Biotechnology Advances 33 (2015) 547–565

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Esterases as stereoselective biocatalysts

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ARTICLE INFO

Available online 10 February 2015

Keywords:
Esterases
Enantioselectivity
Carboxylester hydrolases
Biocatalysis
Protein engineering
Medium engineering
Kinetic resolution

ABSTRACT

Non-lypolitic esterases are carboxylester hydrolases with preference for the hydrolysis of water-soluble esters bearing short-chain acyl residues. The potential of esterases as enantioselective biocatalysts has enlarged in the last few years due to the progresses achieved in different areas, such as screening methodologies, overproduction of recombinant esterases, structural information useful for understanding the rational behind enantioselectivity, and efficient methods in protein engineering. Contributions of these complementary know-hows to the development of new robust enantioselective esterases are critically discussed in this review.

Contents

1. Introduction, definitions and classifications

Carboxylester hydrolases (EC 3.1.1.1) encompass a large and sundry group of enzymes able to catalyse the cleavage and formation of carboxyl ester bonds. These enzymes have been used as biocatalysts due to their good stability, high chemo-, regio- or stereoselectivity, while working without organic cofactors. Traditionally, they have been classified as (carboxyl)esterases and lipases, based on experimental data and theoretical hypothesis, often quite uncertain. Lipases are generally considered as lipolytic carboxylester hydrolases capable of hydrolysing water-insoluble esters, releasing long-chain fatty acids (>8 carbon atoms), whereas esterases have been mostly recognized as enzymes

http://dx.doi.org/10.1016/j.biotechadv.2015.01.006
0734-9750/

acting on water-soluble esters bearing short-chain acyl residues (<8 carbon atoms). Various benchmarks (primary sequence, structural features, kinetics, and use of specific inhibitors) have been proposed to clearly differentiate lipases from esterases, but all the proposed criteria for differentiation have been applied with little success. All the suggested criteria for discriminating lipases from esterases were recently reconsidered and it was concluded that none of them was suitable, as lipases are just a kind of esterases (Ben Ali et al., 2012). Therefore, they pragmatically suggested organizing the world of carboxylester hydrolases in lipolytic esterases (lipases or LEst, proposed EC: L3.1.1.1) and non-lipolytic esterases (NLEst, proposed EC: NL3.1.1.1), although the official nomenclature is still referred to (carboxyl)esterase as EC 3.1.1.1 and lipases (triacylglycerol) as EC 3.1.1.3.

This review deals with the use of non-lypolitic esterases (from here forward simply called esterases) as biocatalysts and is organized according to important concepts related to stereoselective hydrolysis of chiral and prochiral esters developed in the last few years, addressing specific aspects of the interactions underlying the stereoselective action of these proteins and methods for their improvement. Examples where the use of esterases allows the development of chemoenzymatic routes to industrially relevant molecules by obtaining products with high optical purity and high space-time yields are discussed. This review is also written as an update to previous reviews concerning the use of non-lypolitic esterases as biocatalysts (Bornscheuer, 2002a; Bornscheuer and Kazlauskas, 1999; Panda and Gowrishankar, 2005).

2. Structural determinants of activity and enantioselectivity

2.1. Structural determinants of activity

Many studies have attempted to elucidate – on a structural basis – the determinants of stereoselectivity in the various classes of proteins from the hydrolase family. This family is possibly the largest in enzymology, and includes lipases, esterases, amidases, epoxide hydrolases, dehalogenases and hydroxynitrile lyases. From a structural standpoint, all of them are sharing the so-called α/β hydrolase fold, where eight strands in a central β-sheet are connected by α-helixes that surround the protein core. Many α/β-hydrolase fold enzymes also contain cap domains of highly variable structure, typically sitting on top of the active site in the hydrolase domain.

