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Research review paper

Lipases: Valuable catalysts for dynamic kinetic resolutions

Amanda S. de Miranda *, Leandro S.M. Miranda, Rodrigo O.M.A. de Souza

Laboratory of Biocatalysis and Organic Synthesis, Universidade Federal do Rio de Janeiro, Instituto de Química, Cidade Universitária, Rio de Janeiro CEP21941909, Brazil

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ABSTRACT

Dynamic kinetic resolutions have proven to be a useful method for the preparation of enantiopure compounds from racemates, leading to the formation of a single enantiomer in theoretically 100% yield. Because lipases are ubiquitous, versatile, stereoselective and robust biocatalysts, they have been successfully applied as co-catalysts in these reactions, being mostly combined with metals in the chemoenzymatic dynamic kinetic resolutions of alcohols and amines.

Contents

1. Introduction

Chiral molecules that are non-superimposable mirror image of each other, *i.e.* an enantiomeric pair, present the same physicochemical properties in isotropic conditions but are distinguishable in systems that are not isotropic, such as biochemical systems, which are composed of many chiral molecules, such as protein, carbohydrates and nucleic acids (Berthod, 2006). This is the base for the fact that two enantiomers of a chiral substance can have a strikingly different behavior towards a biological system, such as the case of chiral drugs whose enantiopure forms display distinct pharmacological effects. In fact, many bioactive compounds, including pharmaceuticals, agrochemicals, fragrances and nutrients are chiral, many of them sold as a single enantiomer (Wolf, 2008), so that the development of methods to obtain optically pure compounds is crucial for chemical industries.

In order to fulfill the increasing demand for enantiopure compounds, significant progress in asymmetric synthesis and catalysis have been achieved. During the evolution of asymmetric synthesis, the exploitation of the natural "pool" of chiral molecules as source of catalysts and ligands has been a valuable strategy. Among these chiral molecules, the use of enzymes, protein-based macromolecules that catalyze

Abbreviations: AIBN, α,α′-azoisobutyronitrile; AmP-MCF, amino-functionalized mesocellular foam; AP-SiO2, silica functionalized with 3-aminopropyl groups; BCL, *Burkholderia cepacia* lipase; Bz, benzyl; CAL-A, *Candida antarctica* lipase A; CAL-B, *Candida antarctica* lipase B; CRL, *Candida rugosa* lipase; DDKR, dynamic double kinetic resolution; DKR, dynamic kinetic resolution; DMP, 2,4-dimethyl-3-pentanol; DSR, dynamic systemic resolution; IPA, isopropyl alcohol; ISCBCL, ionic-surfactant-coated *Burkholderia cepacia* lipase; KR, kinetic resolution; MCF, mesocellular foam; MPS, mesoporous sílica; NMM, *N*-methylmorpholine; NOV435, Novozyme 435; PPL, porcine pancreatic lipase; RT, room temperature; TBME, *tert*-butyl methyl ether; THF, tetrahydrofurane; TMS, trimethylsilyl; TBAF, tetrabutylammonium fluoride.

* Corresponding author.
E-mail address: a.s.miranda@hotmail.com (A.S. de Miranda).

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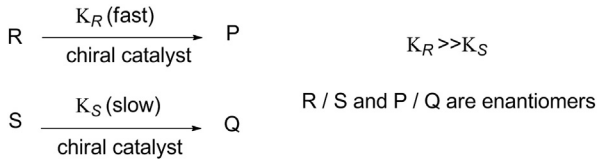

Fig. 1. Kinetic resolution of a racemate.

reactions in living organisms, is particularly advantageous. These catalysts developed by nature are biodegradable, able to work under mild, environmentally benign conditions and usually present high chemo-, regio- and stereoselectivity, thus leading to cleaner reactions. On the other hand, enzymes can present some disadvantages, such as limited substrate scope, requirement of narrow reaction conditions and access to only one enantiomeric form. Furthermore, many of these enzymes require co-factors and their use outside the cell environment is a challenge (Faber, 2011).

Among numerous enzymes that have been applied to organic synthesis, lipases have gained greater prominence over the years. Lipases are hydrolases [E. C. 3.1.1.3] ubiquitous in living organisms, where they catalyze the hydrolysis of esters of fatty acids (Bornscheuer and Kazlauskas, 2005; Kazlauskas and Bornscheuer, 1998). In the laboratory lipases have found to be versatile, being useful for the catalysis of diverse reactions, such as formation or hydrolysis of amides, epoxidation, aldol reactions, Michael additions, among others (Busto et al., 2010), thus finding wide application in organic synthesis. In addition, these enzymes usually present broad substrate scope, do not require co-factors and many of them present good to excellent stereoselectivity, can be easily used outside the cellular environment and are even active in organic solvents (Bornscheuer and Kazlauskas, 2005; Ghanem and Aboul-Enein, 2005; Kapoor and Gupta, 2012; Kazlauskas and Bornscheuer, 1998). These advantages, added to availability of lipases from various commercial sources, make the reactions catalyzed by these enzymes an area of great academic and industrial interest.

