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Design of artificial metalloenzymes†

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Homogeneous and enzymatic catalysis offer complementary means to generate enantiomerically pure compounds. For this reason, in a biomimetic spirit, efforts are currently under way in different groups to design artificial enzymes. Two complementary strategies are possible to incorporate active organometallic catalyst precursors into a protein environment. The first strategy utilizes covalent anchoring of the organometallic complexes into the protein environment. The second strategy relies on the use of non-covalent incorporation of the organometallic precursor into the protein. In this review, attention is focused on the use of semisynthetic enzymes to produce efficient enantioselective hybrid catalysts for a given reaction. This article also includes our recent research results and implications in developing the biotin–avidin technology to localize the biotinylated organometallic catalyst precursor within a well-defined protein environment. Copyright © 2004 John Wiley & Sons, Ltd.

INTRODUCTION

Nowadays, the preparation of enantiomerically pure compounds for the development of pharmaceuticals,[1–3] agrochemicals,[4] flavors,[4,5] and functional materials[6,7] is a very challenging endeavor. Discovery of truly efficient methods to achieve this goal has been a substantial challenge for chemists. In the past 35 years metal-catalyzed enantioselective transformations have enjoyed significant growth, as it was recognized that these are amongst the most efficient ways to produce enantiomerically pure compounds.[8] The Nobel committee recognized this by awarding the 2001 Nobel Prize in Chemistry for achievements in this field.[9–11] However, up to now, relatively few enantioselective catalysts have been used on an industrial scale. One reason for this is that the application of enantioselective catalysts on a technical scale presents some very special challenges.[12,13] Some are due to the special situation for manufacturing chiral products, others to the nature of the enantioselective process. In parallel, enzymatic catalysis has emerged as an important alternative tool for the synthesis of enantiopure compounds in recent years.[14] Table 1 outlines a comparison of some of the most noteworthy features of homogeneous and of enzymatic catalysis.

Biocatalysts are attractive because of their remarkable chemo-, regio-, and stereo-selectivity, their impressive catalytic efficiency, and their reactivity in aqueous media. Although these characteristics can be useful, they can also limit the exploitation of biocatalysts. For example, homogeneous catalysts are more tolerant than enzymes towards variation in size of a substrate because of the inherent single-handed, lock-and-key specificity of biocatalysts:[15] despite their difference in size, acetamidoacrylic acid and acetamidocinnamic acid are often both good substrates for homogeneous hydrogenation catalysts. Biocatalysts have evolved to target a single functionality even in the presence of other, perhaps more reactive, functionalities; enzymes do not require protective groups. In many cases, the industrial application of enzymes is hampered by the instability of the biocatalyst under process conditions. However, some of the inherent limitations of biocatalysts (including small substrate scope, operational stability, and availability of only one enantiomer of the product) have recently been overcome, thus expanding the scope of applications of biocatalysis. In particular, directed evolution (i.e. genetic optimization) combined with high-throughput screening have significantly facilitated the optimization of customized enantioselective enzymes.[16–20] Although genetic engineering techniques can improve the overall stability, the *de novo* design of enzymes remains very challenging. Monoclonal catalytic antibodies opened up many opportunities in this fascinating field.[21,22]

Enzymes exquisitely tailor both the first and the second coordination spheres of their active sites to afford efficient and selective catalytic systems (Table 1). This characteristic is especially difficult to realize for synthetic transition-metal catalysts. In the field of homogeneous catalysis, the steric and the electronic control of a catalytic moiety is primarily limited to the first coordination sphere of the metal. However, the role of the solvent and of the counterion

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Table 1. Comparison of homogeneous and enzymatic catalyses

	Homogeneous catalysis	Enzymatic catalysis
Substrate scope	Large	Limited
Enantiomers	Both enantiomers accessible	Single enantiomer
Functional group tolerance	Small	Large
Reaction repertoire	Large	Small
Turnover numbers	Small	Large
Solvent compatibility	Large	Small (aqueous)
Optimization	Chemical	Genetic
Second coordination sphere	Ill defined	Well defined

