received 12 October 2002; received in revised form 10 September 2003; accepted 15 September 2003

Abstract

High-purity mono- and di-esters of polyethylene glycol (molecular weight: 600) with stearic acid or 12-hydroxystearic acid was afforded using enzymatic catalysis with immobilized *Candida antarctica* lipase B. The reactions were performed without solvent and the formed water was removed with vacuum, driving the reactions to completion. Isolated yields varied between 77 and 87%.

Keywords: 12-Hydroxystearic acid; Polyethylene glycol ester; Enzymatic synthesis; *Candida antarctica* lipase B; Surfactants

1. Introduction

Efficient solubilizers are needed in both pharmaceutical and cosmetic applications to facilitate dissolving active substances. Many of the commercial processes used to produce these solubilizers today are crude and produce highly complex mixtures containing several hundred components as well as having high batch-to-batch variations. Complex mixtures may have beneficial physical properties compared to the pure substances but are not attractive in medical applications because of the strict regulations posed on pharmaceuticals. There is clearly a need for well-defined, efficient solubilizers suitable for pharmaceutical use and processes for their production.

Commercial surfactants based on hydroxy fatty acid esters of polyethylene glycol (PEG) are produced by polymerizing ethylene oxide directly onto the hydroxy fatty acid. The products are often potent solubilizers but also very complex mixtures with additional ester or ether groups attached to the fatty acid hydroxyl group. The high specificity provided by enzymes and the gentle conditions under which they work offer an environmentally friendly means to synthesize pure products [1].

In this work, we present a simple, efficient and solvent-free method using enzyme catalysis to produce well-defined fatty acid esters of PEG (e.g. PEG(600) 12-hydroxystearate, see Fig. 1). We use enzyme catalysis to produce mono- and di-esters of PEG and 12-hydroxystearic acid as well as the corresponding stearate esters. We show that the enzymatic method is efficient and can be used to produce well-defined mono- and di-esters as well as mixtures thereof.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG, average molecular weight of 600), stearic acid (95%) and 12-hydroxystearic acid (>99%) were purchased from Aldrich and used as received. Immobilized lipase B from *Candida antarctica* (Novozym 435) was a kind gift from Novozymes A/S.

2.2. Enzymatic synthesis

Fatty acid (stearic acid and 12-hydroxystearic acid, respectively) and PEG(600) were mixed and heated to 80 °C with stirring under vacuum (<1 mm Hg) for drying. The following amounts of reactants were used: *PEG monostearate*: 70 mmol PEG (42 g) and 7 mmol (2.0 g) stearic acid. *PEG mono-12-hydroxystearate*: 200 mmol PEG (120 g) and

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doi:10.1016/j.molcatb.2003.09.006

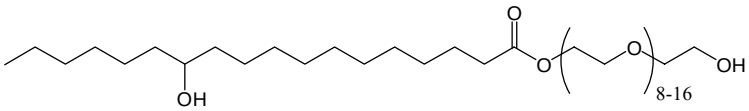

Fig. 1. PEG(600) mono-12-hydroxystearate.

20 mmol (6.00 g) 12-hydroxystearic acid. *PEG distearate*: 5 mmol PEG (3.0 g) and 16 mmol (4.6 g) stearic acid. *PEG di-12-hydroxystearate*: 4.2 mmol PEG (2.5 g) and 13.3 mmol (4.0 g) 12-hydroxystearic acid.

To start the reaction Novozym 435, immobilized lipase B from *C. antarctica*, was added with 35 mg/mmol of expected product. All syntheses were done under vacuum to remove the formed water. In order to have fully melted systems, the stearate ester syntheses were done at 75 °C and the 12-hydroxystearate ester syntheses at 80 °C.

2.3. HPLC analysis

The reactions were monitored using reversed phase HPLC with a Supelco Discovery C18-column (4.6 mm i.d. × 150 mm), 1 ml/min of a 88:12 or 95:5 methanol:water mixture with the addition of 0.5% acetic acid in the separations of the stearate esters. Detection was done with a Sedex 45 evaporative light scattering detector (Sedere, Alfortville, France) at 30 °C and 2 bar air pressure.

2.4. Purification

To remove excess PEG, the esters were purified with repeated extraction between ethyl acetate and NaCl solution (300 g/l) according to the Weibull method [2] but at room temperature. Excess fatty acid was removed with extraction between chloroform and 1 M Na_2CO_3. To remove traces of the free fatty acid from the diester syntheses, preparative silica-gel chromatography was performed with a MPLC system (Baeckstrom Separo AB, Lidingö, Sweden) using a gradient from ethyl acetate to methanol.

