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Towards the Directed Evolution of Hybrid Catalysts

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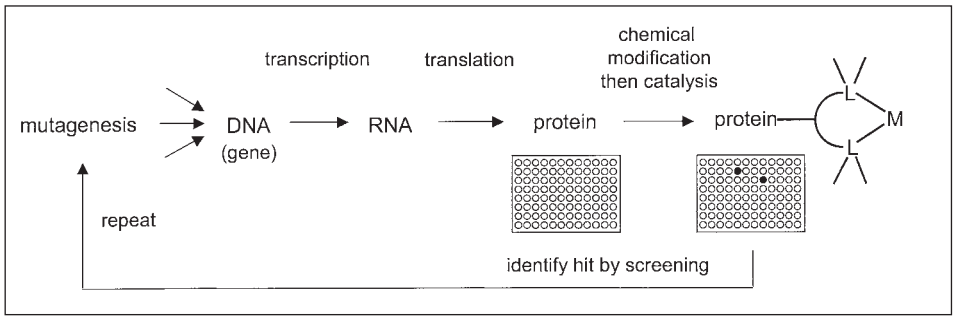
Abstract: The first step in applying the recently proposed concept concerning the application of directed evolution to the creation of selective hybrid catalysts is described, specifically the covalent attachment of Mn-salen moieties and of Cu-, Pd-, and Rh-complexes of dipyridine derivatives as well as the implantation of a diphosphine moiety in a protein, future steps being cycles of mutagenesis/screening.

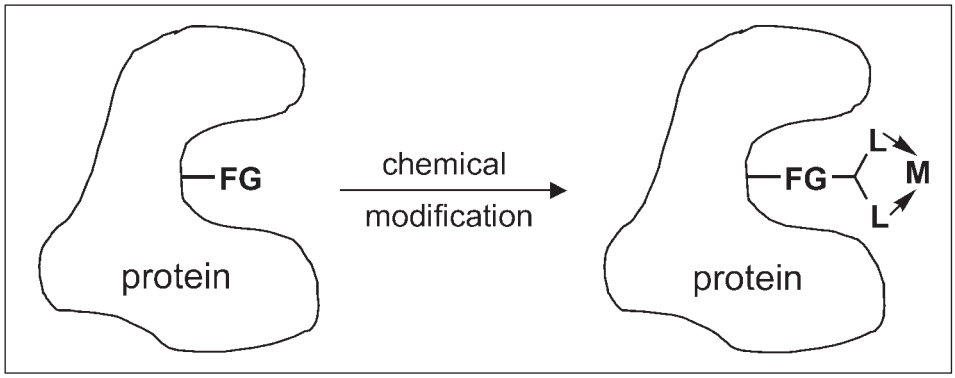
Keywords: Chemical modification · Directed evolution · Enzymes · Hybrid catalysts · Transition metal catalysis

We have previously shown that the molecular biological methods of directed evolution [1] can be applied to the creation of enantioselective enzymes for use in organic synthesis [2]. Although this new approach to asymmetric catalysis offers many perspectives, the method is restricted to reaction types known to be catalyzed by enzymes. Numerous transformations known in the area of transition metal catalysis cannot be catalyzed by enzymes, *e.g.* hydroformylation, olefin hydrogenation and metathesis, allylic substitution, *etc.* [3]. Consequently, directed evolution cannot be used to tune catalysts for such synthetically useful transformations. Recently, we proposed a way to go beyond this natural limitation, the concept comprising 1) random mutagenesis and expression of an appropriate protein (as in conventional directed evolution); 2) chemical modification of the library of mutant proteins with implantation of a ligand/metal moiety in each mutant at a specific site; and 3) en masse screening of a given transition metal catalyzed reaction [4]. Following identification of the best catalyst, the process is repeated and the gene which encodes the mutant protein (*i.e.* the 'host') is subjected once more to mutagenesis. By going through several such cycles, molecular biology is effectively used to tune any catalytic property of interest (activity, selectivity) [4] (Scheme 1).