(typical second coordination-sphere interactions) often play a determinant role in the performance of a catalyst. From these considerations, it appears that homogeneous and enzymatic catalyses are in many respects complementary. For this reason, in a biomimetic spirit, efforts are currently under way in different groups to design artificial enzymes.[23–30]

PREPARATION OF ARTIFICIAL METALLOENZYMES

One growing area in bioinorganic chemistry has been to develop artificial metalloenzymes (Fig. 1). The major aim of these efforts is to design artificial systems by modifying the amino acid sequence of a protein and or chemically modifying a protein to introduce a metal-binding site.[23] This latter approach should allow the attachment

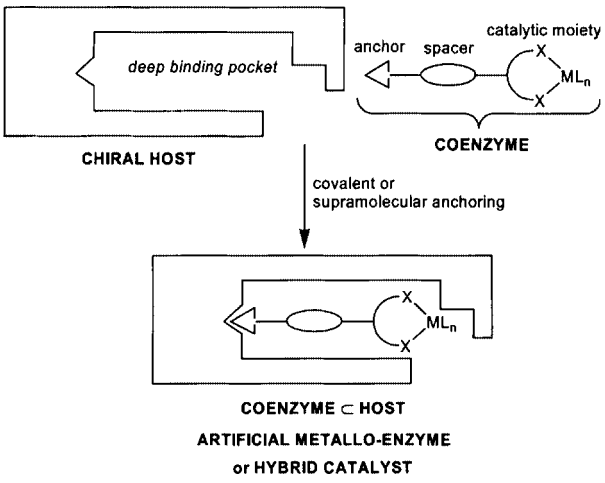


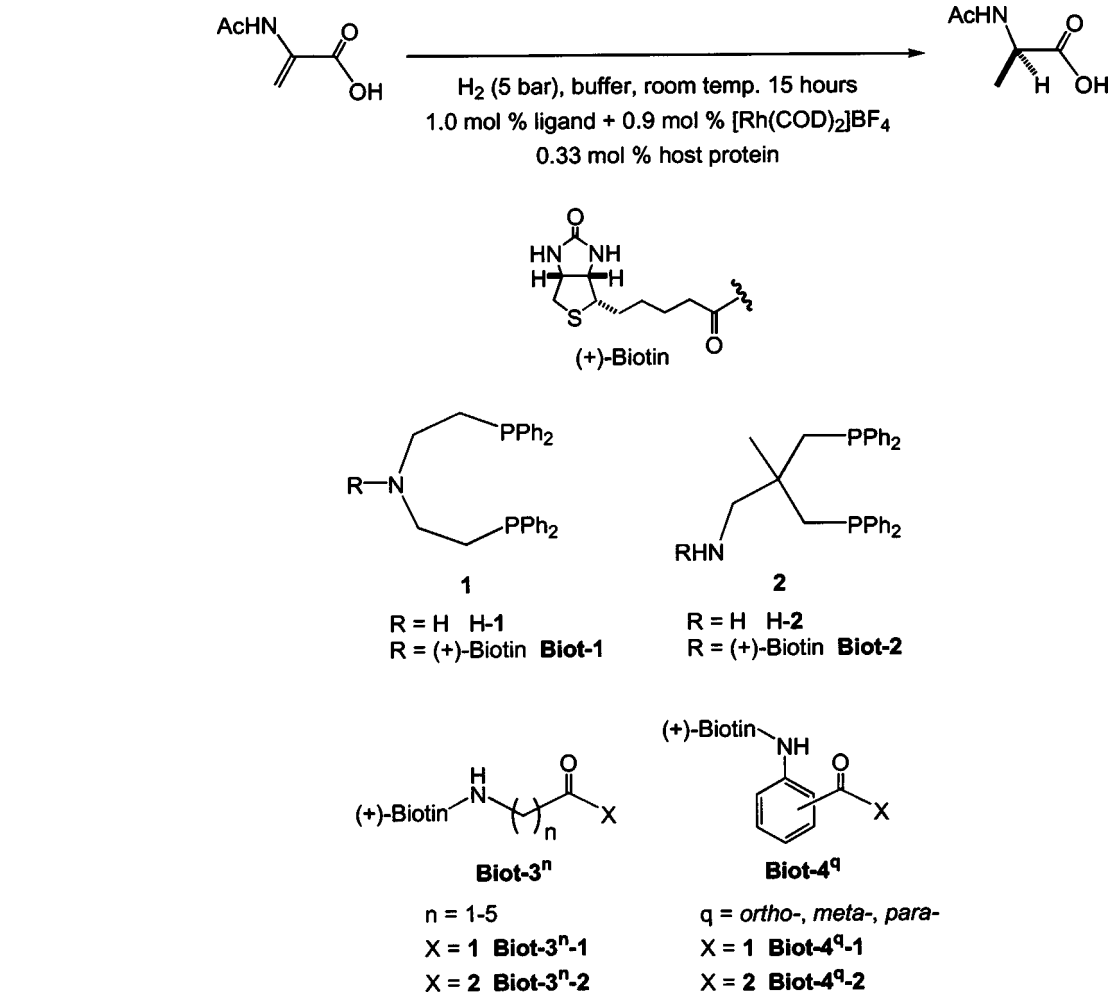
Figure 1. Anchoring of an active catalyst within a chiral host to produce enantioselective catalysts.

