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Enzymatic amidification for the synthesis of biodegradable surfactants: Synthesis of *N*-acylated hydroxylated amines

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Abstract

Glucamide surfactants can be obtained by reacting amino–sugar derivatives with fatty acids in the presence of lipase enzymes in organic media. Reactions are catalyzed with Lipozyme in hexane or with Novozym in 2-methyl-2-butanol as solvents. In hexane, the formation of a salt complex between the fatty acid and *N*-methyl-glucamine allows the efficient acylation of the amine. Fatty acid conversion is limited to 50% due to salt formation. In 2-methyl-2-butanol, conversion yield of the fatty acid up to 100% can be obtained by removing the water coproduct under reduced pressure. Acido–basic conditions allow the control of the reaction chemoselectivity. This reaction can also be completed using various amines and acyl donors.

Keywords: Glucamide surfactants; Lipozyme; Novozym; Organic media; Enzymatic amidification

1. Introduction

Glycamide surfactants are nonionic surfactants in which the hydrophilic moiety (an amino–sugar derivative) and the hydrophobic moiety (a fatty acid) are linked via an amide bond [1]. This results in a chemical linkage that is highly stable under alkaline conditions, which is of key interest for many surfactant applications [2]. Such sugar fatty amide surfactants can be obtained by chemical synthesis, using for example the Schotten–Baumann reaction in aqueous alkaline conditions. One important drawback of this approach is the final coproduction of salts. An alternative approach consists in using an enzymatic synthesis route, which avoids the formation of by-products and salt coproducts. Amide bond synthesis can be catalyzed by proteases. But proteases are generally highly specific for given amino acids and sensitive to organic solvents [3]. Besides proteases, lipases have been proven to be able to catalyze the formation of amide bonds in nonconventional media [4,5] and involved in the synthesis of peptides [3,6,7], fatty amides [8–10], *N*-acyl-

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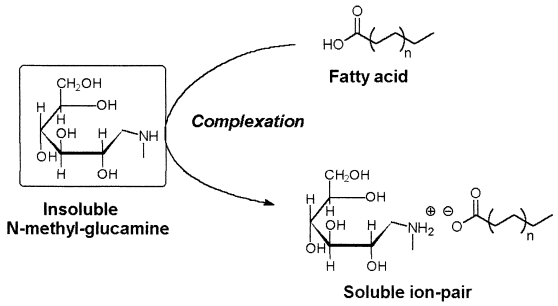

Fig. 1. Ion-pair formation between a fatty acid and *N*-methyl-glucamine.

amino acids [11,12] and acyl-amino-propanol [13].

We thus studied the possibility of catalyzing the amidification of a widely available amino–sugar derivative, *N*-methyl-glucamine, by fatty acids, using commercially available immobilized lipase preparations as catalysts.

2. Synthesis of glucamide surfactants in hexane

Hexane is a nonpolar solvent, in which *N*-methyl-glucamine (**1**) is not soluble. In the presence of a fatty acid **2**, *N*-methyl-glucamine was solubilized by ion-pair formation (Fig. 1), as shown by infrared spectroscopy [14].

When using a 60-mM solution of oleic acid in hexane, the soluble *N*-methyl-glucamine concentration ranges from 50% with an equimolar amine concentration to 100% with a 10-mM amine concentration [14]. The acid/amine ratio not only determines the amount of *N*-methyl-glucamine solubilized in the organic phase, but also the chemoselectivity of the enzyme reaction. Lipase is able to catalyze the synthesis of both amide (**3a**) and ester bonds (**4a**) (Fig. 2):

When immobilized lipase from *Rhizomucor miehei* (Lipozyme, Novo Nordisk) was used in the presence of oleic acid and *N*-methyl-glucamine, the reaction chemoselectivity varied according to the acid/amine ratio [14]. For an acide/amine ratio of 8 (excess acid), ester synthesis was favoured, 100% of *N*-methyl-glucamine transformation yielding the 6-*O*-oleoyl-*N*-methyl-glucamine. When the ratio was lower than 1 (excess amine), oleoyl-*N*-methyl-glucamide was the only product. This result shows the importance of acido–basic conditions even in nonconventional media, particularly when the substrate molecules contain ionic groups. In fact, acido–basic conditions determine the ionic state of both substrate and enzyme catalysts, and thus both the efficiency and the chemoselectivity of the synthesis reaction. For example, the addition of a base (triethylamine) to a reaction mixture results in an increase of the amidification yield, but a decrease in the acid conversion rate [14].

