Directed Evolution of Enzymes

Obtaining clean, efficient, and biodegradable catalysts

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Abstract

This brief technological report presents an overview of techniques and applications in the field of directed evolution of enzyme catalysts. These techniques allow for the creation of modified enzymes that are better adapted to many industrial contexts. Recent applications in organic synthesis as well as commercial, biomedical, and environmental usage of these modified catalysts will be presented.

Résumé

Cette brève fiche technologique présente en survol les techniques d'évolution dirigée permettant la génération de mutants enzymatiques pouvant être par la suite utilisés comme catalyseurs dans un contexte d'intérêt prédéfini. Quelques applications de ces catalyseurs modifiés sont présentées touchant des domaines aussi divers que la synthèse chimique, l'utilisation industrielle et commerciale, la recherche biomédicale et l'environnement. Le lecteur désirant une version française détaillée et en profondeur de ce domaine de recherche est invité à se référer à la fiche BIOTECHNO, vol. 2, n° 2 publiée par le Centre québécois de valorisation des biotechnologies du gouvernement du Québec www.cqvb.qc.ca/publications_home.htm.

Enzymes are among the most powerful catalytic molecules we know, accelerating chemical reactions at a rate of 10^6 to $1,0^{17}$ times faster than the same uncatalyzed reactions. Moreover, they are often stereo-, regio- and chemoselective as well as being entirely biodegradable and environmentally friendly. On the other hand, reactions catalyzed by enzymes are generally confined to mild temperature conditions in aqueous solutions at pH 7. Because of these constraints, it is difficult to integrate enzymatic catalysts into industrial processes where they are prone to denaturation as a result of harsher prevailing conditions. Enzymatic engineering now offers the possibility of improving the robustness as well as the catalytic efficiencies of enzymes by techniques commonly known as "directed evolution." This approach mimics natural evolution in a test tube by introducing randomly distributed mutations on the gene encoding the enzyme of interest. The resulting "library" of mutants is screened for a desired characteristic to attempt to identify a mutant enzyme that exhibits the characteristic of interest. Successful reports published in the last ten years teach us that the introduction of a small number of mutations on a given enzyme is often sufficient to drastically modify its properties. The possibilities of applications are almost endless and have been amply demonstrated in improvement of catalytic efficiency and robustness, modification of optimal temperature and pH, among others. These modified enzymes are now used in a considerable number of chemical fields ranging from chemical synthesis, pharmaceutical and biomedical applications to environmental detoxification and numerous other industrial purposes. A few examples will be presented here. References [1–4] contain detailed examples and thorough investigations of the methods used for directed evolution of biocatalysts.

Mutated enzymes recognize and degrade many stable chemical pollutants

Green chemistry and the environment

Directed evolution of enzymes has found many applications in the fields of environmental protection and green chemistry. Soil detoxification and degradation of toxic chemicals are particularly amenable to enzymatic treatments because their elimination by classical means often generates chemical by-products that are environmentally noxious. Furukawa and collaborators applied the combined approaches of site-directed mutagenesis and Family Shuffling™ of genes to broaden the recognition spectrum of biphenyl dioxygenases [5]. The resulting mutated enzymes recognize and degrade many stable chemical pollutants, notably PCBs but also aromatic hydrocarbons such as benzene and toluene.

Modified enzymes are also increasingly used toward industrial purposes that exploit their clean and environmentally friendly usage compared to other catalysts that are damaging to the environment. Thus, enzymes are perfectly adapted to green chemistry applications. A convincing industrial application in this field is the use of proteases in laundry detergents. Proteases such as subtilisin are constantly improved in order to adapt them to the constantly changing and harsh reaction environments of washing machines. Ness et al. have modified subtilisin by Family Shuffling™ using the commercial enzyme Savinase™ as well as other members of the same family of proteases. By creating 654 different random mutants, they selected modified enzymes that displayed up to four times the activity of the native parent with respect to thermostability, pH dependence and the presence of different solvents [6]. This work demonstrates the capacity for directed evolution studies to produce environmentally friendly catalysts with improved catalytic efficiency, a characteristic that strongly influences their industrial profitability.

