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Directed evolution of selective enzymes and hybrid catalysts☆

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Dedicated to Yoshito Kishi

Abstract—The methods of directed evolution, developed in the 1990s, can be applied successfully to the creation of enantioselective enzymes for use in synthetic organic chemistry. The combination of appropriate molecular biological methods for random mutagenesis and expression coupled with high-throughput screening systems for the determination of ee-values forms the basis of this novel approach to asymmetric catalysis. The principle is illustrated by the dramatic enhancement of enantioselectivity of a lipase as the catalyst in the hydrolytic kinetic resolution of a chiral ester, the selectivity factor improving from E=1.1 to E=51. Reversal of enantioselectivity is also possible. Finally, the concept of directed evolution of selective hybrid catalysts has been delineated.

1. Introduction

The stereoselective synthesis of chiral organic compounds is of substantial academic and industrial interest,[1,2] as evidenced inter alia by the Nobel Prize for Chemistry 2001 to K. B. Sharpless, R. Noyori and W. S. Knowles. Although most industrial syntheses of optically pure intermediates still involve classical antipode separation,[3] asymmetric catalysis can be expected to gain in importance in the future. Chemists have two options, namely chiral synthetic catalysts such as transition metal complexes,[1] or biocatalysts such as enzymes[2] (Fig. 1). In the former case ligand tuning is necessary, which is not a trivial matter. In the case of biocatalysis, many enzymes are commercially available and do in fact work well in catalyzing the enantioselective transformation of a number of (unnatural) compounds. However, the problem of substrate specificity persists, which means that a vast number of substrates are not converted with acceptable degrees of stereoselectivity. In this case site specific mutagenesis can be applied as a type of ligand tuning, but this has not been routinely successful.[4]

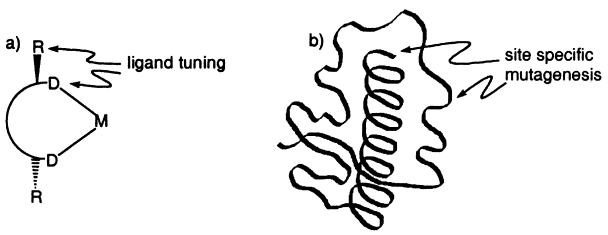

Figure 1. (a) Schematic representation of ligand tuning in the design of a chiral transition metal (M) catalyst (the C_2 symmetry is arbitrarily shown); the arrows symbolize points of potential structural variation and D denotes donor atom. (b) Schematic representation of a de novo design of an enantioselective enzyme: the arrows symbolize the exchange of amino acids on the basis of site-specific mutagenesis.

In view of these problems we have applied the methods of directed evolution of functional enzymes to the creation of enantioselective enzymes for use in synthetic organic chemistry.[5,6] The starting point is a wild-type enzyme which catalyzes a given reaction of interest, A→B, but not enantioselectively. The gene that encodes the wild-type enzyme is first subjected to random mutagenesis using such molecular biological methods as error prone polymerase chain reaction (epPCR),[7] saturation mutagenesis,[8] cassette mutagenesis[9] and/or DNA shuffling.[10] Upon inserting the library of mutant genes in an appropriate microorganism, mutant enzymes (variants) are expressed which are individually screened for activity and enantioselectivity in the reaction of interest. The mutant gene of the optimal enzyme variant is then subjected once more to mutagenesis/expression/screening. This creates an evolutionary pressure, leading to the formation and identification of an even better enzyme. Since the process can be repeated as often as

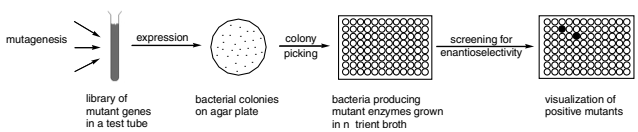

Figure 2. Individual steps in the directed evolution of an enantioselective enzyme.

