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Aminolysis of linoleic and salicylic acid derivatives with *Candida antarctica* lipase B: A solvent-free process to obtain amphiphilic amides for cosmetic application

Ana Maria Mouad[a,b,*], Delphine Taupin[b], Laurent Lehr[b], Florent Yvergnaux[b], André Luiz Meleiro Porto[a]

[a] Universidade de São Paulo, Instituto de Química de São Carlos, Av. João Dagnone, 1100, 13563-120 São Carlos, São Paulo, Brazil
[b] BioEurope-Groupe Solabia, Route d'Oulins-28260, Anet, Eure-et-Loir, France

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ABSTRACT

In this biotechnological process, the fatty amides (9*Z*,12*Z*)-*N*-dodecyloctadeca-9,12-dienamide (**3**) and *N*-dodecyl-2-hydroxybenzamide (**5**), respectively derived from linoleic acid and salicylic acid were synthesized through aminolysis reactions catalyzed by *Candida antarctica* lipase B. These amphiphilic compounds receive great attention from cosmetic industry due to a range of beneficial properties for skin. The aminolysis reactions were performed with the esters ethyl linoleate (**1**) and ethyl salicylate (**4**) as acyl group donors and the fatty compound *N*-dodecylamine (**2**) as the nucleophilic substrate. The aminolysis reactions were carried out in a solvent-free process, which is beneficial from an environmental and economical perspective, at 65 °C and reduced pressure (50 mbar). Parameters as enzyme amount and substrates molar ratios were investigated and the products were monitored by HPLC analysis. The conversion rates were measured through disappearance of ethyl linoleate and ethyl salicylate. The best conversion rates (up to 95%) were obtained by adding an enzyme amount of 5.0 g/mol of acyl donor group substrate and an equimolar substrates ratio (1:1). The products characterization was performed by High Resolution Mass Spectrometry, Infrared spectroscopy and Nuclear Magnetic Ressonance. This work reveals that enzymatic synthesis provides an attractive way for the cosmetic industrial production of fatty amides, which may represent key ingredients to maintain and/or restore a healthy skin.

1. Introduction

Over the last decades, the application of enzymes as catalysts has expanded horizons in several fields of biotechnology. This expansion may be related to a 'greener' way to produce fine chemicals with high degree of selectivity, none or minimal side reactions and relatively milder conditions compared to the chemical counterparts [1].

In this scenario, lipases (triacylglyceride hydrolases; EC 3.1.1.3) constitute the most important enzymes for biotechnological applications [2]. They present commercial availability, stability and usually do not require expensive cofactors [3,4]. These biocatalysts are capable of reacting with several substrates, catalyzing not only the hydrolysis and synthesis of long-chain acylglycerols but also alcoholysis, acidolysis, interesterification and the transfer of acyl groups from esters to amines (nucleophiles), promoting aminolysis reactions [5,6]. The use of lipases in aminolysis of esters in anhydrous media has been successfully described for the synthesis of peptides, fatty acid amides, polymers, surfactants and low cost detergents [7,8].

From the cosmetic sector standpoint, lipases represent great biocatalysts in the synthesis of fine chemicals such as specialty esters, aroma compounds and active ingredients [9]. Notedly, the lipase B from *Candida antarctica* (CALB) has proven to be a versatile catalyst to synthesize cosmetic products for skin care, such as retinol (vitamin A) and ascorbic acid (vitamin C) derivatives, widely used as natural antioxidants in the treatment of photoaging and skin disorders [10].

In a previous work, CALB catalyzed aminolysis reactions in a solvent-free process, leading to the production of ceramides analogous molecules, which are essential for epidermal permeability barrier function and overall condition of the skin [11–13]. In this work, we proposed the employment of CALB as catalyst in

* Corresponding author at: Universidade de São Paulo, Instituto de Química de São Carlos, Av. João Dagnone, 1100, 13563-120 São Carlos, São Paulo, Brazil
E-mail address: ana.m.mouad@gmail.com (A.M. Mouad).

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aminolysis reactions between the esters ethyl linoleate (**1**) and ethyl salicylate (**4**), respectively derived from linoleic and salicylic acids, and the fatty compound *N*-dodecylamine (**2**).

The interest in the application of these compounds as cosmetic ingredients can be described as follows; firstly, the presence of a linoleic acid (LA) derivative is interesting in cosmetic formulations since it represents an important skin constituent, with specific functions in the production and maintenance of epidermal permeability barrier [14–16]. LA has received increased attention in cosmetic market due to its anti-inflammatory, acne reduction and moisture retention properties [12]. Furthermore, this polyunsaturaded fatty acid (PUFA) is featured with 18 carbon atoms and two *cis* double bonds at position 6 (*omega*-6 family), representing an essential fatty acid. Whereas the human body can not synthesize double bonds at position 6, LA must be necessarily obtained from the diet or topical applications [14].

With respect to ethyl salicylate (**4**), salicylic acid (SA) derivatives composes the phenolic acid family, recognized by the antioxidant activity against the highly reactive free radicals, which are involved in adverse skin effects such as photoaging, wrinkling, general loss of elasticity and cancer [17–20]. SA is frequently used in the treatment of *Acne vulgaris* due to its keratolytic properties and is also known as an ultraviolet B sunscreen, reducing sunburn and melasma production [21–23].