2.5. NMR

Structures were confirmed with a 400 MHz NMR spectrometer (Bruker, Rheinstetten, Germany). *PEG monostearate*: [^{1}H] NMR (400 MHz, $CDCl_3$): δ 4.2 (t, 2H); 3.6 (br, 52H); 2.3 (t, 2H); 1.6 (quint, 2H); 1.25 (br, 28H); 0.9 (t, 3H). *PEG distearate*: [^{1}H] NMR (400 MHz, $CDCl_3$): δ 4.2 (t, 4H); 3.7 (t, 4H); 3.6 (br, 46H); 2.3 (t, 4H); 1.6 (quint, 4H); 1.25 (br, 56H); 0.9 (t, 6H). *PEG mono-12-hydroxystearate*: [^{1}H] NMR (400 MHz, $CDCl_3$): δ 4.2 (t, 2H); 3.6 (br, 52H); 3.55 (br, 1H); 2.3 (t, 2H); 1.9 (br, 1H); 1.6 (quint, 2H); 1.4 (q, 4H); 1.25 (br, 22H); 0.9 (t, 3H). *PEG di-12-hydroxystearate*: [^{1}H] NMR (400 MHz, $CDCl_3$): δ 4.2 (t, 4H); 3.7 (t, 4H); 3.6 (br, 46H); 3.55 (br, 2H); 2.3 (t, 4H); 1.8 (br, 2H); 1.6 (quint, 4H); 1.4 (q, 8H); 1.25 (br, 44H); 0.9 (t, 6H).

3. Results and discussion

The lipase-catalyzed reaction of PEG with fatty acids such as stearic acid or 12-hydroxystearic acid is very straight-forward and simple. Pure 12-hydroxystearic acid is crystalline and practically insoluble in PEG at room temperature. The fatty acid melts at 83 °C and is then miscible with PEG. Thus, it becomes possible to perform the reaction without addition of solvents. This increases the reactant concentrations and improves the reaction rate.

The isolated products were all white solids with purities of 98–99% as determined by HPLC (e.g. PEG(600) mono-12-hydroxystearate in Fig. 2). The isolated yields (based on the limiting reactant) were PEG(600) monostearate 87% (5.28 g), PEG(600) distearate 77% (4.34 g), PEG(600) mono-12-hydroxystearate 82% (14.4 g), PEG(600) di-12-hydroxystearate 78% (3.79 g).

The observed reaction rates were similar for reactions with stearic acid and 12-hydroxystearic acid. The syntheses were stopped when the limiting reactant had been completely consumed as determined by HPLC. For the monoester syntheses, 50% conversion was reached after approximately 4 h and the reactions were considered complete after 24 h. The diester reactions reached 70% conversion after approximately 2.5 h and were completed after 6 h. Product loss in the purification steps resulted in somewhat lower isolated yields.

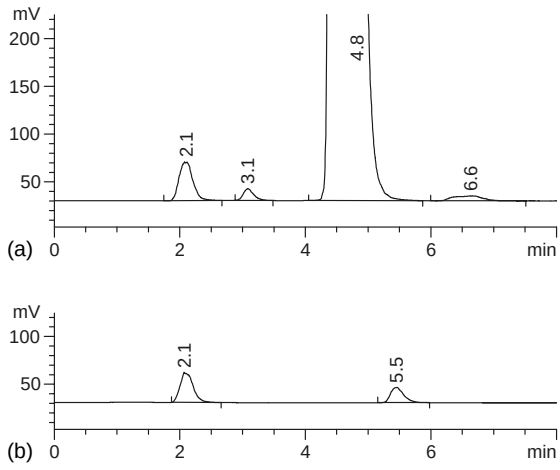

Fig. 2. Reversed-phase HPLC traces of: (a) PEG(600) mono-12-hydroxystearate (10 mg/ml, t_r = 4.8 min) as obtained after the extraction step, (b) a mixture of PEG(600) (t_r = 2.1 min) and 12-hydroxystearic acid (t_r = 5.5 min) each at 0.1 mg/ml, illustrating 1% contamination of sample (a).

