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Research review paper

Cofactor regeneration for sustainable enzymatic biosynthesis

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Abstract

Oxidoreductases are attractive catalysts for biosynthesis of chiral compounds and polymers, construction of biosensors, and degradation of environmental pollutants. Their practical applications, however, can be quite challenging since they often require cofactors such as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These cofactors are generally expensive. Efficient regeneration of cofactors is therefore critical to the economic viability of industrial-scale biotransformations using oxidoreductases. The chemistry of cofactor regeneration is well known nowadays. The challenge is mostly regarding how to achieve the regeneration with immobilized enzyme systems which are preferred for industrial processes to facilitate the recovery and continuous use of the catalysts. This has become a great hurdle for the industrialization of many promising enzymatic processes, and as a result, most of the biotransformations involving cofactors have been traditionally performed with living cells in industry. Accompanying the rapidly growing interest in industrial biotechnology, immobilized enzyme biocatalyst systems with cofactor regeneration have been the focus for many studies reported since the late 1990s. The current paper reviews the methods of cofactor retention for development of sustainable and regenerative biocatalysts as revealed in these recent studies, with the intent to complement other reviewing articles that are mostly regeneration chemistry-oriented. We classify in this paper the methods of sustainable cofactor regeneration into two categories, namely membrane entrapment and solid-attachment of cofactors.

Keywords: Oxidoreductases; Enzyme immobilization; Cofactor regeneration; Biocatalysis; Biosynthesis; Industrial biotechnology

Contents

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1. Introduction

Oxidoreductases represent about one quarter of the known enzymes (Kula and Kragl, 2000). A wide spectrum of applications have been explored so far for this group of enzymes, including synthesis of chiral compounds, such as chiral alcohols, aldehydes and acids; preparation and modification of polymers, especially biodegradable or biocompatible polymers; biosensors for a variety of analytical and clinical applications; and degradation of organic pollutants (Sheldon and Stephen, 1983; Hummel, 1999). Due to their high efficiency and specificity, these enzymes are particularly attractive for biosynthesis. Oxidoreductases generally require a non-protein chemical group, a cofactor, to catalyze reactions. Although certain oxidoreductases possess prosthetic groups to facilitate reactions, the majority of the enzymes explored for biosynthesis needed to interact with cofactors that are not permanently tethered to the enzymes. The most widely involved cofactors are several organic compounds, which are also often referred to as coenzymes, such as NAD(H), NADP(H) and ATP. In particular, NAD(H) and NADP(H) have been examined extensively in recent years for chemical processing applications.

Unlike enzymes, cofactors act as stoichiometric agents in biotransformation reactions and undergo chemical reactions with substrates. Often they are much more expensive than the desired products. Accordingly, efficient regeneration and reuse of the cofactors are essential to large-scale synthetic applications (Chenault et al., 1988; Wichmann and Vasic-Racki, 2005). A total turnover number (TTN) of the cofactors, defined as mol product produced/mol cofactor applied, in the order of hundreds up to thousands is usually desired to make the biocatalytic processes economically viable (Chenault et al., 1988).

Methods including chemical, electrochemical, photochemical, microbial and enzymatic reactions have all been developed for cofactor regeneration (Chenault and Whitesides, 1987). Among others, enzymatic approach is particularly preferred for industrial processes due to its high selectivity and efficiency. It also affords the feasibility of coupling more than one valuable chemical production routes. There are two different ways to achieve enzymatic regeneration (Fig. 1). One is through the use of substrate-coupled reaction systems, in which one enzyme that uses both the reduced and oxidized forms of a cofactor is applied to catalyze both the desired synthesis of the product from one substrate and the cofactor regeneration reaction with a second substrate. One example for that is the alcohol dehydrogenase (ADH)-catalyzed organic synthesis reaction. Acetophenone was reduced enantioselectively into (*S*)-1-phenylethanol by an NADPH-dependent ADH from *Thermoanaerobacter* sp., and the conversion of acetophenone could reach 98% when 2-propanol was used as the secondary substrate to drive the regeneration of NADPH catalyzed by the same ADH in a batch reactor (Findrik et al., 2005). Since the same enzyme is required to catalyze two separated reactions simultaneously, it is usually difficult to achieve thermodynamically-favorite reaction conditions for both reactions in the same reaction medium. The other way is through the use of a second enzyme to catalyze the cofactor regeneration reaction. The use of a second enzyme, which has been adapted for the majority of cofactor regeneration processes, usually affords broader options of substrates for the cofactor regeneration reaction, and thus makes it much easier to achieve large thermodynamic driving

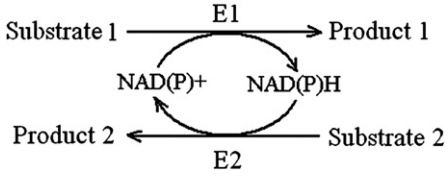

Fig. 1. Enzymatic regeneration of cofactors (for substrate-coupled regeneration, the two enzymes are the same, E1 = E2; for enzyme-coupled regeneration, E1 and E2 represent two different enzymes).