Lipophillic derivatives of salicylic acid also have been proven to present anti-aging, photoprotective, keratolytic and antibacterial properties. In fact, it has been shown that the addition of fatty chains to SA molecule confers superior efficacy on the original molecule [24]. The greater efficacy is related to the skin permeability, which is lower for hydrophilic compounds, since the outermost layer of skin represents a highly lipophilic barrier constituted of ceramides, fatty acids and cholesterol [25]. In this context, the addiction of fatty chains can be extended to LA derivatives, where the increasing of fatty chains in unsaturated fatty acids, particularly those of *cis* configuration, confers greater protective effect against cutaneous dehydration and xenobiotics [26]. Thus, this structural modification represents an attractive way to improve the skin-product compatibility.

Additionally, this reactions may lead to the production of amphiphilic molecules, which presenting an hydrophobic region (soluble in lipophilic medium) and a hydrophilic head (solubility in aqueous environment), represent ideal stabilizers for emulsion formulations, being recognized as emulsifiers or surfactants [27]; once there is a concern about the classical synthesis of surfactants derived from petroleum [28,29], biosurfactants containing amide bonds are potential substitutes, presenting skin tolerance, good biological degradability and significantly lower toxicological effects [30].

Therefore, the structural modification of LA and SA esters (**1** and **4**) via aminolysis reactions catalyzed by CALB with the fatty *N*-dodecylamine (**2**), in a solvent-free process, can be used as a tool to produce amphiphilic amides, which may attract great interest from cosmetic industry and differentiated applications for skin cosmetic formulations.

2. Materials and methods

2.1. Materials

The lipase B from *C. antarctica* (Novozym 435, immobilized) was kindly provided by Novozymes A/S, Bagsvaerd, Denmark. Ethyl linoleate (**1**) was purchased from Stearinerie Dubois (Ciron, France), while *N*-dodecylamine (**2**) and ethyl salicylate (**4**) were purchase from Sigma–Aldrich (St. Louis, MO).

2.2. The reaction parameters

It is important to consider that to run enzymatic reactions under solvent-free conditions, at least one of the reactants has to be kept in the liquid state; thus, the reaction temperature has to be high enough (65 °C) to shift the equilibrium in the right sense. Also, to limit the possible reverse reaction (hydrolysis), the reactions were carried out under vacuum, evaporating thus, the ethanol (by-product) formed during the reaction.

2.3. Enzymatic reactions

In order to obtain the amphiphilic amides **3** and **5** in great yields, two studies were perfomed to evaluate the effect of substrates molar ratio and the biocatalyst amount. In the effect of substrates molar ratio study, the reactants acyl donor groups **1** or **4** and the nucleophile *N*-dodecylamine (**2**) were added in 50 mL flasks in the following values: 0.8: 1.0; 1.0:1.0 and 1.0:2.0 (acyl donor **1** or **4**: nucleophile **2**). Then, after 5 min under vacuum (50 mbar) for gas removal of the system, 5.0 g of *C. antarctica* B/mol of ethyl esters (**1** or **4**) were added to the reaction mixtures.

Also, one sequence of reactions was performed by changing the biocatalyst amount (CALB) in order to find the higher yields. This study was carried out in 50 mL glass reactors, wherein equimolar substrates concentrations [1 acyl donor groups (**1** or **4**): 1 nucleophile (**2**)] were added. After 5 min under vacuum (50 mbar) for gas removal of the system, different CALB amounts were added to the reaction mixtures (0.6; 1.2; 2.5; 5.0; 7.5; 10 g/mol acyl donor reactants **1** or **4**).

All the reactions were conducted under stirring (250–300 rpm) using an overhead stirrer IKA RW 16 Basic, Staufen, Germany, equipped with a plastic propeller, at 65 °C. After 20 h, the products were purified through *flash* chromatography and the rates of conversion of the reactions were monitored through the disappearance of ethyl linoleate (**1**) or/and ethyl salicylate (**4**) using HPLC analysis.

3. Analytical and characterization methods

3.1. High performance liquid chromatography (HPLC)

The aminolysis reactions were monitored by high-performance liquid chromatography (HPLC analysis) carried out in a system (Alliance-Waters) composed of a column (Xterra MS C18 5M, 150 mm × 2.1 mm), a column oven (temperature 40 °C), an autoinjector and UV/vis detector (PDA, W2996 = 210 nm). The compounds were evaluated with an eluent system of methanol (A) and water (B), both containing 0.1% of trifluoroacetic acid, as shown in Table 1.

3.2. Nuclear magnetic resonance (NMR)

The NMR specters of proton and carbon (^{1}H and ^{13}C NMR) of the chemical structure of the products were obtained using a Bruker AM 500 at the Analytical Center of the Institute of Chemistry of São Carlos (IQSC/USP), operating in 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR), using $CDCl_3$ as the deutered solvent and TMS as the internal standart unless otherwise noted. The chemical shifts are given in ppm and the coupling constants (*J*) in Hz.

3.3. High resolution mass spectrometry (HRMS)

The analyses were performed on a high resolution mass spectrometer quadrupole/flight time, operating in the MS mode in the unit: microTOF-QII, detector Daltonics Bruker (Bremen Germany). In these analyzes, the samples were ionized in the positive mode using atmospheric pressure chemical ionization (APCI). The analyses were performed at the Institute of Chemistry of São Carlos, in