Biochimica et Biophysica Acta 1800 (2010) 316–326

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Structure–function relationships of epoxide hydrolases and their potential use in biocatalysis

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ARTICLE INFO

Article history:
Received 3 September 2009
Received in revised form 9 November 2009
Accepted 23 November 2009
Available online 27 November 2009

Keywords:
Epoxide hydrolase
Structure–activity relationship
Mechanism
Stereospecificity
Biocatalysis
New enzymes
Directed evolution

ABSTRACT

Background: Chiral epoxides and diols are important synthons for manufacturing fine chemicals and pharmaceuticals. The epoxide hydrolases (EC 3.3.2.-) catalyze the hydrolytic ring opening of epoxides producing the corresponding vicinal diol. Several isoenzymes display catalytic properties that position them as promising biocatalytic tools for the generation of enantiopure epoxides and diols.
Scope of review: This review focuses on the present data on enzyme structure and function in connection to biocatalytic applications. Available data on biocatalysis employed for purposes of stereospecific ring opening, to produce chiral vicinal diols, and kinetic resolution regimes, to achieve enantiopure epoxides, are discussed and related to results gained from structure–activity studies on the enzyme catalysts. More recent examples of the concept of directed evolution of enzyme function are also presented.
Major conclusions: The present understanding of structure–activity relationships in epoxide hydrolases regarding chemical catalysis is strong. With the ongoing research, a more detailed view of the factors that influence substrate specificities and stereospecificities is expected to arise. The already present use of epoxide hydrolases in synthetic applications is expected to expand as new enzymes are being isolated and characterized. Refined methodologies for directed evolution of desired catalytic and physicochemical properties may further boost the development of novel and useful biocatalysts.
General significance: The catalytic power of enzymes provides new possibilities for efficient, specific and sustainable technologies to be developed for production of useful chemicals.

1. Introduction

Enzymatic hydrolysis of epoxides has been applied to biocatalytic processes for several years and a number of informative reviews on the subject have been published [1–5]. The aim of this review is to summarize the present status of the research field focused on structure–function relationships in relevant enzymes and discuss these in the context of possible biocatalytic applications.

Epoxide hydrolases catalytically adds a water molecule to an epoxide ring so forming a vicinal diol. Several classes of these enzymes exist in nature, both in soluble and membrane-bound forms and they are, with few exceptions [6,7], independent on cofactors. Several examples of heterologous overexpression and mutagenesis have allowed for the production of adequately large enzyme quantities for closer studies of the different wild-type enzymes and for directed enzyme evolution. For biocatalytic purposes a relatively small set of microbial and plant enzymes has been used. This review will therefore focus mainly on these enzymes but attempt more general statements where appropriate.

The importance of epoxide-containing synthetic precursors cannot be overestimated in the production of various fine chemicals and pharmaceuticals [8]. The chiral nature of any substituted epoxide or diol presents these compounds as valuable precursors for use in downstream synthetic steps. Hence, a major focus of the research in this field of biocatalysis has been on identifying and characterizing enzymes capable of converting the biocatalytically interesting epoxides in a stereospecific, including enantio- and regiospecific, manner to efficiently generate relevant epoxides and/or diols. The inherent limitation of a 50% theoretical maximum yield in kinetic resolution of racemic epoxides has also been addressed and reports on dynamic kinetic resolutions, with a theoretical maximum yield of 100%, have also been presented during the last years [9,10].

2. The enzymes

2.1. Enzyme classes

Epoxide hydrolases are found in organisms populating every branch of the evolutionary tree. Physiological roles include detoxification of noxious epoxides taken up as xenobiotics [11] or endogenously produced either from oxidative stress [12] or in the

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doi:10.1016/j.bbagen.2009.11.014

bioactivation of polyaromatic hydrocarbons [13,14]. In addition, epoxide hydrolases have in mammals been shown to be involved in blood pressure regulation [15–17] and in inflammatory responses, influencing the metabolism of leukotriene A4 and hepoxilin A3 [18]. The leukotriene A4 hydrolase reaction, notably, does not produce a vicinal diol product and a catalytic mechanism distinct from other isoenzymes has been proposed [6]. A specialized enzyme acting on cholesterol-5,6-oxide is also present. Epoxide hydrolases also fulfill roles in the secondary metabolism of various soil bacteria, and in plants, these enzymes contribute to pathogen defense systems.

With the exceptions of the cholesterol epoxide hydrolase (EC 3.3.2.11), the fosfomycin resistance protein FosX [7] and the leukotriene A4 and hepoxilin A3 hydrolases (EC numbers 3.3.2.6 and 3.3.2.7, respectively), the large majority of epoxide hydrolase isoenzymes can be grouped into two main structural families. One of these families is populated by microbial enzymes with the limonene-1,2-epoxide hydrolase (EC 3.3.2.8) from *Rhodococcus erythropolis* as the archetype [19,20] (Fig. 1). The other, larger family is formed by enzymes with an α/β-hydrolase fold [21] (Fig. 2).

2.2. Enzyme structure and catalytic mechanisms

This review will discuss only the present knowledge of the structure–function relationships in catalysis of enzymes belonging to the limonene epoxide hydrolase and the α/β-hydrolase fold families. Much progress has been made in understanding the function of the leukotriene A4 and FosX hydrolases but due to the very strict substrate specificities of these enzymes they present limited biocatalytic potential and will not be discussed here.

