Bioorganic & Medicinal Chemistry 22 (2014) 5638–5656

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Artificial enzymes with protein scaffolds: Structural design and modification

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ARTICLE INFO

Article history:
Received 22 April 2014
Revised 9 June 2014
Accepted 11 June 2014
Available online 20 June 2014

Keywords:
Biocatalyst
Artificial enzyme
Oxidation
Reduction
C–C bond formation/cleavage

ABSTRACT

Recent development in biochemical experiment techniques and bioinformatics has enabled us to create a variety of artificial biocatalysts with protein scaffolds (namely 'artificial enzymes'). The construction methods of these catalysts include genetic mutation, chemical modification using synthetic molecules and/or a combination of these methods. Designed evolution strategy based on the structural information of host proteins has become more and more popular as an effective approach to construct artificial protein-based biocatalysts with desired reactivities. From the viewpoint of application of artificial enzymes for organic synthesis, recently constructed artificial enzymes mediating oxidation, reduction and C–C bond formation/cleavage are introduced in this review article.

1. Introduction

Biocatalyst-based organic synthesis has undoubtedly become a recognizable subject among synthetic chemists, because naturally occurring enzymes mediate chemical reactions under mild conditions in water. Consequently, investigation of biocatalysts contributes to the development of eco-friendly chemical synthesis protocols in both laboratory-scale experiments and industrial plant processes.[1–3] Substrate-specificity and product-selectivity (including regio-selectivity and stereo-selectivity) in naturally occurring enzymes bring about a possibility to yield pure chiral building blocks required in the synthesis of bio-active compounds, because employment of these compounds in racemic mixtures sometimes causes serious problems as seen in the 'thalidomide disaster'. The chemo-selectivity of enzymes result from (i) the optimized arrangement/orientation of functional groups in highly-ordered structures featured by α-helices and β-sheets and (ii) the chiral environment provided by L-amino acid polymer matrix in proteins. Furthermore, biocatalysts can be easily separated from low-molecular products in the purification procedure, because most of biocatalysts have high-molecular weight structures. This can be a merit in food and pharmaceutical plants, where contamination of by-products (especially, originated from heavy metal catalysts) in products should be strictly avoided.

Biocatalyst-based synthesis has been conducted by using microbes (e.g., baker's yeast) or isolated enzymes from them. In synthesis using microbes, we sometimes need to regulate the reactivities of unnecessary enzymes among many kinds of enzymes in microbes (so-called 'a bag of enzymes') to suppress formation of undesirable products.[1–3] Using isolated enzymes may help to overcome this problem, although their strict substrate-specificities sometimes result in narrow scope and limitation of applicable substrates. This is also a serious problem in the employment of biocatalysts for general organic synthesis. Furthermore, non-natural types of chemical reactions cannot be achieved by using naturally occurring enzymes. However, the aforementioned structural features of enzymes are still attractive because these features are difficult to obtain by low-molecular synthetic catalysts.

Owing to recent development in biochemical experiment techniques (e.g., protein expression,[4] chemical modification of proteins[5–8] and/or cofactor,[9,10] etc.), a lot of research examples on the creation of protein-based artificial biocatalysts (namely, 'artificial enzymes') have been reported. In fact, the number of papers describing artificial proteins has been growing in the last decades, which have been summarized in several review articles.[9–18]

Construction of artificial enzymes involves (i) modification of natural and easy-obtainable enzymes to improve their original functions and (ii) conversion of non-enzymatic proteins into enzymes. The construction strategies include genetic mutation, chemical modification and their combination methods. Especially, in the construction of metal-containing artificial enzymes, a combination of genetic and synthetic approaches is versatile to create

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http://dx.doi.org/10.1016/j.bmc.2014.06.021
0968-0896/

unique biocatalysts with a variety of catalytic functions. Furthermore, X-ray crystallographic structural analysis and computational prediction of designed protein structures have been popularly conducted for fine-tuning the structures of proto-type artificial enzymes, enabling us to effectively re-design and construct improved artificial enzymes based on structural and mechanistic knowledge, not by a trial-and-error procedure.

From the aspect of synthetic chemistry, this review article focuses on artificial enzymes mediating (i) oxidation, (ii) reduction and (iii) C–C bond formation/cleavage, which are basic reactions and often conducted in synthetic chemistry. Because the fusion of metal ion reactivity and protein matrix environment often shows unique characteristics with respect to resultant functions, most of the research examples described are related with metal ion cofactor-containing enzymes (metalloenzymes), although some non-metal enzymes with important and attractive functions in organic synthesis are also included.

2. Oxidation

2.1. Construction of artificial metallooxidases by cofactor replacement

In numerous protein engineering works, hemoproteins (heme-containing proteins) have often been targeted because of the accumulation of much structural and mechanistic information. Especially, heme *b* (protoheme IX, 1)-containing proteins have often been employed as a protein scaffold for construction of artificial metalloenzymes, because the heme cofactor is non-covalently fixed in the protein matrices and can be easily replaced with a synthetic metalloporphyrin-derivative cofactor (so-called 'reconstitutional strategy', Fig. 1). A variety of research examples using hemoproteins to create artificial oxidases have been reported, and some of them were introduced in previous review articles.[9,10]