The acid conversion yield did not exceed 50%. This was attributed to the ion-pair formation between the acid and the amine, which

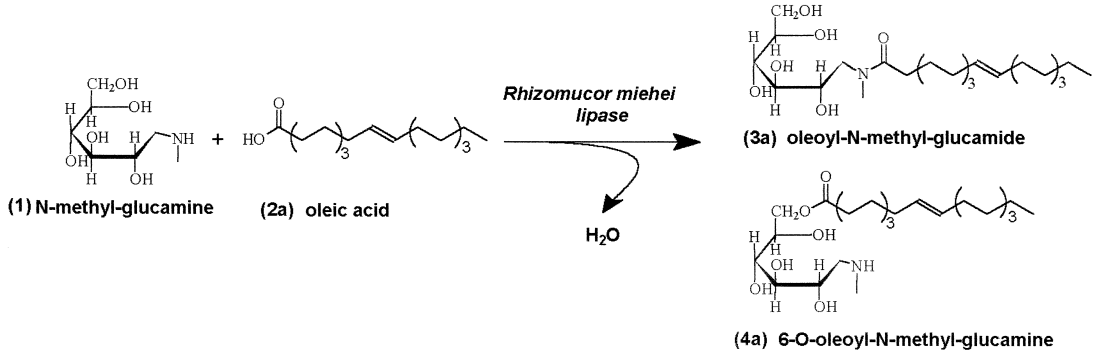

Fig. 2. Enzymatic synthesis of amide and ester derivatives of *N*-methyl-glucamine in the presence of oleic acid.

determines the possibility of reaction. In fact, when methyl oleate was used as an acyl donor, no solubilization of *N*-methyl-glucamine was observed, and thus very limited acylation occurred, due to the impossibility to form an ion pair [14]. This ion-pair formation, which is highly stable in hexane, limits the possibility of acid conversion. This was confirmed by infrared spectroscopic characterization of the reaction mixture: the reaction stopped due to the lack of free acid in the reaction medium [14].

In order to improve the acid conversion yield, a less nonpolar reaction solvent, 2-methyl-2-butanol, was used to solubilize the *N*-methyl-glucamine substrate more efficiently.

3. Synthesis of glucamide surfactants in 2-methyl-2-butanol

2-Methyl-2-butanol is a polar protic solvent which is not a substrate for lipase and which is not toxic. It has been used for sugar ester synthesis catalyzed with immobilized lipase [15]. *N*-Methyl-glucamine solubility in this solvent is 6 g/l at 55°C. In this case, the most efficient immobilized lipase preparation was found to be the commercially available (Novo Nordisk) enzyme from *Candida antarctica* adsorbed onto an acrylic resin: Novozym 435 [16]. This enzyme is highly stable in organic media and can be easily recovered from the reaction mixture.

When Novozym is operated at 55°C under atmospheric pressure in the presence of a stoichiometric mixture (175 mM each) of oleic acid and *N*-methyl-glucamine, 40% of the fatty acid (**2**) is converted after 130 h to yield a product mixture containing 80% amide and 20% mono-ester derivatives [16].

When the reaction was run at 90°C and under atmospheric pressure, 100% conversion of oleic acid was reached in only 50 h and a 97% amide yield was obtained. Under these reaction conditions, a new product was obtained with a 3% yield, corresponding to the diacylated amide–ester (**5**) derivative (Fig. 3). This product was formed from the mono-ester (**4**) which was produced as an intermediate and which had completely disappeared at the end of the reaction [16].

When the reaction was run at 90°C and 500 mbar pressure, 100% conversion of oleic acid was obtained in 20 h, but a final mixture containing 75% amide, 10% amide–ester, 5% ester and 10% unreacted amine was observed [16]. Amine substrate was not completely transformed in such conditions.