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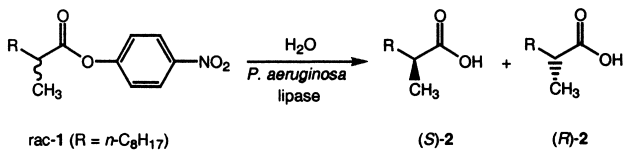
needed, a type of 'Darwinistic' principle holds, without the necessity of knowing anything about the structure or mechanism of the enzyme.[5,6] Thus, the method goes beyond normal combinatorial asymmetric catalysis.[11] Moreover, high diversity is possible, while the problem of deconvolution is not relevant. This is because the bacterial colonies are plated out on agar plates and harvested individually using an automated colony picker[6] (Fig. 2). Each colony originates from a single cell and thus produces only one mutant enzyme. Therefore, the enzyme variants and the corresponding mutant genes are spatially addressable.

Directed evolution had previously been applied in the generation of enzymes showing higher activity and/or thermal and chemical stability.[7–10,12–16] In this paper we summarize the data concerning the first example of the use of directed evolution to stepwise enhance the enantioselectivity of an enzyme in a given reaction. Moreover, we discuss the limits of the method, and show how to overcome them. Specifically, the concept of directed evolution of hybrid catalysts is delineated.

2. Directed evolution of (*S*)- and (*R*)-selective lipases starting from a single wild-type

As a model reaction we initially chose the hydrolytic kinetic resolution of the ester **1**,[5] catalyzed by the bacterial lipase from *Pseudomonas aeruginosa*.[17] The selectivity factor, reflecting the relative rate of reaction of the (*S*)- and (*R*)-substrate **1**, amounts to only E=1.1 in slight favor of the (*S*)-acid **2** (Scheme 1).



Scheme 1.

The *p*-nitrophenol and not the methyl ester was chosen because hydrolysis releases *p*-nitrophenol, a compound that can be monitored by UV/Vis spectroscopy at 410 nm. Of course, if the racemate is used in the wells of a 96-format microtiter plate, only total activity but not enantioselectivity would be accessible. Therefore a simple trick was developed, namely the use of (*S*)- and (*R*)-**1** separately pairwise.[5] Thus, 48 enzyme mutants could be screened using a 96-well microtiter plate equipped with a standard plate reader. This rather crude assay allowed about 500 samples to be tested per day. Later more efficient ee-assays were developed for the kinetic resolution of chiral esters and for other types of asymmetric reactions.[18]

We first had to consider the mutation rate, which has to do with the problem of exploring protein sequence space. The lipase from *P. aeruginosa* has 285 amino acids. Complete randomization would result in 20^{285} different enzyme-variants, which is more than the mass of the universe, even if only one molecule of each enzyme were to be produced.[5,6] The other extreme entails the minimum amount of structural

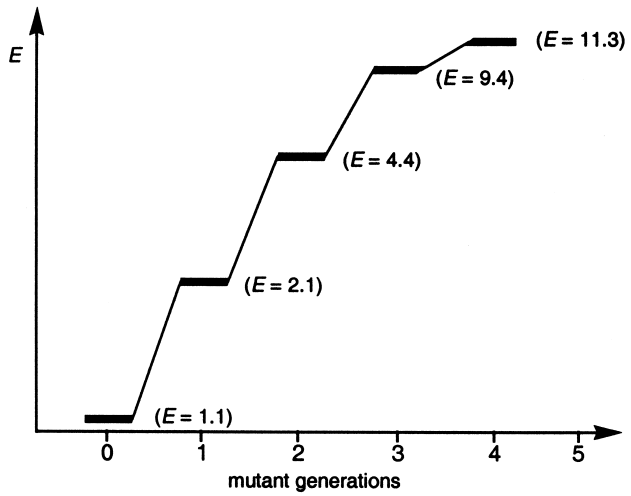


Figure 3. Increasing the enantioselectivity of the lipase-catalyzed hydrolysis of the model ester **1**.[5,6]

change, namely the substitution of a single amino acid per molecule of enzyme by one of the other 19 naturally occurring amino acids. In this case, on the basis of the algorithm $N=19M\times285!/[(285-M)!M!]$, where M= number of amino acid substitutions per enzyme molecule, the library of variants would theoretically have 5415 members. However, when using epPCR as the random mutagenesis method, a library of 5000–6000 members is not expected to contain all theoretically possible permutations. This is because the genetic code is degenerate. If two amino acids are exchanged per enzyme molecule (M=2), then the number of enzyme-variants increases dramatically (about 14 million!). In the case of M=3, it is more than 52 billion.