of a wide range of functionality that either does not exist in natural proteins or cannot be easily introduced using genetic methods. Inspired by the seminal work of E. T. Kaiser,[24] several groups have recently developed methods to modify proteins covalently by incorporating transition-metal catalysts to yield hybrid catalytic systems with promising properties. Among these, Distefano and coworkers have reported the preparation of an adipocyte lipid-binding protein–1,10-phenanthroline conjugate and its ability to bind copper(II). The resulting construct was found to catalyze the enantioselective hydrolysis of ester and amide substrates.[25] Rana and Meares[26,27] have investigated the use of ferrous-EDTA as metal chelate for incorporating a transition metal into proteins. Interestingly, it was found that iron-EDTA–protein conjugates were not only able to cleave DNA or RNA, but were also capable of peptide bond hydrolysis. Reetz[17,18] first proposed the concept of applying the methods of directed evolution to hybrid catalysts.

To the best of our knowledge, Wilson and Whitesides[29] were the first to convert a protein into a homogeneous hydrogenation catalyst. That work featured the use of non-covalent incorporation (i.e. supramolecular) of the organometallic precursor into the protein, taking advantage of the affinity of the avidin for the *N*,*N*-bis(2-diphenylphosphinoethyl)biotinamide to anchor a chiral chelating phosphine ligand. In this example, the asymmetric induction in hydrogenation of prochiral enamides using the rhodium(I) catalyst prepared was not high enough to be practical (*ca* 40–44% ee), but this work showed that the enantioselectivity was a result of a specific macromolecule–catalyst interaction (second coordination sphere). In the same spirit, Watanabe and coworkers[31] reported on the use of apomyoglobin as a host for {(Salophen)Cr(III)} complexes for the enantioselective sulfoxidation of thioanisole (*ca* 0.3–13% ee).

