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Research review paper

Advances in lipase-catalyzed esterification reactions

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ABSTRACT

Lipase-catalyzed esterification reactions are among the most significant chemical and biochemical processes of industrial relevance. Lipases catalyze hydrolysis as well as esterification reactions. Enzyme-catalyzed esterification has acquired increasing attention in many applications, due to the significance of the derived products. More specifically, the lipase-catalyzed esterification reactions attracted research interest during the past decade, due to an increased use of organic esters in biotechnology and the chemical industry. Lipases, as hydrolyzing agents are active in environments, which contain a minimum of two distinct phases, where all reactants are partitioned between these phases, although their distribution is not fixed and changes as the reaction proceeds. The kinetics of the lipase-catalyzed reactions is governed by a number of factors. This article presents a thorough and descriptive evaluation of the applied trends and perspectives concerning the enzymatic esterification, mainly for biofuel production; an emphasis is given on essential factors, which affect the lipase-catalyzed esterification reaction. Moreover, the art of using bacterial and/or fungal strains for whole cell biocatalysis purposes, as well as carrying out catalysis by various forms of purified lipases from bacterial and fungal sources is also reviewed.

Contents

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1. Introduction

Lipases are highly stable enzymes, which remain active even under unfavorable conditions. They are obtained in satisfactory yields from animals, plants, and natural or recombinant microorganisms, and have found a plethora of applications in food and pharmaceutical industries and technologies, as significant biocatalysts (Pandey et al., 1999). The physiological role of lipases (water-soluble triacylglycerol acylhydrolases, EC 3.1.1._) is the catalytic conversion of tri-glycerides into di-, or mono-glycerides, fatty acids and glycerol. A number of lipases are unable to hydrolyze ester bonds at secondary positions, as most of microbial lipases do, while another group of these enzymes hydrolyzes both primary and secondary esters. A third group of lipases exhibits fatty acid selectivity, and cleaves ester bonds of particular types of fatty acids (Krishna and Karanth, 2002). Lipases, which are serine hydrolases, are of considerable industrial potential, and catalyze esterification, interesterification and transesterification reactions in non-aqueous media (organic solvents and supercritical fluids), usually for biofuel production. Lipases catalyze also alcoholysis, acidolysis and aminolysis reactions, as well as hydrolyze organic carbonates (Pandey et al., 1999). In any case, the course followed by a lipase depends strongly on the aqueous content of the reaction medium, as absence of water eliminates the competing hydrolysis reaction. Conversely, there is a variety of reports on lipases catalyzing synthetic reactions in non-aqueous, or in low water content systems (Knežević et al., 2004; Krishna and Karanth, 2002; Hasan et al., 2009).

Lipases perform catalysis via a motif comprising three residues (serine, histidine and aspartate or glutamate); however, existing evidence indicates a convergence of the catalytic motifs of serine proteases and lipases (Kokkinou et al., 2012; Matsumura et al., 2008). An essential catalytic feature of lipases is a surface loop, the lid domain, which covers their active site (Aloulou et al., 2006; Meier et al., 2007), although a few lipases do not display a lid structure (Krishna and Karanth, 2002). Different reaction mechanisms describe lipase-catalyzed hydrolysis, esterification, and transesterification reactions, depending also on the specific used medium; thus, mostly non-Michaelis–Menten kinetic models have been suggested, which are applied in non-isotropic media and comprise steps leading to lipase activation and the formation of the corresponding enzyme–substrate complexes (Aloulou et al., 2006; Papamichael et al., 2012). Nevertheless, it should be emphasized that any lipase-catalyzed process (including synthesis) is influenced by the lipase stability, selectivity, mass transfer and other factors (Tufvesson et al., 2011), and one might choose from a variety of lipase-forms in order to use it as a biocatalyst, i.e. as: (a) whole-cell catalysis (lipases kept inside the host cell), in either a free or immobilized form, concomitantly taking into account the cost of side reactions, (b) liquid formulated lipases (lipases dissolved in aqueous solutions), and (c) immobilized lipases (lipases immobilized in solid matrices) either by cross-linking, or encapsulation, or adsorbing and/or covalent linking onto a matrix. In addition it should be taken into account that lipases should be able to retain water, since these enzymes may need the interface to work (Nielsen et al., 2008).

Finally, as most of the articles dealing with the lipase-catalyzed esterification focus mainly on the developed techniques and the yield optimization, herein, we not only report on the available mechanistic principles of the esterification reaction and its inherent difficulties due to fact that it is performed in organic solvents and/or in biphasic media (Kvittingen, 1994 and references therein), but also in contrast deal with the key factors and courses affecting the lipase-catalyzed esterification for biofuel production, their trends, challenges and future perspectives.

2. The main reactions

2.1. Hydrolysis

In general, enzymatic hydrolysis is considered important in science, technology and industry. Specific hydrolases, e.g. lipases, degrade lipids and other esters in a variety of scientific and industrial processes (Papamichael et al., 2012). The hydrolysis of natural and artificial esters is an unusual reaction, more likely due to the opposite polarities of hydrophobic substrates, and hydrophilic catalysts and products; this reaction is mainly occurring at the aqueous/organic solvent interface, although the interfacial composition is a matter of further research concerning the reaction microenvironment. Since many years ago, an array of works has been published reporting that the feedback mechanism of ester hydrolysis, including the digestion of triglycerides, could offer important information concerning the understanding and control of this reaction course (Aloulou et al., 2006; Reis et al., 2009).

2.2. Esterification

Enzyme-catalyzed esterification acquired increasing attention in many applications, due to the significance of the derived products. More specifically, the lipase-catalyzed esterification reactions attracted research interest during the past decade, due to an increased use of organic esters in biotechnology and the chemical industry (Torres and Castro, 2004). For this reason, esterification by lipases was developed a few decades ago (Okumura et al., 1979) and various microbial lipases have been employed in experiments using either primary or secondary alcohols, or both, free-solvent systems, or organic solvents. Among the important factors which influence the ester yield are the concentrations of enzyme and substrates, their molar ratio, the reaction pH-value and temperature, the mixing rates, and the water content (Zaks and Klibanov, 1988 and references therein).

3. Lipases as biocatalysts — the kinetics

Lipases, as hydrolyzing agents are active in environments, which contain a minimum of two distinct phases, where all reactants are partitioned between these phases, although their distribution is not fixed and changes as the reaction proceeds. Furthermore, the complications of phase heterogeneity and its temporal changes should be also considered. As a consequence, the level of understanding of the regulation of the catalysis by lipases has lagged behind that for the homogeneous one (Pandey et al., 1999; Reis et al., 2009).

In recent reports, numerous enzymes were classified according to their folding pattern. Lipases from different sources are normally categorized in the α/β-hydrolase folding group, sharing the same or at least similar folding patterns with esterases. Lipases, as hydrolyzing enzymes possess specific sequences of α-helices and β-strands. Detailed information on these structures has been reported previously (Jaeger et al., 1999, and references therein; Joseph et al., 2008 and references therein). In addition, a tool has been described to differentiate between lipases and esterases based on their protein surface electrostatic

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