Therefore a low mutation rate was chosen so as to induce an average of only one amino acid exchange per enzyme molecule.[5] Thus, in the case of the kinetic resolution of the ester **1**, epPCR was adjusted to cause about 1–2 base substitutions per 1000 base pairs of the gene, resulting in an average of one amino acid exchange. Typically, 2000–3000 enzyme-variants per generation were screened. Following expression in *E. coli*/*P. aeruginosa*, the screening system based on the UV/Vis absorption of the liberated *p*-nitrophenolate was employed. As a consequence of the first round of mutagenesis and screening, a variant displaying a selectivity factor of E=2.1 was identified. The corresponding mutant gene was then subjected once more to mutagenesis, and the process was repeated several times. The result after four generations led to a variant (A) having an E-value of 11.3 (Fig. 3).[5,6]

Although these remarkable results constitute proof of principle,[5] a selectivity factor of 11.3 is not yet practical. Thus, a fifth round of mutagenesis was performed, and indeed the usual library of about 3000 mutants contained several slightly improved variants. In spite of this advancement it became clear that we needed methods which allow for more efficient ways to explore protein sequence space with respect to enantioselectivity. Accordingly, DNA-analyses leading to amino acid sequence determinations of the variants were carried out as a first step. For example, the best mutants of the first four generations turned out to have

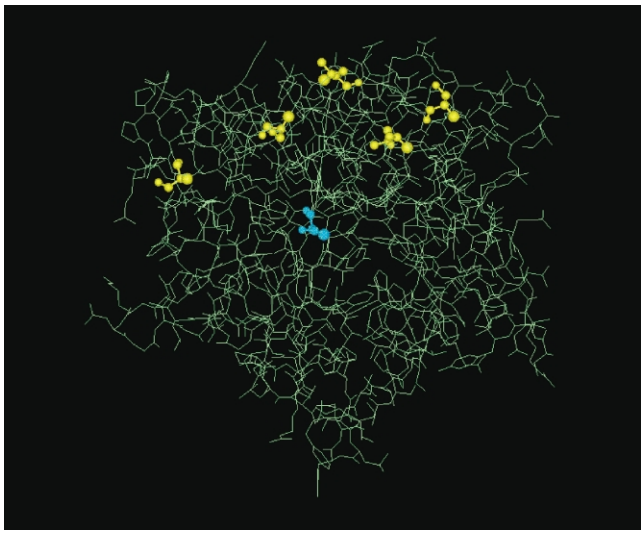


Figure 4. The crystal structure of the wild-type lipase from *P. aeruginosa*;[21] the blue marking denotes the active site (serine), while the yellow markings define the mutations of mutant C.

the following amino acid substitutions, whereby in each round of mutagenesis one new mutation was added to the existing one(s):[5,6]

Variant with $E = 2.1$:	$Ser_{149} \rightarrow Gly_{149}$ (S149G)
Variant with $E = 4.4$:	$Ser_{149} \rightarrow Gly_{149}$ (S149G)
	$Ser_{155} \rightarrow Leu_{155}$ (S155L)
Variant with $E = 9.4$:	$Ser_{149} \rightarrow Gly_{149}$ (S149G)
	$Ser_{155} \rightarrow Leu_{155}$ (S155L)
	$Val_{47} \rightarrow Gly_{47}$ (V47G)
Variant with $E = 11.3$:	$Ser_{149} \rightarrow Gly_{149}$ (S149G)
	$Ser_{155} \rightarrow Leu_{155}$ (S155L)
	$Val_{47} \rightarrow Gly_{47}$ (V47G)
	$Phe_{259} \rightarrow Leu_{259}$ (F259L)

We then drew the following conclusions:[6,19]

1. The process of random mutagenesis/screening identifies sensitive positions ('hot spots') in the enzyme which are responsible for improved enantioselectivity.
2. Such positions are likely to be correct, but the particular amino acid identified may not be optimal.
3. Saturation mutagenesis at the hot spots can be expected to generate improved mutants.