Myoglobin (Mb, an O_2-transport protein) and horseradish peroxidase (HRP, a peroxidase) commonly have heme *b* cofactor 1. In these proteins, the heme cofactor is fixed by the coordination of a histidine residue (His93 for Mb and His170 for HRP). Both proteins have another histidine residue at the opposite side of the coordinating histidine (His64 for Mb and His42 for HRP). In spite of the structural similarity, the peroxidase activity of Mb is much lower than that of HRP. One of the reasons is that Mb lacks a specific domain for substrate binding. Another reason is that the position of His64 at the active site is not suitable for assisting the activation of H_2O_2 to generate Fe(IV)-oxo porphyrin π-radical (so-called 'Compound I'), a high-valent reactive intermediate, on the heme iron. In order to overcome the problem caused by the former structural feature of Mb, Hayashi and co-workers constructed the Mb with cofactor 2 ('double-winged cofactor', see the structure in Fig. 2) using the reconstitutional strategy, hoping that the hydrophobic moiety at the terminal of the heme propionates of 2 contributes to the facilitation of substrate access.[19] The reconstituted Mb showed the enhanced catalytic activity for the oxidation of 2-methoxyphenol using H_2O_2 as an oxidant (see entries 1 and 2 in Table 1). This is mainly caused by the facilitation of substrate binding (decrease in K_m). The next step in constructing a superior artificial enzyme was to improve the chemical process (increase in k_{cat}). According to a work reported by Watanabe and co-workers, the mutation of His64 to Asp enhances the peroxidase activity of Mb because Asp64 in the mutant works as a general acid-base catalysis of H_2O_2 activation.[20] Based on this fact, cofactor 2 was inserted into the apo-H64D Mb mutant. This is, namely, a combination approach of amino acid residue replacement ('genetic mutation') and cofactor replacement ('chemical mutation'). The strategy was, as expected, found to be effective for creating a Mb-based artificial enzyme with an enhanced activity by ca. 400-fold from native Mb (Compare the kinetic parameters in entries 1 and 4 in Table 1).[19] However, the k_{cat} value of the H64D Mb with 2 decreased (entries 3 and 4 in Table 1), which was thought to be an undesirable situation in catalysis chemistry. Accordingly, Matsuo, Hayashi and co-workers examined the utility of cofactor **3** (mixture of **3a** and **3b**, 'single-winged cofactor'), where one

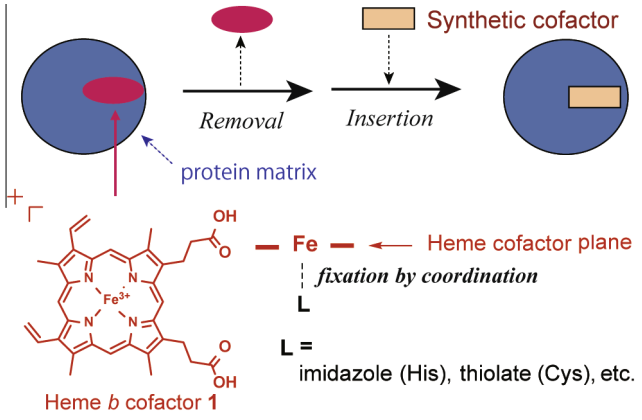

Figure 1. Replacement of cofactor in heme *b*-containing proteins.

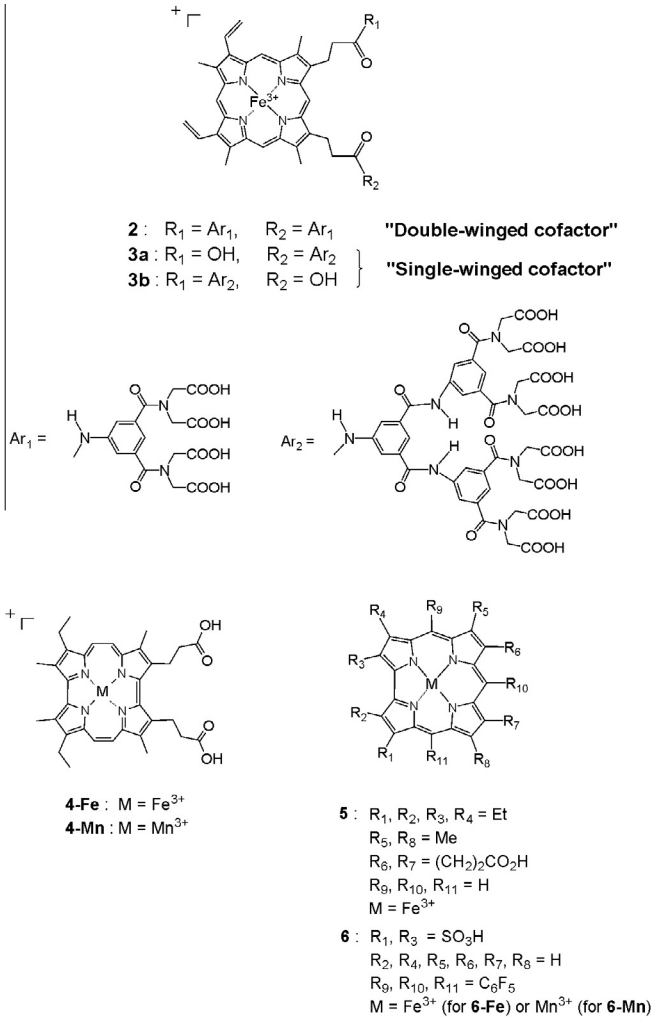

Figure 2. Structures of representative heme analogs used for reconstitution of heme *b*-containing proteins.