Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Chemical Biology

Enzyme catalysed tandem reactions

Isabel Oroz-Guinea and Eduardo García-Junceda

To transfer to the laboratory, the excellent efficiency shown by enzymes in Nature, biocatalysis, had to mimic several synthetic strategies used by the living organisms. Biosynthetic pathways are examples of tandem catalysis and may be assimilated in the biocatalysis field for the use of isolated multi-enzyme systems in the homogeneous phase. The concurrent action of several enzymes that work sequentially presents extraordinary advantages from the synthetic point of view, since it permits a reversible process to become irreversible, to shift the equilibrium reaction in such a way that enantiopure compounds can be obtained from prochiral or racemic substrates, reduce or eliminate problems due to product inhibition or prevent the shortage of substrates by dilution or degradation in the bulk media, etc. In this review we want to illustrate the developments of recent studies involving *in vitro* multi-enzyme reactions for the synthesis of different classes of organic compounds.

Address
Departamento de Química Bio-Orgánica, Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

Corresponding authors: Oroz-Guinea, Isabel (isabel.oroz@iqog.csic.es) and García-Junceda, Eduardo (eduardo.junceda@csic.es, eduardo.junceda@iqog.csic.es)

Current Opinion in Chemical Biology 2013, **17**:236–249

This review comes from a themed issue on **Biocatalysis and Biotransformation**

Edited by **Nicholas J Turner** and **Matthew D Truppo**

For a complete overview see the Issue and the Editorial

Available online 13th March 2013

http://dx.doi.org/10.1016/j.cbpa.2013.02.015

Introduction

Chemists have always been inspired by Nature, not only due to the vast diversity of compounds that the living organisms are capable of creating, but also due to the extraordinary synthetic strategies deployed. Inside a cell, thousands of different chemical reactions take place to support cellular growth and survival. Enzymes, the catalysts used by living organisms, have evolved to perform these diverse chemical transformations with high selectivity and specificity. This extraordinary synthetic effectiveness that enzymes display is due in part to their work in multistep reactions, or cascade processes, which give rise to the complex metabolic networks found in biological systems. This synthetic strategy allows building complex structures from simple elements, can make a reversible process irreversible, eliminates inhibition problems caused by product excess, or prevents the lack of substrate [1••].

Biocatalysts have adopted Nature's strategy to reproduce such tandem reactions and apply them in organic synthesis as a powerful tool to efficiently produce structurally complex molecules. Two major approaches have been made: the use of whole microorganisms (*in vivo*) or the use of isolated enzymes (*in vitro*) [2••]. *In vivo* approaches exploit the metabolic pathways of the microbial hosts, which often have been engineered to produce the desired product, and to minimize the diversion of substrate for their normal metabolic requirements. The use of microorganisms has some advantages: first, there is no need for enzyme isolation or purification; second, enzymes show a high stability due to the fact that they are surrounding their natural environment and third, cofactor regeneration is typically provided by the cell's own metabolic machinery. Although the complexity of the cellular system provides all these benefits, it has also some drawbacks: first, engineering microorganisms is labor-intensive and time consuming; second, lower selectivities are achieved owing to the competition from other similar enzymes present in the cell; third, a balance between the toxicity of the substance produced and the product yield is required and fourth, control and maximization of the synthetic processes is difficult. In contrast, *in vitro* approaches are less complex and, therefore, reaction conditions are easier to control and can be optimized by, for example, varying the concentration of the enzyme or the substrate, adding cosolvents or with variation of pH or temperature. In addition, a higher purity product is usually obtained due to the absence of competing side reactions and metabolites which are present in *in vivo* systems. Despite these advantages there are some limitations: first, isolation, characterization and obtaining sufficient quantities of the enzymes for the reaction are required; second, the enzymes have to be stable and functional under reaction conditions; third, cofactor dependent enzyme reactions need a continuous cofactor regeneration system to avoid the use of stoichiometric amount of usually expensive cofactors. Alternatives to overcome these limitations included the use of recombinant DNA techniques to identify, produce and modified proteins; enzyme immobilization that can enhance their stability and allow the possibility to compartmentalize synthetic routes incompatible among them and the use of enzymatic cofactor regeneration systems.

In this review we want to illustrate the developments of recent studies involving *in vitro* multi-enzyme reactions for the synthesis of different compounds.

Tandem reactions for the preparation of alcohols

Alcohols are useful intermediates or building blocks for the synthesis of many pharmaceuticals and chemicals. These compounds are produce in Nature following several pathways and using different classes of enzymes like oxidoreductases (OR), hydrolases and lyases.