Saturation mutagenesis is a molecular biological method with which mutations at a given position of an enzyme can be introduced, a small library of only 300–400 variants being necessary to ensure that all of the remaining 19 amino acids have been introduced.[8] Upon applying this strategy at one of the hot spots (e.g. at position 155), it was discovered that phenylalanine (F) is the best amino acid. Saturation mutagenesis using the best gene in the third generation led to the identification of a variant (B) which showed a selectivity factor of E=20, phenylalanine again 'showing up' as the best amino acid at position 155. Thereafter, epPCR was applied again, which resulted in E=25 (variant C). Clearly, the combination of mutagenesis methods, namely epPCR and saturation mutagenesis, constitutes an efficient method to explore protein sequence space with respect to enantioselectivity.[5,19,20]

The best mutant (C) showing an E-value of 25 turned out to have five mutations, namely:[19]

$Ser_{149} \rightarrow Gly_{149}$ (S149G).
$Ser_{155} \rightarrow Phe_{155}$ (S155F).
$Val_{47} \rightarrow Gly_{47}$ (V47G).
$Ser_{164} \rightarrow Gly_{164}$ (S164G).
$Val_{55} \rightarrow Gly_{55}$ (V55G).

Although it has not yet been possible to obtain crystals of this (S)-selective mutant, the X-ray crystal structure of the wild-type lipase from *P. aeruginosa* was determined recently by Dijkstra.[21] It is depicted here in Fig. 4 together with the five points of mutations (hot spots as shown in yellow) introduced successively as described above. The active site is the hydroxy function of serine shown in blue, which is part of the usual catalytically active triad of a lipase composed of serine, histidine and aspartate. At serine a covalent acyl-enzyme intermediate is formed. Surprisingly, the hot spots are not really close to the active site.[5,19,20] Remote effects have been uncovered in other cases regarding activity and stability of mutant enzymes.[12,13,21] However, this is the first case of remote effects influencing the enantioselectivity of an enzyme. All previous attempts to enhance enantioselectivity on a rational basis by applying site specific mutagenesis have focused on amino acid substitutions close to the active site,[4] keeping Emil Fischer's principle of 'lock and key' or Koshland's improved model based on 'induced fit' in mind. Thus, the present results may appear to be a serious contradiction. However, this is not the case. The picture in Fig. 4 is static in nature and therefore does not reflect the true structure of the mutant enzyme. Indeed, molecular modeling suggests that the specific amino acid substitutions at remote positions cause the enzyme to take on a slightly different shape which induces higher enantioselectivity. It is also conspicuous that glycine is introduced several times, which can be expected to increase the conformational flexibility of the enzyme.

Although an E-factor of 25 begins to have practical value, it was important to continue to explore protein sequence space with respect to enantioselectivity using other mutagenesis methods. For example, the question arose as to the mutation rate.[6] Would it make sense to repeat the project using a relative high epPCR-based mutation rate corresponding to an average of three mutations per enzyme molecule? Upon putting this into practice interesting results were observed.[22] Out of a library of 15,000 mutant enzymes several were identified showing improved S-selectivity, the two best ones, variants D and E leading to E-values of 3.0 and 6.5, respectively (Fig. 5).

It can be seen that in each case three amino acids were indeed exchanged and that the hot spots occur in regions very different from those of the previous mutants.[5,6] At this

Variant D $E = 3.0$

$Ser_{53} \rightarrow Pro_{53}$ (S53P)

$Cys_{180} \rightarrow Thr_{180}$ (C180T)

$Gly_{272} \rightarrow Ala_{272}$ (G272A)

Variant E $E = 6.5$

$Asp_{20} \rightarrow Asn_{20}$ (D20N)

$Ser_{161} \rightarrow Pro_{161}$ (S161P)

$Thr_{234} \rightarrow Ser_{234}$ (T234S)

Figure 5. Amino acid exchange events in variants D and E.

stage we decided to apply combinatorial multiple-cassette mutagenesis (CMCM)[23] in modified form.[22] This was accomplished by performing DNA shuffling[10,23] with mutant genes encoding the two best enzyme variants obtained at high mutation rate (mutants D and E, Fig. 5) and an oligocassette with simultaneous saturation at previously identified hot spots 155 and 162 (Fig. 6). Position 162 had previously been identified as a hot spot by cassette mutagenesis in the hot region of 160–163.[22] Indeed, this strategy turned out to be successful because several highly enantioselective enzymes were found, among them variant J with six exchanged amino acids (D20N, S53P, S155M, L162G, T180I, and T234S).[22] The *E*-value turned out to be >51 (ee>95%), which underlines the value of this approach.