2.2.1. Structure and catalytic function of limonene-1,2-epoxide hydrolase and related enzymes

Enzymes in this class are homodimeric proteins where each subunit is constituted of a six-stranded β-sheet flanked by three α-helices [20,22] (Fig. 1A). *Bona fide* epoxide hydrolases belonging to this family identified to date are the founding *R. erythropolis* epoxide hydrolase (LEH) and Rv2740 from *M. tuberculosis* [23]. Sequence database queries result in numerous putative proteins belonging to the same family but these putative enzymes have yet to be firmly characterized.

The catalytic site is built up by acidic and basic residues with an Asp-Arg-Asp catalytic triad that is essential for catalysis [20] (Fig. 1B). The proposed catalytic mechanism is assumed to follow an S_N2 pathway, i.e., without formation of an intermediate carbocation based primarily on the fact that the hydrolysis product displays an inversion of configuration [24,25]. The proposed mechanism differs from that of enzymes in the α/β-hydrolase fold family, discussed below, in that no covalent enzyme-intermediate is formed. The reaction depends on general acid/base catalysis as depicted in Fig. 1C. The leaving group oxirane oxygen is protonated by Asp^{101} (LEH numbering) in its protonated form. In concert, the reacting water, activated by a general

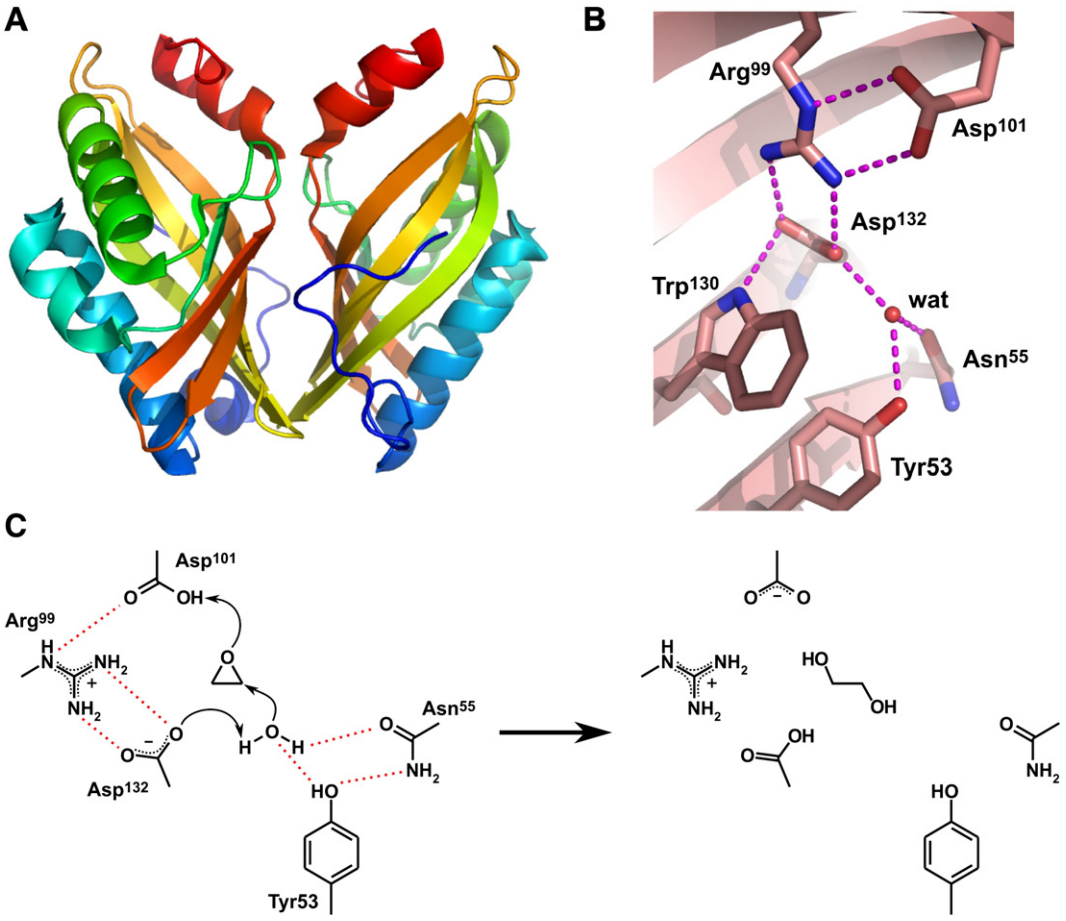

Fig. 1. (A) Ribbon diagram of the *R. erythropolis* limonene-1,2-epoxide hydrolase. Each subunit in the dimer has been color-coded from blue (N-terminal) to red (C-terminal). The image was created in PyMol (DeLano Scientific; http://www.pymol.org) from the atomic coordinates in 1nww [20]. (B) The catalytic site of the limonene epoxide hydrolase. Catalytic residues are shown in stick representation together with a water molecule suitably posed for nucleophilic attack of a bound epoxide substrate. Dotted lines represent hydrogen bonds. Image created with PyMol from the 1nww coordinates. (C) Proposed catalytic mechanism of limonene epoxide hydrolase. Asp^{101} functions as an acid protonating the leaving group epoxide oxygen. Asp^{132}, acting as a general base, abstracts a proton from a bound water molecule facilitating nucleophilic attack. The formed diol product dissociates from the active site and the catalytically active protonation states of the two Asp residues are restored by proton transfer via Arg^{99}.