From a merely mechanistic standpoint, all proteins in this vast family seem to share a common fundamental mechanism, having at their active site a catalytic triad consisting of a nucleophile (serine, aspartate or cysteine), a histidine and a carboxylic acid (aspartate or glutamate). These residues occur on conserved locations in loops, and the protein fold brings them together to form the active site. The catalytic mechanism for carboxylesterases (representing the most investigate members of the family) starts with nucleophilic attack by the serine hydroxyl on the substrate carbonyl. The serine hydroxyl group is activated by the catalytic histidine/aspartate, which takes up the proton from the Ser–OH group. A transient tetrahedral intermediate is formed, which is stabilized by two peptide nitrogen atoms that form the so-called "oxyanion hole". The proton is then transferred from the histidine to the leaving group, and the acid group of the substrate becomes covalently bound to the serine. The histidine then activates a water molecule, which hydrolyses the covalent intermediate via nucleophilic attack on the carbonyl carbon of the intermediate. After the hydrolysis, the histidine donates a proton to the serine, releasing the acyl component of the substrate. This "consensus" two-step mechanism has been challenged very recently (Aranda et al., 2014) on the basis of computational studies. A four-step mechanism was suggested, which includes the formation of two tetrahedral intermediates: the first one involves a Ser residue bounded to the previous carbonyl group of the substrate as in the classical mechanism. The second tetrahedral intermediate of the mechanism is formed upon attack of a histidine-activated water molecule (or alcohol molecule) to the acyl–enzyme complex. Next step involves proton transfer to the Ser–OH group followed by product release, as in the "consensus" mechanism.

It has been suggested that different conformations of the oxyanion loop in esterases (and acyltransferases) may also control which nucleophile (water or alcohol) is favoured in the attack at the C═O of the acyl–enzyme complex, activating or deactivating the attacking water via a second water molecule (Jiang et al., 2011). The hypothesis is based upon the comparison of X-ray structures of a number of esterases, where a carbonyl oxygen points towards the active site (conformation A of Fig. 1), whereas in acyltransferases a –NH– of polypeptide chain points towards the active site (conformations B and C of Fig. 1).

Databases of elucidated 3D-structures of α/β hydrolase fold proteins are available, being quite helpful for structure analysis and predictions. The α/β-Hydrolase Fold Enzyme Family 3DM Database (ABHDB or 3DM) is a structure-based classification of most of the available sequences of α/β-hydrolase fold enzymes, thought as a tool for the analysis of structure–function relationships and the mechanistic determinants of substrate specificity (Kourist et al., 2010). Another helpful tool is the ESTHER database (http://bioweb.ensam.inra.fr/esther), which collects information related to this superfamily (from genes to protein sequences, including structural and applicative data (Hotelier et al., 2004)). Esterases can be classified using the so-called superfamily-based approach, which generates a superfamily based on structural and sequence similarity; furthermore, 3DM has also been proven to be suitable for the recruitment of esterases based on similarity in sequence–structure alignments of known esterases, and for understanding/predicting rational modification of proteins.

2.2. Enantioselectivity and the active site structure

Given the structure of the active site and of the surrounding substrate binding pocket, two major hypotheses have been put forward to provide some basis for explaining enantioselectivity. One relates to specific geometric features of the active site itself, as specific distances between some substrate atoms and groups in the active site sidechain have a different impact on k_{cat} and K_m for different substrates, thus providing a simple rationale for enantioselectivity as determined by the reaction kinetics (Ema, 2004; L. Zhang et al., 2014). Most relevant to the case of the esters of tertiary alcohols, a role has been suggested also for the "oxyanion hole" pocket (as relevant to substrate orientation), and for the so called "nucleophilic elbow" around the catalytic serine residue (as relevant to catalysis proper) (Bassegoda et al., 2010). This "elbow" surrounds the active site serine residue in a conserved pentapeptide sequence motif Gly-X1-Ser-X2-Gly, and results in the formation of a very sharp turn between strand β5 and the following α-helix.

Enzymes from the hormone-sensitive lipase-like family share the GGG(A)X motif in the oxyanion hole and a highly conserved GDSAGG motif close to the catalytic serine, whereas acetylcholine esterases and mammalian liver esterases contain a GESAGA consensus motif in their "nucleophilic elbow". The results of mutagenesis studies indicated that the consensus motif is of considerable plasticity and can be varied to a certain extent without compromising conversion of tertiary alcohol (Bassegoda, 2010). However, the second glycine seems to be a key position for the enantioselectivity of these esterases. As a matter of fact, hydrolases bearing the GGG(A)X-motif (e.g., *Candida rugosa* lipase, *Candida antarctica* lipase A, pig liver esterase (PLE), an acetyl choline esterase from banded krait and a recombinant esterase from *Bacillus subtilis*) proved to be active against tertiary alcohol esters (Henke et al., 2003). Enzymes having the common GX-motif are not able to accommodate these sterically demanding compounds. Due to the GGG(A)X-motif, the space in the oxyanion hole pocket seems to be enlarged enough to allow a quaternary carbon to enter the active site (Kourist and Bornscheuer, 2011).