Fig. 7 summarizes these and further efforts regarding the exploration of protein sequence space with respect to the enantioselectivity of the model reaction catalyzed by lipase mutants from *P. aeruginosa*.[22] A total of only 40,000 mutant enzymes were screened. It is clear that presently no algorithm can be presented according to which the optimal search strategy can be predicted. Rather, at this stage it is necessary to move in protein sequence space in relatively small steps and to make decisions for further action as the results unfold. It may appear disturbing that in some cases continuous improvements upon performing a certain type of mutagenesis in repeating cycles are not observed (e.g. third cycle of epPCR at high mutation rate, Fig. 7). However, this is really not surprising. DNA-shuffling, which ensures high diversity,[10] needs to be applied in such cases. The

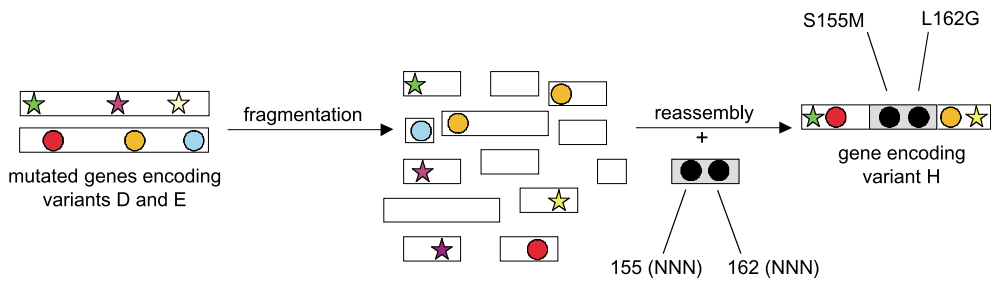


Figure 6. Extended CMCM in the evolution of an (*S*)-selective lipase variant (green star: position 20; purple star: position 161; yellow star: position 234; red circle: position 53; orange circle: position 180; blue circle: position 272).[22]

