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Towards regioselective enzymatic hydrolysis and glycerolysis of tricaprylin in miniemulsion and the direct preparation of polyurethane from the hydrolysis products

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ABSTRACT

Miniemulsion droplets are used as the reaction environment for the enzyme catalyzed hydrolysis and glycerolysis of tricaprylin. The reaction was conducted at slightly elevated temperatures in aqueous dispersion without any organic solvent. Lipase PS from *Pseudomonas cepacia*, known as a lipase without site preference (unspecific), and lipase from *Rhizopus arrhizus* (RAL), a lipase with sn-1,3 preference, were used for the reactions. The time courses of the conversions were studied in detail with NMR and HPLC. RAL exhibited sn-1,3 preference and Lipase PS clearly showed significant reaction in sn-2 position during hydrolysis of the triglyceride as well as during glycerolysis. From the hydrolysis products, polyurethane-based polymers were synthesized by directly adding isophoronediisocyanate to the miniemulsions after different reaction times. The properties of the products, e.g. the T_g, can be controlled by time-dependent addition of the isocyanate.

1. Introduction

Products derived from natural oils or fats are widely used ingredients in cosmetics, functional foods, pharmaceuticals, and many other fields. Typically, these products are produced by sequential hydrolysis of natural oil, which is a tri-ester of glycerol (triacylglycerol, triglyceride, TG) and long chain carboxylic acids (fatty acids, FFA). FFA, monoacylglycerols (MG) and diacylglycerols (DG) are used as emulsifiers in cosmetics and pharmaceuticals, nutritional additives or building blocks (FFA and MG) in organic and polymer synthesis [1–3].

Chemical hydrolysis of triacylglycerols in aqueous emulsions with inorganic alkaline catalysts at high temperatures (>100 °C) is one of the oldest chemical processes known to mankind. This process, known as soap boiling, leads to the metal salts of the free fatty acids and glycerol but not to monoacylglycerols or diacylglycerols. For the synthesis of MG, natural oils can be reacted with glycerol. This so called glycerolysis is nowadays used as a continuous process for MG-production on an industrial scale. High temperatures (250 °C) are required to ensure sufficient solubility of the fat or oil in glycerol in order to perform the reaction in reasonable time. Typically, chemical glycerolysis has to be conducted in the absence of water (<0.01 wt% H_2O) to minimize competitive hydrolysis. Moreover, this process is energy consuming, provides low yields on MG and distillation is necessary when purity and flavor quality are required, as e.g. as food additive [4,5]. However, with the use of large volumes of glycerol produced from biodiesel production, the glycerolysis of triacylglycerols is an interesting alternative to obtain products which could be directly applied as monomers (e.g. monoacylglycerols) for polymerization reactions [6] and production of bioplastics based on renewable resources [7].

Several alternatives for hydrolysis or glycerolysis at milder and less energy consuming reaction conditions were evaluated, among which the lipase catalyzed reactions have received considerable attention and several reaction systems were reported in

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literature [8–10]. Typically, enzymes show their highest activity at temperatures around 40 °C, which reduces the reaction temperature considerably from the high temperature required for chemical hydrolysis (>100 °C) and glycerolysis (>200 °C).

In contrast to the conventional, chemically catalyzed hydrolysis or glycerolysis of triacylglycerides, different lipases can preferentially catalyze the hydrolysis in distinct positions of the glycerol ester [11–13]. The possible reaction sequences are illustrated in Scheme 1. The scheme also shows that some of the possible products can be obtained with lipases of different selectivities and the acyl migration, i.e. intramolecular transesterification of 1,2-diacylglycerol and 2-monoacylglycerol to the thermodynamically more stable 1,3-diacylglycerol and 1-monoacylglycerol, respectively [14]. Most of the known lipases exhibit sn-1,3 specificity or catalyze reactions in any position (unspecific lipase) with no distinct preference. Very few lipases, however, exhibit preferential catalysis in the central (sn-2) position [12,13]. External factors, as e.g. the solvent [15] or the reaction environment [16], are known to affect the selectivity and specificity of lipases. However since the conversion is not 100%, a mixture of acylglycerols, free fatty acid and glycerol is obtained.

The application of lipases for the production of monoacylglycerols (MG) was reviewed by Bornscheuer [17] and more recently by Feltes et al. [18]. Many approaches have been investigated in the enzymatic synthesis of MG, in particular, using 1,3-regiospecific lipases for selective hydrolysis, esterification of fatty acids or transesterification of fatty esters with glycerol, and the glycerolysis of fats or oils [19]. The general focus of biotransformation research is to find commercial and economical feasible reaction conditions by using enzyme modification techniques [20] searching for new sources of lipases of microbial [21–24] and vegetable [25] origin, studying the use of adequate bioreactors [26], and by optimization of the reaction conditions [27], preferably in the absence of organic solvents [28].

In contrast to esterases, which are mainly capable of catalyzing reactions of water soluble substrates, lipases are enzymes which can catalyze the hydrolysis and esterification reactions of hydrophobic substrates. Although lipases act on hydrophobic substrates, the natural environment of the enzymes is water. Thus, the enzyme is located at the interface between water and the hydrophobic substrate to catalyze the reaction there. The direct consequence of this phenomenon is that the interfacial area will have a decisive influence on the conversion rate of the lipase-catalyzed reaction, as esterification [29] or hydrolysis [30].

