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Synthesis of (-)-menthol fatty acid esters in and from (-)-menthol and fatty acids – novel concept for lipase catalyzed esterification based on eutectic solvents

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ABSTRACT

Deep eutectic solvents (DESs) based on (-)-menthol and fatty acids (octanoic, decanoic and dodecanoic acid) were investigated as reaction media for the lipase catalyzed esterification of the DES compounds itself to synthesize (-)-menthol fatty acid esters. The DES acts as reaction medium and substrate pool simultaneously without the need of adding any solvent. *Candida rugosa* lipase was active in the neat (-)-menthol:fatty acid DESs to synthesize (-)-menthol fatty acid esters. This example shows for the first time that a valuable product can be enzymatically produced using both components of a DES without any co-solvent. The addition of water to the DESs enhanced the reaction outcome likely due to interfacial activation of the enzyme. In biphasic reaction systems with an addition of 10 wt% of water to the DES phase, the conversion (7 d) of octanoic, decanoic and dodecanoic acid reached 50%, 83% and 71%, respectively. This corresponds to a batch productivity of 133 $g\,L^{-1}\,d^{-1}$ (24 h) and a final (-)-menthyl dodecanoate concentration of 957 mM (7 d) in the (-)-menthol:-dodecanoic acid DES. Closer investigation of this DES reaction system revealed that water addition and stirring speed are interacting parameters to optimize the process. The developed DES reaction systems represent neat reactant mixtures enabling the lipase catalyzed esterification under solvent-free conditions.

1. Introduction

Biocatalysis has become a major tool for the selective transformation of industrially relevant molecules. Especially since it was discovered that enzymes are active in non-aqueous media [1], synthetic biocatalysis involving sparingly water-soluble substrates has evolved. However, the transformation of hydrophobic substrates remains challenging in terms of compromising enzyme activity and/or stability and high substrate loadings. Often aqueous reaction mixtures are chosen to maintain enzyme activity at the expense of higher substrate concentrations. If a non-aqueous reaction medium is selected instead, it should be mentioned that the choice of the reaction medium strongly influences the environmental impact of a reaction system. In general, the best solvent would be no solvent [2]. This is especially true if the most or all reaction components end up in the final product, resulting in high atom economy. The experimental-based screening of a suitable reaction medium that fulfils both requirements, i.e. low environmental impact and high atom economy, can be laborious, because numerous reaction medium engineering strategies exist [3–5]. In the past decades, novel solvent classes such as ionic liquids (ILs) and deep eutectic solvents (DESs) have emerged and even augmented the opportunities in reaction medium engineering. ILs and DESs represent promising alternatives to conventional, organic solvents for enzyme catalyzed reactions [6] owing to their tuneable physicochemical properties. DESs are eutectic mixtures of at least two initial compounds that form a hydrogen bond network. The hydrogen bond acceptor is mixed with a hydrogen bond donor in a certain molar ratio and usually mixing for a certain period of time at elevated temperatures is required to from a DES. The resulting liquid is characterized by having a considerably lower freezing point than its initial constituents at room temperature [7]. Depending on their initial compounds, there are also so-called natural DES (NADES), which are synthesized solely from natural precursors [8]. Unlike ILs, DESs are considered as potential “greener” solvents due to their potential biocompatibility, biodegradability and lower toxicity [9]. To date several promising results have been published demonstrating the application of DESs as reaction media in biocatalysis. However, the potential of these solvents has not yet been fully explored. In 2008 Gorke et al. reported a proof-of-principle study

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for the activity of lipases in DESs, describing transesterification and aminolysis reactions catalyzed by an immobilized CALB lipase in different choline chloride based DESs [10]. By using DESs as transesterification media undesired side reactions, e.g. hydrolysis, may be avoided, owing to limited amounts of water present in DESs. Especially, hydrophobic DESs are characterized by a low water content, which decreases with increasing carbon chain length and hence increasing hydrophobicity of the hydrogen bond donor [11]. However, water is essential for enzyme catalysis and can enhance the enzymatic activity in a DES [12]. Although other enzymes were also investigated using DESs as sole reaction media or as co-solvents [13–15], most studies deal with lipases [16–19]. Lipases are accepted as robust biocatalysts and their application in non-aqueous reaction media is widespread, also in an industrial environment [20]. Recently, lipase-catalyzed reactions have been described, in which the DES acts as solvent and substrate at the same time [21–23]. However, in these studies choline chloride is used to form a DES with only one of two substrates. Since choline chloride is not a reactant it can be regarded as a liquefying additive, which makes product recovery more difficult and eventually more cost-intensive. Inspired by Martins' work on natural terpene:monocarboxylic based DESs [24], the lipase-catalyzed esterification of (-)-menthol with octanoic, decanoic or lauric acid was studied in the corresponding eutectic (-)-menthol:fatty acid reactant mixtures. Menthol is a poorly water soluble monoterpene alcohol with eight stereoisomers. Of these isomers, (-)-menthol is commercially most relevant as aroma compound due to its characteristic mint flavor and its refreshing cooling effect with a wide range of applications in cosmetics, pharmaceuticals, food and tobacco. Furthermore, the strong flavor of (-)-menthol can be extenuated by esterification with short-chain fatty acids [25]. Consequently, we screened different lipases to synthesize (-)-menthyl octanoate, (-)-menthyl decanoate and (-)-menthyl laurate directly from the corresponding DESs without the addition of an organic solvent (Scheme 1). Since some lipases are activated by a phase interface, the addition of water to the hydrophobic water immiscible (-)-menthol-based DESs was examined as a key parameter on the reaction yield. As mentioned before several authors used one component of a DES as a reaction partner [21–23]; the aim of this work was to investigate whether it is possible to use both components of a DES as substrate in an enzymatic conversion without the addition of other solubilizing agents.