In general, the use of OR allows the transfer of electrons from a reductant to an oxidant, catalysing a redox process and producing alcohol through the reduction of a carbonyl compounds. Ketoreductases (KREDs), provide alcohols via ketone reduction. Baeyer–Villiger monooxygenases (BVMOs) are another type of redox enzymes which catalyse the oxidation of ketones, sulfides, and other heteroatoms by atmospheric oxygen. All these enzymes are ubiquitous in Nature and the number of new identified members of these families is continually increasing. The use of OR presents a main limitation, since most of them are dependent on the nicotinamide cofactors β-1,4-nicotinamide adenindinucleotide (NADH), β-1,4-nicotinamide adenine dinucleotide phosphate (NADPH) or rare cofactors from the group of the flavines (FAD) or methoxatines. Thus, cofactor regeneration is needed to counter the expensive cost of these compounds. Effective cofactor regeneration methods such as chemical, electrochemical, photochemical or enzymatic methods have been developed. However, the methodology that is most often exploited for *in situ* cofactor regeneration is the multienzymatic system approach. Excellent revisions about this subject have been published [3,4] therefore, in this paper enzymatic strategies for cofactor regeneration will be not discussed.

There are several examples for the use of KREDS in literature. Wada *et al.* [5] obtained the enantiopure alcohol (4*R*,6*R*)-actinol (4-hydroxy-2,2,6-trimethylcyclohexanone) from ketoisophorone [3,5,5-trimethyl-2-cyclohexene-1,4-dione] by a two-step enzymatic asymmetric reduction system, which started with the asymmetric reduction of the C=C bond followed by the asymmetric reduction of (6*R*)-levodione to (4*R*,6*R*)-actinol using the old yellow enzyme from *Saccharomyces cerevisiae* and the (6*R*)-levodione reductase of *Corynebacterium aquaticum* M-13. Glucose dehydrogenase was also used for cofactor regeneration in both steps (Figure 1a). Ma *et al.* [6] synthesized the ethyl (*R*)-4-cyano-3-hydroxybutyrate, a key intermediate in the synthesis of the cholesterol-lowering drug atorvastatin (Lipitor). This compound can be prepared by KRED catalysed reduction of a β-keto ester coupled with a GDH catalysed conversion of glucose to gluconate for cofactor regeneration. A third enzyme, halohydrin dehalogenase (HHDH) is employed for the subsequent conversion of the chlorohydrin product to the corresponding cyano compound (Figure 1b). In addition, enantiomerically pure diols can be achieved combining the use of two different KREDs by two consecutive reduction reactions: a stereoselective diketone reduction and a hydroxy ketone reduction [7].

One-pot deracemization of secondary alcohols can be performed by sequential ADHs oxidation and reduction processes. An artificial reaction pathway combining simultaneous concurrent tandem oxidation and reduction cycles with opposite cofactor and stereopreference in one-pot was designed by Voss *et al.* [8••] for the resolution of alcohol racemates. Both enantipure (*R*)-alcohols and (*S*)-alcohols were obtained combining different estereoseltive ADHs with an oxidase and a formate dehydrogenase for the cofactor regeneration. This approach can be extended to stereoinversion of enantioenriched *sec*-alcohols (Figure 1c). Another way for the deracemization of secondary alcohols is the enantioselective oxidation of several *sec*-alcohols by an ADH combined with the stereoselective oxidation of different ketones or sulfides by a BVMO. Both reactions are productive and connected through internal cofactor recycling, allowing a parallel interconnected kinetic asymmetric transformation (PIKAT) and maximizing the redox efficiency. In this manner, it was possible to obtain simultaneously up to three enantioenriched derivatives [9,10•]. Another example of the utilization of these enzymes is the synthesis of 12-ketoursodeoxycholic acid that was achieved by alternating oxidative and reductive steps catalysed by hydroxysteroid dehydrogenases (HSDHs) with different cofactor specificity, using the regeneration systems as the driving force. The enzymes used in this synthesis were the NADH-dependent 12α-HSDH and 7α-HSDH in the oxidative step and a NADPH-dependent 7β-HSDH in the reductive one. Lactate dehydrogenase (LDH) from rabbit muscle and pyruvate were used for NAD^+ regeneration and NADPH was recycled by the glucose/glucose dehydrogenase system from *Thermoplasma acidophilum* [11] (Figure 1d). An example of a multistep oxidative cascade involving a cytochrome P450 monooxygenase (TamI) and an FAD-dependent oxidase (TamL) have been described by Carlson *et al.* [12]. These enzymes act codependently through repeated exchange of substrates for postassembly line tailoring of tirandamycin antibiotics.

Lyases and hydrolases can also be employed for the synthesis of alcohols. Lyases catalyse the C–C bond formation coupling a ketone and an aldehyde and forming a diol [13••] or coupling a ketone with hydrogen cyanide giving an acid derivative [14], some examples will be explained subsequently. Hydrolases catalyse the hydrolytic cleavage of the carbon–oxygen single bonds by lipases or esterases in esters or analogous carboxylic derivatives to produce acids and alcohols (or rarely starting from sulphate or phosphate esters as a substrate for phosphatases or sulphatases, respectively) or by epoxide hydrolases, which catalyse the opening of epoxides [15].