In order to limit the number of coproducts, fatty-acid esters were preferred to triglycerides [16]. When operating at 90°C under 500 mbar pressure, with 350 mM oleic acid ester, 350 mM *N*-methyl-glucamine mixture as substrate, in the presence of 10 g/l Novozym, 100% acid conversion was obtained in 20 h with the ethyl

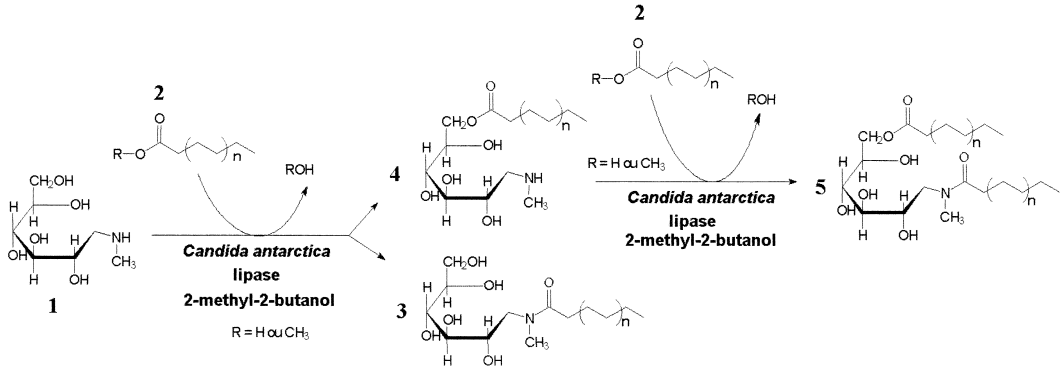

Fig. 3. Enzymatic synthesis of amide, ester and amide–ester derivatives of *N*-methyl-glucamine in the presence of oleic acid methyl ester.

Table 1
Effect of fatty-acid methyl ester/*N*-methyl-glucamine molar ratio on the chemoselectivity of the acylation reaction

Fatty-acid methyl esters/ *N*-methyl-glucamine (mM/mM)	Amide–ester **5** (%)	Ester **4** (%)	Amide **3** (%)	Total amine conversion (%)
350/175	30	20	50	100
350/350	10	0	80	90
350/700	4	0	96	50

Reaction conditions: 10 g/l of Novozym at 90°C under 500 mbar pressure in 2-methyl-2-butanol. Reaction time: 10 h.

ester and in 15 h with the methyl ester used as acyl donors. In this case, water is no longer the reaction coproduct but the alcohol corresponding to the ester is coproduced. From this point of view, methyl esters are of key interest as (i) fatty-acid methyl esters are industrially available as diesel substitutes obtained by acidic alcoholysis of plant oils (rapeseed oil, sunflower oil) and (ii) methanol can be easily removed from the reaction medium at 90°C under reduced pressure. At 90°C and 500 mbar with an equimolar mixture containing 350 mM fatty-acid methyl esters (Diester®, Sidobre Sinnova: 59.1% C18:1; 22% C18:2; 11% C18:3; 5.2% C16:0; 1.9% C20:1; 0.5% C22:1; 0.1% C14:0) and 350 mM *N*-methyl-glucamine as substrates, 100% fatty-acid methyl ester conversion was reached after 10 h reaction, with an amide yield of 80% [17]. The ester derivative (**4**) was produced during the first hours of reaction, but completely disappeared at the end of the reaction acting both as an acyl acceptor to give the amide–ester derivative (**5**) and as an acyl donor [17] (Fig. 3). In this case the acid/amine ratio also controls both the efficiency and the chemoselectivity of the synthesis reaction (Table 1). This underlines the importance of substrate and enzyme ionization even in non-aqueous organic media.

4. Conclusion

In conclusion, an efficient method has been developed for the enzymatic synthesis of amide derivatives from a secondary amine substrate using a lipase as catalyst. It involves low-cost and largely available renewable raw materials. It was established that the process can be extended to various sources of amine and acyl donors [16]. This process is presently under development [18].

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