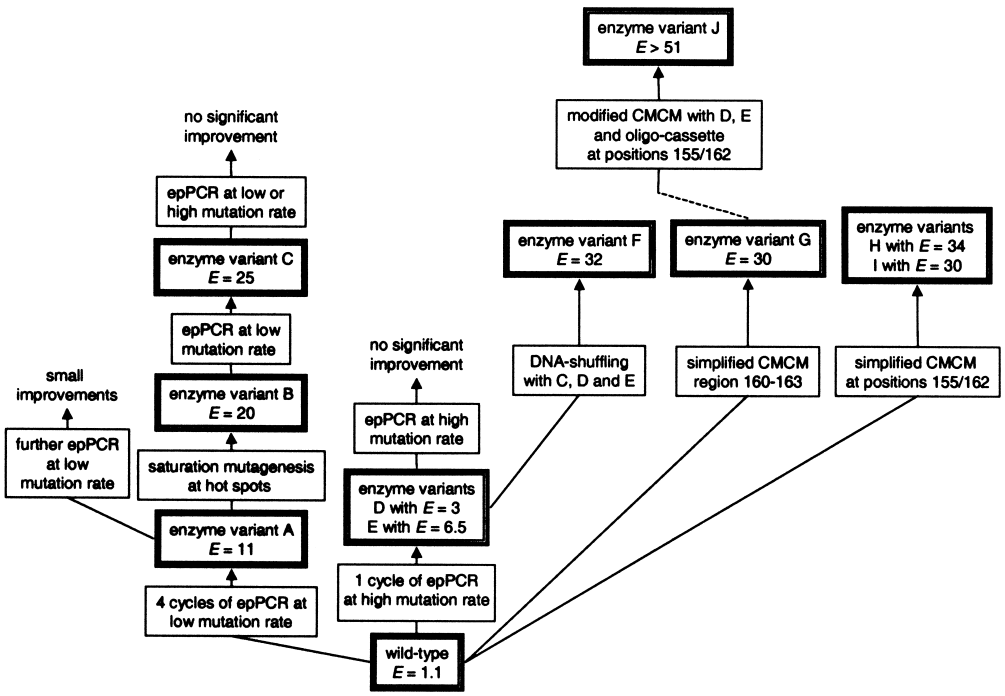


Figure 7. Schematic representation of the directed evolution of enantioselective enzymes (lipase variants) catalyzing the hydrolytic kinetic resolution of ester **1**.[22]

study described here is the first example of the use of DNA-shuffling in the quest to increase the enantioselectivity of an enzyme,[24,27] and we are certain that this technique will be of help in other cases as well.

These studies have taught us that protein sequence space with respect to enantioselectivity is best explored by undertaking the following steps:[22]

1. Generation of mutants by epPCR at high (or low) mutation rate;
2. Identification of hot spots and application of saturation mutagenesis;
3. Identification of hot regions and application of cassette mutagenesis; and
4. Extension of the process of CMCM to cover a defined region in protein sequence space.

As already outlined, the enzyme variant J showing an *E*-value of >51 has six amino acid exchanges relative to the wild-type. In contrast to the five mutations of the variant C showing an *E*-value of only 25 as previously discussed[19] (Fig. 7), the six exchanges occurring with the new variant are not all remote.[22] Rather, about half of them are close to the active site and the rest are remote. The analysis of this phenomenon has not yet been performed, nor have crystals been obtained for an X-ray analysis of variant J. However, kinetic studies show that this variant is highly efficient.[25] In going from the wild-type to variant J, the k_{cat}/K_m-value increases significantly: for (*S*)-**1**, $k_{cat}/K_m=9.0\times10^2$ [$M^{-1}s^{-1}$] (wild-type) and 3.7×10^5 [$M^{-1}s^{-1}$] (variant J); for (*R*)-**1**, $k_{cat}/K_m=3.5\times10^2$ [$M^{-1}s^{-1}$] (wild-type) and 8.4×10^3 [$M^{-1}s^{-1}$] (variant J).

Finally, we were interested in reversing the direction of enantioselectivity,[6,26,27] i.e. in evolving (*R*)-selective variants starting from the same wild-type lipase from *P. aeruginosa*. This was achieved by screening for (*R*)-selective variants. In the initial library of lipase mutants created at high mutation rate (in which two (*S*)-selective variants were found as described above), the process of screening 15,000 mutants also revealed the presence of several variants showing a stereochemically opposite trend.[6,26] For example, two (*R*)-selective mutants were identified characterized by a single mutation V232I (*E*=2.0) and four mutations S112P, S147N, T150A and T226A (*E*=1.1). The former one was used for another cycle of high error mutagenesis resulting in the identification of two variants displaying *E*-values of 3.0 and 3.7. A third cycle afforded a mutant with *E*=7.0 in favor of (*R*)-**1**.

At this stage another cycle of epPCR-based mutagenesis failed to produce further improvements.[26] Therefore, DNA-shuffling[10] was applied. These efforts culminated in the creation of an enzyme variant having 11 mutations and showing a selectivity factor of *E*=30 in favor of (*R*)-**1**.[26] Although the source of enantioselectivity has not been illuminated, it was shown that the hot spots are very different from those of the (*S*)-selective mutants. Moreover, some of the mutations are near the active site, while others occupy remote positions.[26] The ultimate goal is to obtain the X-ray crystal structures of the most (*S*)- and (*R*)-selective mutants, which would allow a more quantitative analysis. Work along these lines is continuing.