Recently, Ward and coworkers[32] described the incorporation of achiral biotinylated rhodium–diphosphine complexes into (strept)avidin. Compared with avidin, streptavidin possesses a similar affinity for biotin ($K_a \approx 10^{14}$ M^{-1}) but is endowed with a deeper binding pocket. This has led to the screening of the diphosphine ligands **Biot–1** and **Biot–2** and the corresponding rhodium complexes (Scheme 1). Catalyst precursor [Rh(COD)(**Biot–1**)]$^+$ displayed good enantioselectivity, in favor of the (*R*)-enantiomer (92% ee). The catalyst derived from [Rh(COD)(**Biot–2**)]$^+$ afforded acetamidoalanine in 20% ee (*S*) with only 55% conversion after 15 h. Achiral alkylamino acid spacers $\mathbf{3}^n$ ($n = 1–5$) and arylamino acid spacers $\mathbf{4}^q$ (q = *ortho-*, *meta-*, *para-*) were inserted between the biotin anchor and the amino-diphosphine moieties **1** and **2** to afford ligands **Biot–**$\mathbf{3}^n$**–1**, **Biot–**$\mathbf{3}^n$**–2**, **Biot–**$\mathbf{4}^q$**–1** and **Biot–**$\mathbf{4}^q$**–2** (Fig. 2). By contrast, the sense of enantioselection for catalysts [Rh(COD)(**Biot–**$\mathbf{3}^1$**–1**)]$^+$ and [Rh(COD)(**Biot–**$\mathbf{3}^1$**–2**)]$^+$ in streptavidin was inverted compared with the catalysts devoid of spacer (24% ee (*S*) and 42% ee (*R*) respectively).

These artificial metalloenzymes are also amenable to a chemogenetic optimization procedure. Having identified by



Scheme 1. Enantioselective hydrogenation of the acetamidoacrylic acid using biotinylated complexes in (strept)avidin.

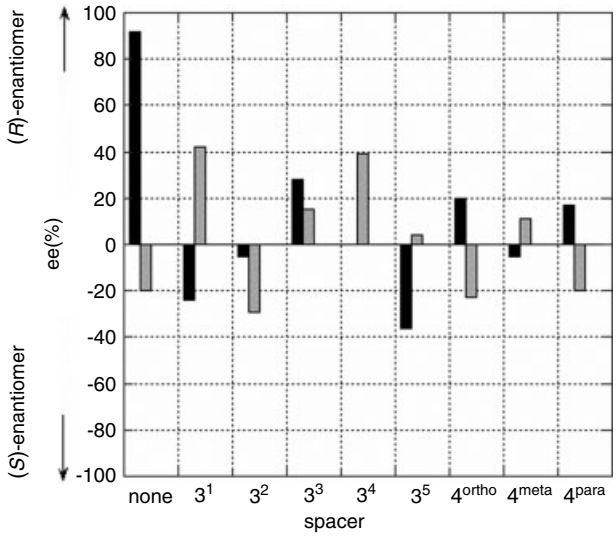


Figure 2. Graphical summary of the results for the enantioselective reduction of acetamidoacrylic acid with ligand scaffold **1** (black) and ligand scaffold **2** (gray) in streptavidin.

chemical modification the most promising organometallic fragments, the streptavidin was subjected to site-directed mutagenesis. Substitution of serine 112 by a glycine residue in the loop of streptavidin (S112G) yields an improved host protein for the reduction of acetamidoacrylic acid with $[Rh(COD)(\mathbf{Biot-1})]^+ \subset$ streptavidin S112G (96% ee (*R*)). The artificial metalloenzymes presented in this work offer an attractive way to exploit the second coordination sphere provided by a host protein to produce versatile enantioselective catalysts with features reminiscent both of enzymatic and of organometallic catalysts. Ward and co-workers[32] have demonstrated that the enantioselectivity may be optimized either chemically and/or genetically (i.e. chemogenetic), thus offering an ideal scaffold for high-throughput optimization of enantioselective catalysts.

