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Catalytic Machinery of Enzymes Expanded

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Enzymes are exceptionally powerful catalysts that recognize molecular substrates and process them in active sites. They are generally built from just 20 types of amino acid, and their catalytic machinery is typically assembled from chemical groups in the amino-acid side chains, often with additional bound metal ions or cofactors. This raises the question of whether the catalytic repertoire of enzymes could be expanded by using an extended 'alphabet' of amino acids that offers a wider range of side chains for catalysis. On page XX, Burke et al.[1] report the construction of an enzyme that exploits such unnatural catalytic functionality.

The amino-acid side chains found in enzymes contain at most one chemical group, and are crucial for molecular recognition. But less than half of the amino acid side chains contain groups that can act as acids, bases or nucleophiles (electron-pair donors), and can therefore have an active role in enzyme catalytic cyclestic cycles. None of the side chains can act as electrophiles (electron-pair acceptors), which could also be useful for catalysis. The introduction of unnatural amino-acid residues that bear potentially catalytic side chains could therefore open up a wide range of new enzymatic reactions.

Conventional catalysts are a fertile source of inspiration for chemical groups that would expand the catalytic repertoire of enzymes: both small-molecule organic catalysts (organocatalysts) and transition-metal catalysts can activate substrate molecules in ways that enable a variety of reactions that are useful for organic synthesis. To enable enzymes to access this exciting reactivity, methods are required for the efficient site-specific incorporation of amino acids bearing new chemical groups. Methods for the directed evolution of the resulting modified enzymes are also required to optimize catalysis within active sites.

Artificial enzymes have previously been constructed by attaching transition-metal catalysts to a small molecule known as biotin, which in turn binds non-covalently with extremely high affinity to the protein streptavidin, thus anchoring the catalyst within a protein framework[2,3]. Metal catalysts have also been covalently attached to the side chains of unnatural amino -acid residues that have been incorporated into proteins using genetic-engineering approaches[4]. With both of these strategies, directed evolution was used to greatly improve the catalytic efficiency and turnover (the average number of reactions catalysed by each enzyme) of the initially produced artificial enzymes, and, for example, to increase the selectivity of the enzyme

for a particular mirror-image isomer of the product (the enantioselectivity). Artificial enzymes have thus been produced that catalyse reactions not found in nature, including the formation of silicon–carbon[4] and carbon¬–carbon bond-forming reactions known as cyclopropanations[4] and ring-closing metathesis reactions[2].

Burke et al. took a different approach. They started from an enzyme[5] (BH32) that had been computationally designed to catalyse a particular type of carbon–carbon bond-forming reaction, but which also weakly catalyses an unrelated transformation: the hydrolysis of compounds known as 2-phenylacetate esters. The authors therefore decided to remodel the enzyme to make it an effective catalyst for these hydrolyses.

The researchers determined that a histidine amino-acid residue (His23) in BH32 forms an intermediate called an acyl–enzyme compound during the catalytic cycle. This intermediate is then hydrolysed to yield the product of the enzymatic reaction. However, the catalytic turnover was poor because the hydrolysis of the acyl–enzyme intermediate was slow. To address this issue, Burke and colleagues replaced His23 by the residue of a genetically encodable, unnatural amino acid, Nδ-methylhistidine (Me-His) (Fig. 1). Me-His is an analogue of histidine in which a methyl group is attached to one of the nitrogen atoms in the side chain. The authors observed that the turnover of the modified enzyme (OE1) was higher than for BH32, an effect that they ascribed to more-rapid hydrolysis of the resulting acyl–enzyme intermediate.

Burke et al. then used directed evolution to optimize the function of Me-His within the enzyme's active site. A wide range of strategies was used to introduce mutations, ultimately resulting in the discoveryproduction of a variant, OE1.3, that had improved catalytic efficiency. This variant differed from OE1 by having six point mutations (changes in which anone amino -acid residue in OE1 has been replaced withby another). The authors found that OE1.3 hydrolyses a range of analogues of 2-phenylacetate esters in which only hydrogen atoms were attached to the carbon atom adjacent to the carbonyl (C=O) group in the molecules. However, analogues in which a methyl group is attached next to the carbonyl group were poor substrates. The authors therefore carried out further directed evolution to generate OE1.4, an enzyme that has improved catalytic activity with this class of substrates, and which predominantly hydrolyses one of the two mirror-image isomers of each substrate.

The Me-His residue in the modified enzymes acts as a nucleophilic catalyst that is broadly analogous to the nucleophilic residues found in the naturally occurring enzymes serine hydrolase and cysteine hydrolase enzymes. But how might organocatalysis[6] in general inspire the discovery of enzymes that are more distant to those found in nature? Organocatalysts catalyse many different reactions using just a few generic mechanisms (activation modes), but the catalysis is often inefficient, requiring rather high catalyst loadings (typically 5¬–

20 mole percent). Some of these activation modes are also widely used by enzymes, such as enamine catalysis, which is used by Class I aldolases. But other activation modes, such as known as iminium catalysis, are less widely used by enzymes, despite the fact that they provide a generic route to the activation of α,β-unsaturated aldehydes towards nucleophilic attack and cycloaddition reactions can be used to enable many different a variety of potentially useful synthetic reactions.

Organocatalysts have been introduced into proteins in various ways, for example by using an attached biotin group as an anchor that binds to streptavidin[7], or by chemically modifying genetically encoded, unnatural amino -acid residues[8]. However, to realize the full power of an expanded range of catalytic chemical groupsfunctionality, substantial optimization is likely to be needed to generate catalytically efficient active sites. Burke et al. have shown that directed evolution can improve enzymes that contain an unnatural organocatalytic groups. Their approach might also provide a route to efficient enzymes that use activation modes that are not found in nature.

Figure legend: Complementary approaches for the introduction of novel catalytic functionality into enzymes. Panel A: Anchoring of a biotinylated catalyst to streptavidin. Panel B: Chemical modification of a genetically-incorporated unnatural azido amino acid. Panel C: Genetically-incorporation of an amino acid bearing novel catalytic functionality.

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