3. Directed evolution of hybrid catalysts

Although the directed evolution of functional enzymes offers many exciting perspectives for future applications in organic synthesis, one obvious limitation is the fact that the reaction types amenable to this type of optimization are restricted to enzymatic processes. This means that the majority of organic transformations catalyzed by transition metals[1] are outside of the realm defined by enzyme catalysis.[2] For example, enzymes cannot catalyze such reactions as hydroformylation, olefin metathesis, allylic substitution, hydrosilylation, etc. If they could, then it would be possible to apply the methods of directed evolution in order to tune activity as well as chemo-, regio- and stereoselectivity. We have therefore proposed the concept of directed evolution of hybrid catalysts.[28] It involves the following steps:

1. Choice of an enzyme (or any protein) which ideally fulfills the following criteria
   (a) is stable
   (b) can be overexpressed in appreciable amounts
   (c) has a reasonably sized cavity (enzyme pocket)
   (d) has a reactive amino acid such as cysteine in the cavity.
2. Application of mutagenesis methods such as epPCR, saturation mutagenesis, cassette mutagenesis and/or DNA shuffling to produce libraries of mutant enzymes (variants).
3. En masse chemical modification of each mutant enzyme so that a transition metal center is implanted at the reactive site (e.g. thiol function of cysteine) in the enzyme cavity.
4. En masse screening of the mutant hybrid catalysts in a given transition metal catalyzed reaction.
5. Repeating *X*-times the process of mutagenesis/expression/chemical modification/screening using the best catalyst identified in each step.

Of course, if the host enzyme has no cysteine, it can be introduced by site-directed mutagenesis, or if it has more than one cysteine, they can be substituted.

Chemical modification of enzymes at reactive cysteine

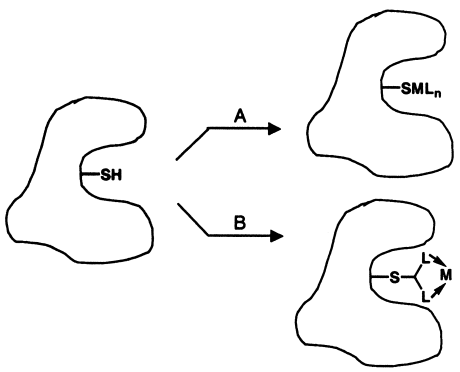


Figure 8. Implantation of transition metal centers in a protein.

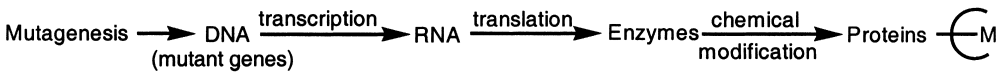


Figure 9. Flow of genetic information from DNA (gene) to organometallic chemistry (M=metal).

moieties has been performed previously for other reasons, Kaiser's work being seminal.[29] In our case the implantation of a transition metal at a defined position can occur by one of two ways (Fig. 8). Direct coordination with an appropriate transition metal salt at the reactive functional group, e.g. cysteine (case A, Fig. 8), or attachment of an achiral ligand system bearing the transition metal (case B).

In exploratory work we have utilized both pathways using wild-type papain as the protein.[28,30] Papain is a protease occurring in the papaya fruit (*Carica papaya*). Expression systems have been described.[31] At this stage the reader is reminded of the fact that protease activity is of no interest in our endeavor, because the protein simply acts as a host and thus as a ligand which can be manipulated structurally by directed evolution. Other proteins may be better.

As far as path B (Fig. 8) is concerned, we have developed building blocks which permit the ready introduction of such ligands as pyridines, dipyridines, and diphosphines as well as mixed bidentate systems in the model protein papain.[28,30] The catalytic profiles of the respective metal complexes are of limited interest, because any beneficial effects in a catalytic reaction would be purely fortuitous. It is the possibility of optimizing any desired catalytic property (or properties) by means of directed evolution which is the basis of our concept.[28] Thus, out of a library of thousands of mutants the 'best' protein for catalyst implantation and catalyst performance can be identified as a consequence of screening. The mutant gene can then be subjected once more to mutagenesis, followed by chemical modification and screening. It can be anticipated that this Darwinistic procedure will induce amino acid exchange processes which will lead to catalysts displaying the desired catalytic profile with respect to activity and selectivity in a given transition metal catalyzed reaction. This means that the flow of genetic information is being extended from the gene to organometallic chemistry (Fig. 9).