Chemical modification of enzymes has been previously performed for a variety of reasons [5], *e.g.* covalent attachment of cofactors as studied by Kaiser [6]. In our case we envisioned a protein with an appropriate pocket (cavity) bearing a reactive group such as a thiol from cysteine, which can be used as a chemical anchor point for introducing synthetic catalysts (Scheme 2). The implantation of a catalytically active metal center in the wild-type protein (*e.g.* some enzyme acting as a host) is of limited interest because it generates a single catalyst



Scheme 1. Concept of directed evolution of hybrid catalysts showing the flow of genetic information from the gene to transition metal hybrid catalysts.



Scheme 2. Implantation of ligand/metal moieties in proteins (FG = functional group such as thiol).

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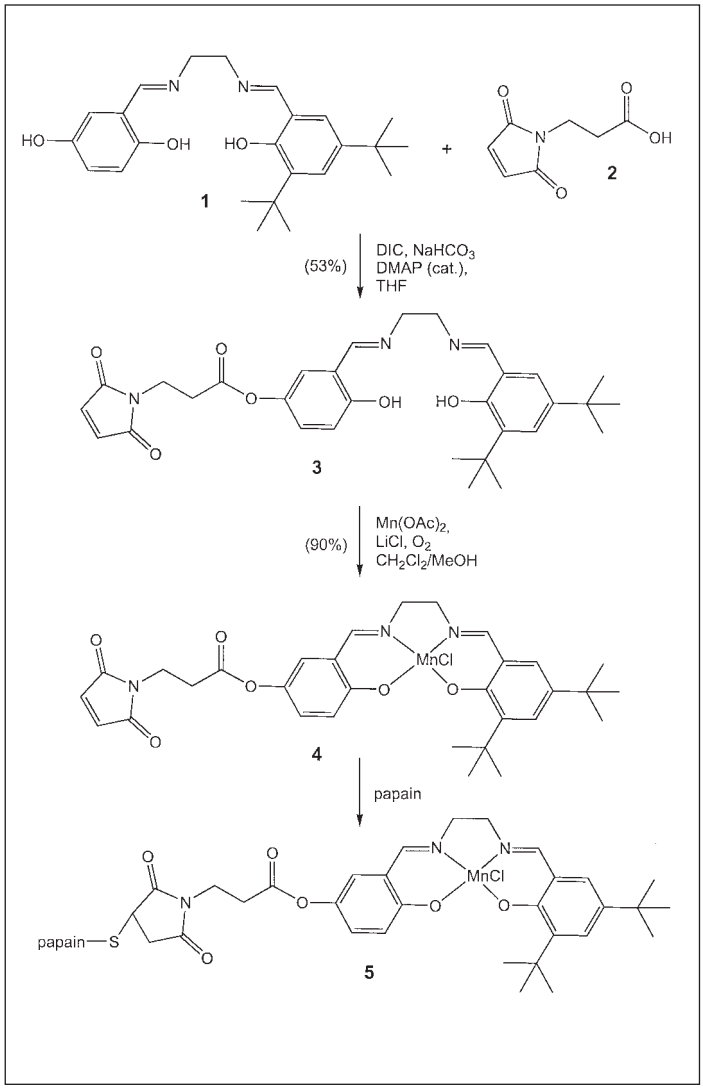
having an unpredictable catalytic profile, in contrast to our concept (Scheme 1) in which a 'Darwinistic' pressure is exerted using thousands of mutant hybrid catalysts. Nevertheless, as a first step the chemistry of introducing ligand/metal systems needs to be developed, which is the subject of this publication.

Since metal complexes of salen and dipyridine are standard catalysts for a variety of reactions [3], we decided to focus on the covalent attachment of these ligands to a suitable protein. The enzyme papain (a cysteine protease having a reactive functional group, *i.e.* thiol of cysteine embedded in a relatively large pocket) was chosen as the host protein [7]. Scheme 3 shows the preparation of a manganese-salen complex **4** bearing a Michael acceptor, designed to react with the (single) free thiol moiety present in papain. Indeed, quantitative modification with **4** was observed forming the hybrid Mn-catalyst **5**.