The droplets of a conventional emulsion typically have an average diameter larger than 5 μm. When the droplet size in an emulsion is decreased from 5 μm to 500 nm the total droplet surface area increases by a factor of 10. Stable heterophase systems featuring droplets of this size range are referred as “miniemulsions”. Here, a hydrophobic phase is dispersed by high shear forces (e.g. ultrasound) in water and stabilized against coalescence using a surfactant. Ostwald ripening in direct (oil-in-water) miniemulsions can be suppressed by the addition of a hydrophobic co-stabilizer (e.g. hexadecane) [31].

Several authors have already shown the efficiency of performing enzyme catalyzed reactions in miniemulsion [16,17,29,32–34]. Considering the huge interface provided by the finely divided droplets, the miniemulsion approach seems to be an ideal technique for the fast enzymatic hydrolysis and glycerolysis of triglycerides. In contrast to a solution system, where the substrate concentration is limited by the solubility, emulsion systems can tolerate a substrate load up to 50 wt% of the emulsion [16]. This reaction system avoids the use of expensive and toxic organic solvents to overcome the limited solubility of hydrophobic reagents (e.g. oils and fatty acids). For that reason miniemulsion can be considered as a “green” and low cost technology.

In this work glycerol trioctanoate (tricaprylin) was chosen as model substrate for enzymatic hydrolysis and glycerolysis in miniemulsion using lipases from *Rhizopus arrhizus* (RAL) and *Pseudomonas cepacia* (PS), exhibiting distinct regiospecificity. In conventional macroemulsions these enzymes exhibit different regiospecificity: RAL catalyzes in sn-1,3 position, while Lipase PS has no preferential site and is thus referred to as an unspecific enzyme (Scheme 1) [12,13]. The conversion to the products of the hydrolysis and glycerolysis were monitored during 24 h. The aim of the glycerolysis experiments was to shift the product mixture to a larger amount of monoglycerides, in particular, 2-monoglyceride. Pure glycerol cannot be used as continuous phase of a miniemulsion, as the viscosity of glycerol is too high to allow the system to be homogenized efficiently. Thus, glycerol/water mixtures were used as continuous phase, and the products were evaluated.

The hydrolysis products were evaluated as a renewable source of reactants for the chemical synthesis of polyurethane/polyurea, which has been directly prepared from the miniemulsions at different hydrolysis stages [35]. These samples have been analyzed by thermal analysis and IR spectroscopy. The results underline that the properties of the resulting polymer can be adjusted by simply adding isocyanate to the reaction mixture with different ratios of hydrolysis products.

2. Materials and methods

2.1. Materials

Rhizopus arrhizus Lipase RAL (molecular weight 43,000 Da) was purchased from Fluka, *Pseudomonas cepacia* Lipase PS (molecular weight 31,700 Da) provided by Amano, tricaprylin (glycerol trioctanoate, TRI, >99%) and glycerol (99%) from Sigma Chemical, deuterated chloroform (99.9%) from Deutero. Hexadecane (HD; 99%), caprylic acid, monocaprylin, isophoronediisocyanate (IPDI), acetonitrile and tetrahydrofuran were purchased from Aldrich. Dicaprylin was obtained from Merck. Lutensol AT50 (poly(ethyleneoxide) hexadecyl ether) was a donation from BASF. All the chemicals were used without further purification. Water of MilliQ quality was used throughout the experiments.

2.2. Hydrolysis and glycerolysis in miniemulsion

For the hydrolysis in miniemulsion, 3 g of tricaprylin (6.374 mmol, molar concentration in the emulsion: $[TRI]_0 = 0.386\ mol\ L^{-1}$), 125 mg of hexadecane and 11.875 g of a 1.0, 2.0 or 3.0 wt% aqueous Lutensol AT50 solution were stirred ($2000\ min^{-1}$) for 1 h at room temperature. Then, the miniemulsion was prepared by ultrasonicating the mixture with a Branson Sonifier W450 digital (1/2 in. tip) at 90% amplitude continuously for 2 min under ice cooling. The density of the miniemulsion was measured as $1.1\ g\ mL^{-1}$.

Glycerolysis was performed with different molar ratios of TG and glycerol. For a molar proportion of 1:2 or 1:5 triglyceride/glycerol, 1.188 (12.90 mmol) or 2.935 g of glycerol (31.87 mmol), respectively were mixed with 3.0 g of tricaprylin with 125 mg of hexadecane, and 11.875 g (~660 mmol) of a 1.0, 2.0 or 3.0 wt% Lutensol AT50 solution in water. The miniemulsion was prepared by ultrasonicating the mixture with a Branson Sonifier W450 digital (1/2 in. tip) at 90% amplitude continuously for 2 min at 80 °C. The elevated temperature is required to reduce the viscosity of the glycerol/water mixture. After sonication, the temperature was decreased to 40 °C before adding the enzyme and starting the reaction. NMR spectroscopy confirmed that the elevated temperature during miniemulsification did not induce (chemical) hydrolysis in significant amounts.