This touches on a fundamental question in catalysis that has been addressed and answered decades ago, namely: what is the fundamental difference between a synthetic catalyst (e.g. a conventional transition metal complex) and an enzyme? Pauling[32] and thereafter the majority of the scientific community[33] postulate that it is the transition state of an enzyme-catalyzed reaction which is stabilized (E·TS). As the geometry of the reacting substrate changes upon reaching the transition state, various stabilizing interactions such as hydrogen bonding, van der Waals effects and electrostatic attractions are maximized. Thus, it is not just the effects exerted by the actual catalytically active center which are important, but also and specifically those arising in the environment around the transition state. Of course, solvation and desolvation also play a role. An alternative view centers around the postulate that (many) enzymatic reactions take place through so-called near attack conformers (NACs) that resemble the transition state, these being brought about by enzyme–substrate interactions (E·NACs).[34] Although this is quite different from the Pauling postulate, hydrogen bonding, van der Waals and electrostatic interactions again play a pivotal role, i.e. the protein microenvironment is crucial.

In contrast, in a reaction mediated by a synthetic catalyst, the energy of the transition state is defined solely by the electronic and steric interactions between the active site (e.g. a metal center) and the substrate (or other reaction partners), the whole entity (and thus the transition state) being intimately surrounded by the solvent. Thus, it makes a difference whether a transition state or a short-lived complex is surrounded by a protein capable of exerting various activating effects, or whether it is surrounded solely by solvent molecules.[33]

The intriguing situation now arises when placing a synthetic catalyst (e.g. metal center) in the cavity of an enzyme, as we are doing. Obviously, the usual electronic and steric factors expected of the catalyst in the absence of a protein environment will operate. However, now 'enzyme-like' interactions of the type described above can also exert an influence. As already indicated, there is no reason to believe that such interactions will already be at a maximum in the case of the hybrid catalyst prepared from the wild-type protein. Indeed, there may be no effect. However, the process of directed evolution makes possible the optimal sequence of amino acids necessary for additional activation. Thus, one and the same synthetic catalyst can be expected to display different activity in a given reaction, depending upon the nature of the mutant protein that surrounds it.

In addition to enhancing catalyst activity with respect to the wild-type hybrid catalyst, it should also be possible to apply directed evolution in the quest to tune chemo- and stereoselectivity. Once achieved, it will be of considerable theoretical interest to uncover the source of improved catalyst activity and/or selectivity. Among the synthetic challenges are the control of regio- and stereoselectivity in Rh-catalyzed hydroformylation and hydrogenation, Pd- or Ir-catalyzed allylic substitution as well as *cis*/*trans*-selectivity in Ru-catalyzed ring-closing olefin metathesis, in addition to stereoselective redox processes.

4. Conclusions and perspectives

Directed evolution of enantioselective enzymes constitutes a fundamentally new approach to asymmetric catalysis.[5,6,18–20,22,24,26,27] Rather than relying on site directed mutagenesis based on molecular modeling, the method is independent of any knowledge of the structure and mechanism of the particular enzyme being studied. In a certain sense the strategy is nevertheless rational, because it is based on the evolutionary (Darwinistic) principle. Of course, the combination of directed evolution and site-directed mutagenesis is also promising.

We are in the process of applying the methods of directed evolution to other enzymes and substrates. Attention also needs to be paid to developing alternative methods to search protein sequence space for enantioselective mutants of a given enzyme. For example, performing saturation mutagenesis systematically at every (or nearly every) position of a given enzyme one by one creates small libraries of mutants which constitute good starting points for beginning detailed directed evolution studies.

Finally, a fundamentally new way to tune any desired property of a man-made transition metal catalyst such as activity, *cis*/*trans*-selectivity or regio- and stereoselectivity may be possible on the basis of the directed evolution of hybrid catalysts. From a present perspective it appears that the major challenges in putting this novel concept into industrial practice are primarily of a technical nature. Efficient overexpression/secretion systems as well as high-throughput parallel reactor types are one of the truly difficult prerequisites. A successful implementation would constitute a practical fusion of molecular biology and transition metal chemistry.[35]

Acknowledgments

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35. Hybrid catalysts as presented here are not limited to transition metal complexes. Metal-free organo-catalysts can also be implanted in proteins.[28,30]