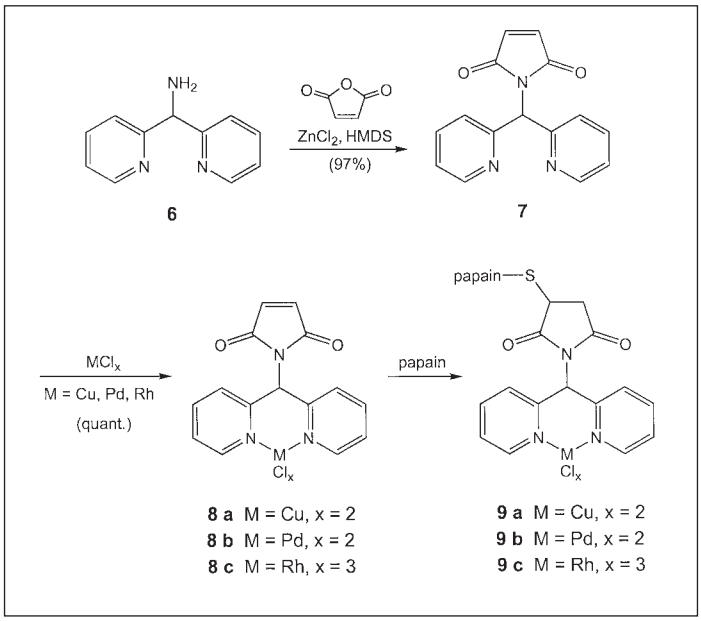
Starting with the known amine **6** [8] the Michael acceptor **7** was synthesized using a known protocol [9] and reacted with various transition metal salts to produce **8a**–**8c**. These reacted smoothly with papain with formation of the hybrid catalysts **9a**–**9c** (Scheme 4).

Finally, in order to incorporate a diphosphine moiety, a different strategy was developed. The diphosphine moiety was directly attached to a phosphonate inhibitor, a class of compounds which are known to react at the catalytically active site of serine proteases or hydrolases [10]. In our case we prepared phosphonate **12** which reacted smoothly with a number of lipases to produce diphosphines **13** (Scheme 5).

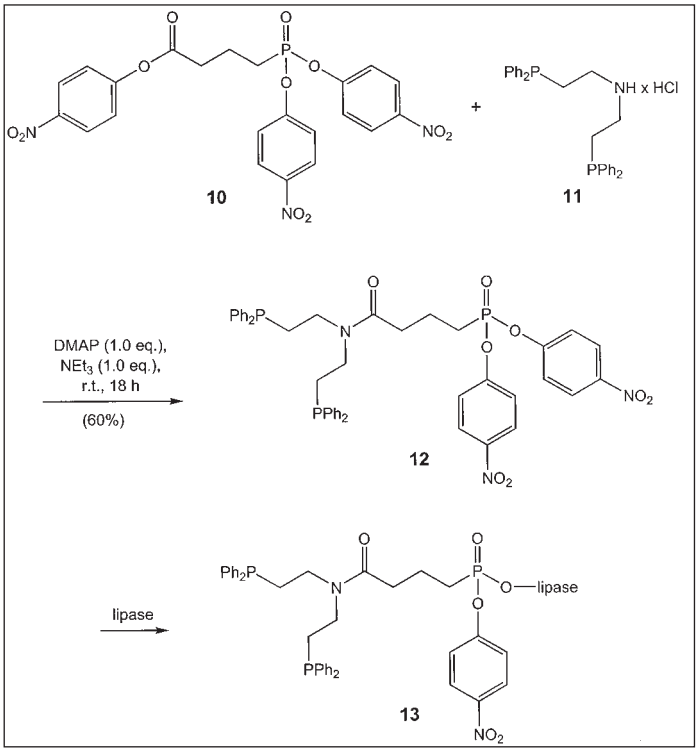
In order to check whether the envisioned reactions of **4** and **8** had taken place at the thiol function, which is part of the catalytically active site of papain, peptidase



Scheme 3.