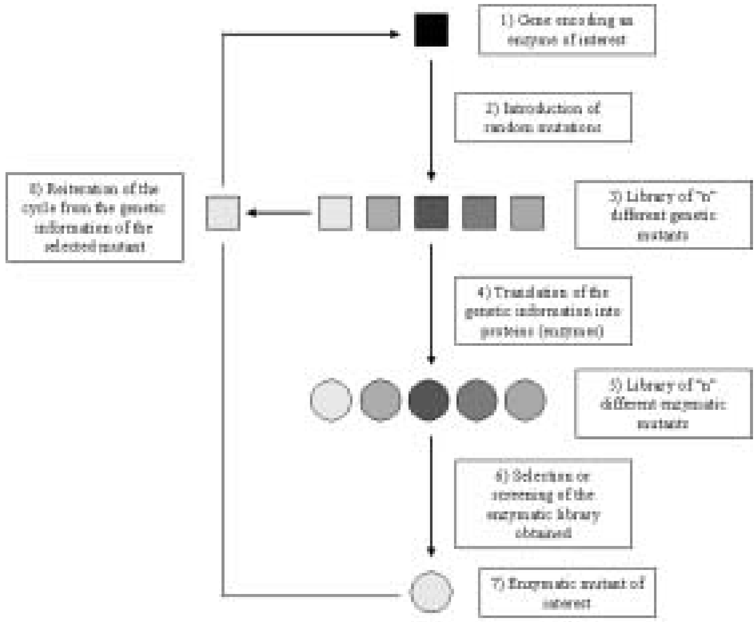

Figure 1. General strategy for the directed evolution of enzymes (adapted from ref 10)

Pulp and paper, food, and other industrial applications

The pulp and paper industry also benefits from enzymatic catalysts, especially in the bleaching and delignification processes undertaken with the fungal enzyme laccase. Laccase expression in systems more practical than fungi is inefficient, hampering its production and its industrial profitability. Nevertheless, using directed evolution studies, Bulter et al. [8] expressed laccase in yeast at levels 8-fold higher than had been previously obtained. Moreover, under the conditions tested, they isolated mutants with a 170-fold increase in specific activity with respect to the native enzyme. Since laccase is also useful in the food industry for fruit juice clarification and may be useful for environmental applications in the degradation of polycyclic aromatic hydrocarbons, this progress may prove important for many industrial purposes.

Enzymes such as α-amylase, glucoamylase and isomerase are heavily used to convert starch into fructose for the production of corn syrup. However, the necessary steps for this conversion require temperature and pH changes that are not well supported by *a*-amylase. Directed evolution successfully improved its stability at pH 4.85, a pH so acidic for the native enzyme that its denaturation rate in these conditions is not even accurately measurable [7]. The capacity to create or modify a specific characteristic of an enzyme demonstrates the power of directed evolution techniques for modulating the properties of these catalysts toward different requirements.

Organic synthesis and pharmaceutical applications

To date, organic synthesis has been refractory to use of enzyme catalysts, particularly because of their lack of robustness in presence of organic solvents. Nevertheless, Chen and Arnold [9] have adapted enzymes to these environments by improving the activity of Subtilisin E by three cycles of error-prone PCR to obtain an enzyme that is 256 times more efficient than the native parent in a solution containing 60 percent DMF. This highlights the efficiency of directed evolution in the modification of enzymes for use in reaction media that are considerably different from their natural environment. Modified enzymes can also be applied to organic synthesis for the resolution of racemic mixes, which is very attractive for synthesis of biologically-active compounds in the pharmaceutical and biomedical industries. Reetz and collaborators modified the selectivity of a bacterial lipase for 2-methyldecanoic acid *p*-nitrophenyl ester using the combined methods of Error-prone PCR and saturation mutagenesis [10]. While the native enzyme gives a two percent enantiomeric excess in favour of the *(S)*-isomer, one of their mutated enzymes provided a 93 percent enantiomeric excess in favour of the same enantiomer. These two examples highlight the fact that directed evolution of enzymes is paving the way toward their use in organic synthesis. Mutated enzymes might eventually be used to catalyze reactions that are currently hard to perform or simply inaccessible using classical synthesis approaches.

Conclusion

The examples presented here provide a glimpse of the numerous applications where enzymes have been adapted to industrial purposes. Originally confined to biological systems, enzymes are now efficiently modified by directed evolution, which makes them an interesting alternative for environmentally clean industrial processes and green chemistry. Considered of marginal industrial utility even ten years ago, enzyme applications are now growing exponentially because of directed evolution, which makes their application much more flexible, profitable and efficient.

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