SCREENING AND OPTIMIZATION

Combinatorial and high-throughput screening techniques, which have revolutionized the search for new drug molecules,

are now finding broader application for the development of new catalysts.[33,34] In many cases, the correlation between their features (structural, electronic) and their performance (activity, selectivity, lifetime) is not easily established. Therefore, an iterative process of 'design', synthesis and testing is usually followed to improve catalyst performance. The high-throughput screening approach can be described as a three-step process, with a feedback loop: fast preparation of catalyst samples with systematically varied properties, testing of the catalytic properties of the produced samples, processing and evaluation of the experimental data, to be used for modification and improvements when preparing the next generation of catalyst samples. The loop is repeated until certain criteria are fulfilled and the materials selected will then be prepared in larger quantities for detailed evaluation and characterization.

The approach described above is well suited for the design and production of large libraries of hybrid catalysts. Indeed, both chemical and genetic diversity-generating methods can be exploited to screen diversity space. An iterative screening and optimization procedure is presented in Fig. 3.

(1) Both the catalyst moiety and the spacer may be produced and coupled by parallel synthetic procedures. Along the same lines as for organometallic catalysts, the donor moieties X, as well as the chelating skeleton, the metal M and its co-ligands L_n, may be varied to produce chemical diversity. Introduction of a spacer between the anchor and the catalyst moiety will allow one to vary the stereochemical environment around the organometallic moiety. High-throughput screening technology can accelerate this process considerably, allowing for the simultaneous evaluation of a large number of candidates.

(2) Existing approaches to improve proteins, or parts thereof, include modeling-based point mutagenesis, cassette library mutagenesis, and random point mutagenesis (including error-prone polymerase chain reaction, and UV mutagenesis), which introduce partially random mutations either into a selected small region of a gene or throughout a gene.[16] Having produced libraries of coenzymes and chiral hosts, these can be combined and screened for their catalytic activity and selectivity. Again here, automation will allow an increase in the efficiency and the reliability of the screening.

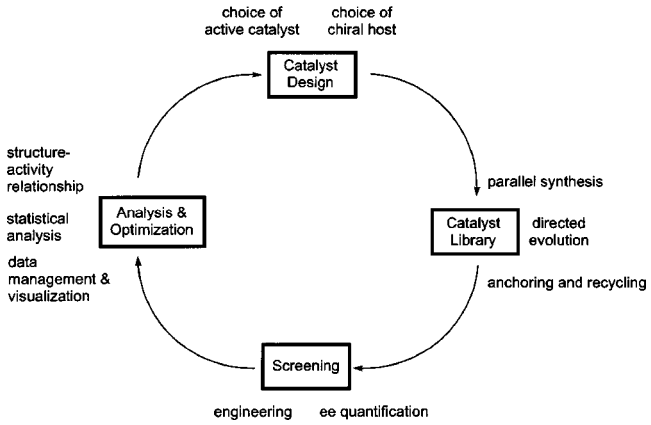


Figure 3. Integrated and iterative procedure for the optimization of hybrid catalysts.

PERSPECTIVES AND OUTLOOK

The major aim of the field of semisynthetic enzymes is to produce efficient enantioselective hybrid catalysts for a given target in a limited time frame. The hydrogenation of *N*-protected dehydroamino acids represents a landmark in enantioselective homogeneous catalysis. Similarly, the hydrolysis of esters is a landmark in enzymatic catalysis. These classical reactions allow one to establish a proof of concept of a new methodology. The capacity to improve artificial metalloenzymes provides the opportunity to focus on more challenging reactions. For example, it should be interesting to test the power of the hybrid catalyst methodology in oxidation reactions: the kinetic resolution of alcohols via oxidation, as well as the epoxydation of prochiral olefins.

New chemical methods promise further increases in the range of functionality that can be incorporated into proteins. These results suggest that semisynthetic approaches will play a key role in the development of future biocatalysts. The potential for cooperation between chemists and biologists to design and implement these catalysts will become clear in the near future. The potential for libraries of metalloenzymes that are rapidly available and the further development of targeted and specific catalysts for a desired reaction are intriguing and exciting developments for the future of chemical synthesis.

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