Scheme 4.



Scheme 5.

activity (using the conventional test based on hydrolysis of N-benzoyl-L-arginine-*p*-nitroanilide) [11] of all hybrid catalysts was determined. In the case of the hybrid diphosphine ligand **12**, esterase activity (using the *p*-nitro phenylacetate assay) was tested. In the case of **4** and **8**, the corresponding saturated succinyl analogs, which by nature have no Michael acceptor properties, were also tested in control experiments. Typical results obtained from **8c** and its succinyl analogs are shown in the Fig.

It can be seen that enzyme activity is inhibited by **8c** but not at all by its succinyl analog. This is a clear indication that the desired Michael addition of papain to **8c** had occurred. Similar curves were obtained for **8a**–**8b** and **4**. In the case of the phosphonate **12**, enzyme inhibition was also observed. However, enzyme activity was largely restored within a day, clearly indicating gradual undesired hydrolytic release of the phosphonate. Such a phenomenon has precedence in other systems [10], especially if phosphorus bears a hydrolytically labile leaving group such as *p*-nitro-phenolate. Therefore, we are currently preparing the analogous ethoxy derivative in order to ensure stability.

Preliminary experiments concerning catalysis show that **5** and **9c** are epoxidation and hydrogenation catalysts, respectively, although the ee-values turned out to be less than 10%, which is no surprise.

In summary, we have devised several synthetic strategies for preparing hybrid ligand/metal systems. The stage is now set for putting our concept of directed evolution of hybrid catalysts into practice [4]. Several expression systems for papain are known [7]. However, other robust proteins may be more suitable as scaffolds, *e.g.* subtilisin E normally used as a detergent, avidin or even thermophiles. The real challenges lie in the parallelized (over) expression and purification of the mutant proteins followed by en masse transition metal catalyzed reactions, high-throughput screening systems already being available [12]. Hopefully, the underlying concept will allow molecular biology to be applied in tuning any catalytic profile of a given transition metal catalyst.

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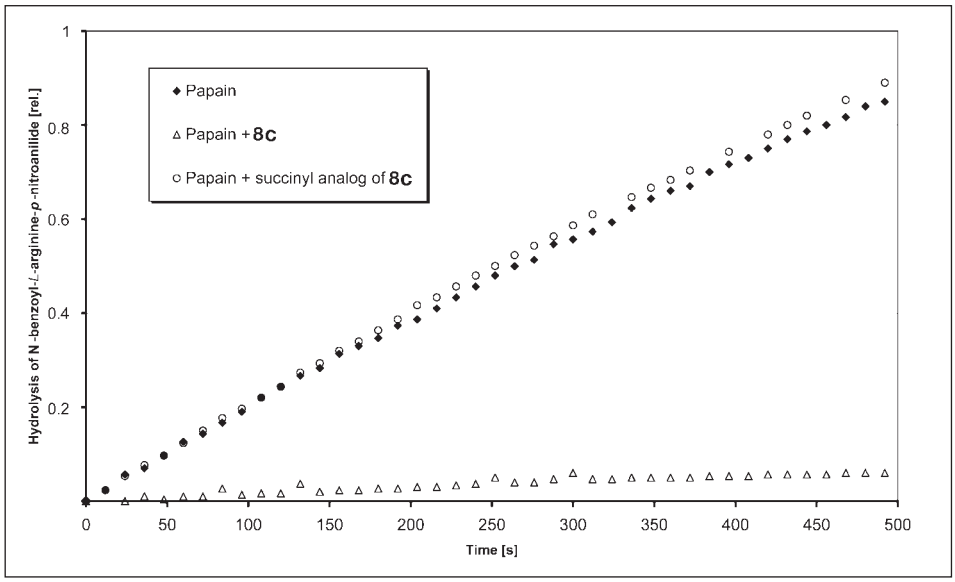

Fig. Inhibition experiments.

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