2. Experimental section

2.1. Materials

Candida rugosa lipase type VII ≥ 700 U/mg (CRL), Amano lipase PS from *Burkholderia cepacia* ≥ 30,000 U/g (BCL), lipase from *Pseudomonas cepacia* (today renamed as *Burkholderia cepacia*) ≥ 30 U/mg (PCL), Amano lipase *Pseudomonas fluorescence* ≥ 20,000 U/g (PFL) were purchased from Sigma-Aldrich. Immobilized *Candida antarctica* lipase B ≥ 5000 U/g (N435) was either purchased from Sigma-Aldrich or provided by c-LEcta GmbH (CALB). (-)-Menthol (purity ≥ 98.5%), octanoic acid (C8:0) (purity ≥ 99%), decanoic acid (C10:0) (purity ≥ 98%) and dodecanoic acid (C12:0) (purity ≥ 97.5%) were obtained from Sigma-Aldrich. (-)-Menthol lauric acid ester was gratifyingly synthesized by the Institute of Applied Synthetic Chemistry (Research Group of Prof. Marko D. Mihovilovic, Vienna University of Technology). All chemicals were used without any additional purification or dehydration step.

Table 1
Composition of (-)-menthol:fatty acid DESs [26].

	Compound 1	Compound 2	Ratio [mol%]
Ment:OA	(-)-Menthol	Octanoic acid (OA)	55:45
Ment:DA	(-)-Menthol	Decanoic acid (DA)	65:35
Ment:DDA	(-)-Menthol	Dodecenoic acid (DDA)	75:25

2.2. Preparation of DESs

Menthol:fatty acid DESs, namely (-)-menthol:octanoic acid (Ment:OA), (-)-menthol:decanoic acid (Ment:DA) and (-)-menthol:dodecanoic acid (Ment:DDA), were generally prepared by mixing the starting compounds in a certain molar ratio (Table 1). The mixtures were placed in an incubator at 42 °C and 250 rpm orbital shaking (Biosan environmental shaker-incubator ES-20) until a homogeneous liquid was obtained, which usually took up to 2 h.

2.3. Karl-Fischer titration

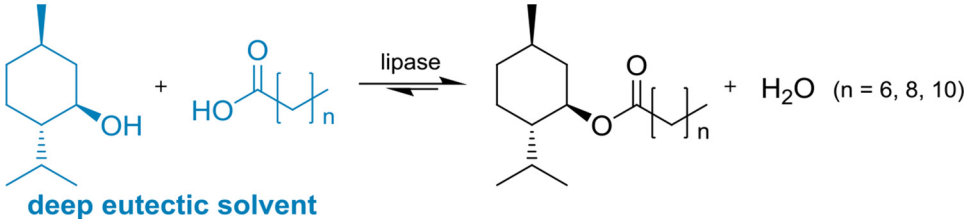
The water content of (-)-menthol:fatty acid DESs was determined by volumetric Karl Fischer titration (KF titrator DL38, Mettler Toledo) using a one-component reagent with a titre of 2 mg mL^{-1} (Hydranal®-Composite 2, Fluka). For the determination of the water uptake 2.5 g of DES were weighed into a 4 mL glass vial containing a stirring bar and 0, 1, 5 or 10 wt% of water were added. The samples were then incubated at 35 °C for 24 h at 1100 rpm. The initial water content was compared with the water content after incubation based on KF titration of the DES phase.

2.4. DES and lipase screening

Six different lipases were evaluated in each DES (Table 1) for the synthesis of (-)-menthol fatty acid esters, when the DES acts as substrate and solvent simultaneously. Two immobilized lipases, N435 and c-LEcta CALB, and four powdered lipases, CRL, BCL, PCL and PFL, were used. 0.5 g of each DES was weighed into a 2 mL reaction tube containing a stirring rod and the reaction was started by the addition of 5 mg immobilized beads or powder of the respective enzyme (i.e. for screening reasons the lipases were simply used in equal amounts). A negative control was prepared for each DES containing no enzyme. The reaction was allowed to proceed for 120 h at 35 °C with constant stirring (Velp Scientifica Multistirrer 6 Digital, 700 rpm). Samples were taken prior to the addition of the lipases and after 120 h reaction time. 10 µL of the reaction solutions were diluted with 990 µL ethanol and analysed by HPLC.

2.5. General procedure for CRL catalyzed esterification in DES

5 mg CRL powder was weighed into 2 mL reaction tubes and either no or different amounts of water were added. Negative controls containing no enzyme were prepared for each DES and water amount. The reaction was started by the addition of the 0.5 *g* DES. The reaction was



Scheme 1. Solvent-free lipase catalyzed (-)-menthol fatty acid ester synthesis in DESs.