Among reactions catalyzed by lipases, formation and cleavage of esters are especially important in the area of asymmetric synthesis, since the high enantioselectivity displayed by many of these enzymes allows the preparation of enantiopure compounds through kinetic resolution (KR) and dynamic kinetic resolution (DKR) of racemates.

In this review, we highlight the use of lipases as co-catalysts in DKR reactions. Most examples include reactions co-catalyzed by lipases and metals, though combination of lipase with other catalysts are also covered.

2. Lipases as biocatalysts in dynamic kinetic resolutions

In the KR of a racemate, one enantiomer interacts in a matched and the other in a mismatched form with the chiral catalyst (chiral recognition), so that the activation energy of the reaction becomes lower for one of them, i. e. in the presence of the chiral catalysts one enantiomer is converted to the product at a higher rate than its antipode (Fig. 1) (Ahmed et al., 2012; Faber, 2011; Pàmies and Bäckvall, 2002a; Rouf and Taneja, 2014).

Kinetic resolutions catalyzed by lipases are mostly based on a stereoselective reaction of nucleophiles with esters or their derivatives. Under physiological conditions, these enzymes catalyze hydrolysis of esters of fatty acids. Since lipases are also stable in non-aqueous media, such as organic solvents, they can also catalyze the reverse reaction as well as reaction of esters with other nucleophiles than water, like alcohols and amines, thus leading to transesterifications and aminolysis of esters. When a racemic nucleophile (Fig. 2a) or an ester (Fig. 2b) is employed, the biocatalyzed reaction can be stereoselective, so that a kinetic resolution takes place. In fact, lipase-catalyzed kinetic resolutions have been described as a useful method for producing compounds with high optical purity, including alcohols, amines, amino acids, carboxylic acids and esters (Ahmed et al., 2012; Bornscheuer and Kazlauskas, 2005; Ghanem, 2007; Ghanem and Aboul-Enein, 2005; Kamaruddin et al., 2009).

In the catalytic site of serine hydrolases, the reaction occurs through a bi-bi ping-pong mechanism and a nucleophilic attack on the carbonyl group promoted by a serine, an histidine and an aspartate residue (also referred as "catalytic triad") (Fig. 3a). The resulting "acyl enzyme" intermediate can, in turn, react with a nucleophile, such as water, alcohols or amines, regenerating the enzyme (Fig. 3b) (Faber, 2011; Pàmies and Bäckvall, 2003).

Most lipases show the same enantiopreference towards esters derived of secondary alcohols, the fast-reacting enantiomer featuring the displacement of groups in the stereogenic center as depicted in Fig. 4. This model, known as the Kazlauskas' rule (Kazlauskas et al., 1991), was originally used for predicting the reactivity in KR of alcohols and has also been found to be useful for KR of amines.

During the course of a kinetic resolution, the enantiomeric purities of substrate and product vary as the reaction proceeds. In a lipase-catalyzed KR, if the product is irreversibly formed, the values of the enantiomeric excess of the product (ee_p) and the substrate (ee_s), which can be experimentally determined, may be used to estimate the enantioselectivity inherent to the process by calculating the *enantiomeric ratio* (E), whose value is the ratio of the rate constants of the reaction for each enantiomer (Chen et al., 1982; Faber, 2011; Pàmies and Bäckvall, 2003). Thus, an enantiomeric ratio of 1 ($E = 1$) is the result of a reaction which presents no enantioselectivity, *i.e.* its chiral catalyst cannot distinguish the enantiomers present in the racemate and the rate of the reaction is the same for both enantiomers. On the other hand, in reactions with $E \geq 100$ the chiral catalyst discriminates efficiently the enantiomers present in the racemate. This situation resembles the ideal situation in which by the end of the reaction 50% of the racemate would have been converted in the product and both product and remaining substrate would present enantiomeric excess higher than 99%.

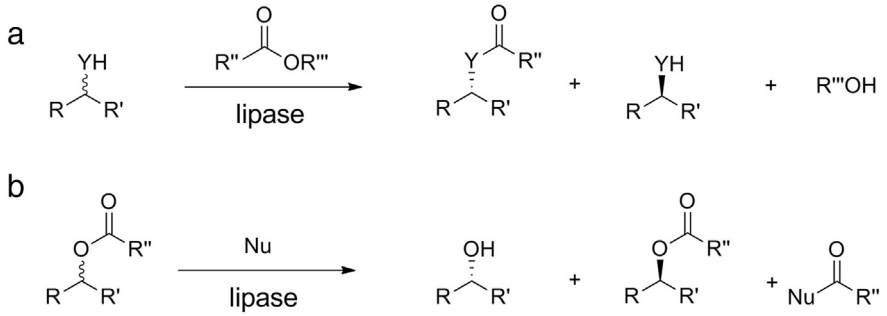

Fig. 2. Lipase-catalyzed kinetic resolution reactions. (a) KR of a racemic nucleophile; (b) KR of a racemic ester. Y = NH, OH, Nu = OH, OR, NH_2.

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