To obtain pure esters when using 12-hydroxystearic acid it is necessary to prevent the formation of esters on the 12-hydroxyl groups. Commercial production of PEG ester surfactants is done via alkali-catalyzed ethoxylation of fatty acids at 110–165 °C. The alkaline reaction conditions causes rapid transesterification and the product mixture contains approximately 30% free PEG, 30% PEG diesters and very small amounts of free fatty acid [3]. In the ethoxylation of 12-hydroxystaric acid, the 12-hydroxyl group or its ethoxylated derivative can be esterified by other 12-hydroxystearic acid moieties causing very complex product mixtures.

Performing the reaction with a regioselective lipase like *C. antarctica* lipase B leaves the hydroxyl group on the fatty acid untouched. It is well known that *C. antarctica* lipase B has very low activity towards secondary hydroxyl groups with propyl groups or longer extending on both sides [4] and that the hydroxyl group on hydroxy fatty acids is not acylated with 1,3-specific lipases [5]. In accordance with this, we did not detect any secondary esters in the product.

Vacuum-driven esterifications of PEG with hydroxy fatty acids have also been carried out using the liquid ricinoleic acid (12-hydroxyoleic acid) and *Rizomucor miehei* lipase [6]. They used stoichiometric amounts of reactants yielding a surfactant mixture, whereas we produce the pure mono- and di-esters of stearic and 12-hydroxystearic acid using excess of one of the reactants.

The driving force in the reaction is the removal of the water that is formed. As there is no solvent that can evaporate, it is advantageous to apply high vacuum to efficiently remove the water. The relatively high temperature, which is needed to melt the reactants, further improves the evaporation of the formed water.

3.1. Controlling mono- or di-ester distribution

A problem with PEG monoester synthesis, for both traditional methods and enzyme-catalyzed reactions, is that there are two independent hydroxyl groups on each PEG molecule that can react with the acid. The easiest way of controlling the ratio of mono- and diesters is to use one reactant in excess. We have used a 10-fold excess of PEG in the monoester syntheses and an approximately three-fold excess of fatty acid in the diester syntheses. Rao et al. [7] used a chemical method based on protecting the PEG borate esters to synthesize monoesters. Another approach to get pure monoester would be to use PEG monomethyl ether. This was not tested as it has been shown that the free hydroxyl group on the PEG affects the physical properties of PEG surfactants [8].

The two hydroxyl groups on the PEG chain are chemically equivalent and the equilibrium constants for esterification of the first and second hydroxyl can be expected to be equal. The effects of controlling the ratio of mono- to di-ester by using one of the reactants in excess can easily be simulated. Using a model with low water activity and reasonable equilibrium constants, a ratio of PEG:fatty acid of M:1 results in approximately M times more monoester than diester in the product. In the diester synthesis virtually no monoester is expected using only a small excess of fatty acid.

Given the equilibrium equations, it is thus inherently easier to push the reaction towards diester formation than to stop at the monoester. We used 10 times excess of PEG in the monoester syntheses and reached a sufficient excess of monoester. In the diester syntheses, we have used three times more acid than PEG (which equals 1.5 acid molecules per hydroxyl group) and found it easy to push the equilibrium with a vacuum pump to obtain the pure diester.

The PEG mono-12-hydroxystearate contained less than 0.25% free acid after the extraction step (Fig. 2) but the PEG monostearate contained 2% stearic acid. Occasionally, small amounts of unidentified side products were also found (e.g. $t_r = 3.1$ and 6.6 in Fig. 2a). To avoid negative effects of the surface-active free fatty acids in later surface-chemical and pharmaceutical characterization, preparative silica-gel chromatography was used to remove the free stearic acid, yielding >99% PEG monostearate. It was also found that the polydisperse mixture of PEG diesters could easily be separated into fractions of very narrow PEG chain-length distributions using silica-gel chromatography. This was not seen for the monoesters. However, for all syntheses, a simple extraction step was enough to remove the excess reactant and isolate the product in high yield (77–87%) and purity (>98%).

In a commercial surfactant, a controlled mixture of mono- and di-esters may be desired in order to engineer the surfactant properties of the product mixture. This can easily be obtained by balancing the amounts of PEG and fatty acid as well as controlling the water activity, e.g. with vacuum to get the desired mono- to di-ester ratio.

The products had the same chain length distribution as the PEG reactant. There was no discrimination based on PEG chain length as seen with MALDI-TOF MS.

Acknowledgements

This work was supported by the Competence Centre for Surfactants Based on Natural Products, SNAP.

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