HYBRID ENZYMES AS DESIGNER CATALYSTS

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INTRODUCTION

Despite the development of robust organometallic catalysts for organic synthesis, the ability to mimic biology's capacity for synthetic cascades remains out of reach. Hybrid enzymes, designed to combine protein scaffolds and organometallic catalysts, offer the benefits of both catalytic systems and have the potential to advance the development of biologically compatible synthetic cascades. Recent advances in chemogenetic optimization have greatly improved the enantioselectivity and reactivity of hybrid enzymes, especially Diels-Alderases and artificial transfer hydrogenases (ATHases).

COPPER(II)-FUNCTIONALIZED PROTEINS AS DIELS-ALDERASES

Hybrid enzymes prepared from copper(II) catalysts have the potential to bring the Diels-Alder reaction into a biological context. Artificial Diels-Alderases have been designed to endow enantioselectivity to achiral Lewis acid catalysts. Reetz and coworkers mutated residues within a protein to act as the primary coordination sphere for the metal center.[1] Enantioselectivity was low, suggesting that the protein was not as well suited as synthetic ligands to act as the primary coordination sphere of the metal. Building on this work, Roelfes and coworkers engineered a highly enantioselective Diels-Alder hybrid enzyme by creating new catalytic centers at the LmrR protein dimer interface (Figure 1).[2] By varying the location of the phenanthroline-Cu(II) complex, Roelfes showed that the protein environment directed access to the catalyst and was responsible for reaction enantioselectivity. The LmrR hybrid enzyme showed that high selectivity is achieved with a tightly controlled binding location for the metal catalyst. Future efforts may be focused on acceptance of a wider range of substrates and obtaining crystal structures of hybrid enzymes to better understand the role of the protein in reaction enantioselectivity.

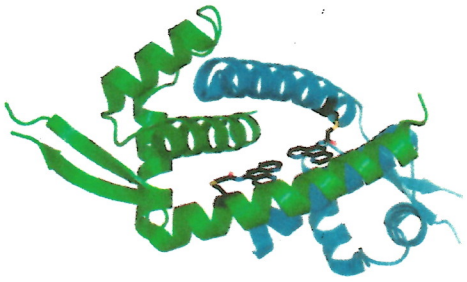

Figure 1. The LmrR dimer modified with phenanthroline ligands binds Cu(II) and catalyzes Diels-Alder reactions.

ARTIFICIAL TRANSFER HYDROGENASES IN CONCERT WITH NATIVE ENZYMES

Wilson and Whitesides were the first to utilize the strong noncovalent interaction between biotin and avidin to embed an organometallic catalyst within a protein.[3] The system has since been optimized

to accomplish a variety of reactions, including ATH (Figure 2).[4] Ward and coworkers conducted a three-dimensional screen of biotinylated catalyst derivatives, protein mutants, and ketone and imine substrates to better understand the roles of the protein and the organometallic catalyst in the reaction.[5,6] These studies showed that through chemogenetic optimization, a reactive and selective catalyst-protein complex could be developed. Whereas the metal center is responsible for hydrogen transfer, the protein environment dictates substrate approach and controls the enantioselectivity of the reaction.[7] Ward and coworkers designed synthetic cascades by coupling ATHases to native oxidases, combining up to four enzymes and accomplishing redox cascades with remarkable enantioselectivity.[8] Although the current substrate scope is limited, these synthetic cascades provide a starting point for developing more complex cascades using hybrid enzymes.

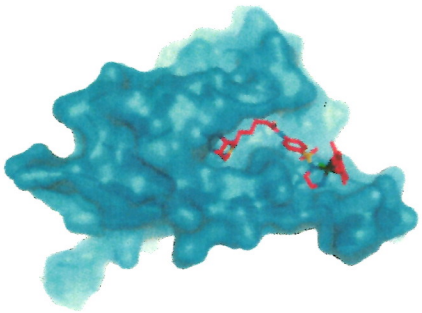

Figure 2. The crystal structure of a streptavidin monomer with a biotinylated catalyst able to carry out selective ATH.

SUMMARY AND OUTLOOK

The past decade has seen significant optimization of reactions catalyzed by hybrid enzymes. The separation of chemical reactivity from chemical selectivity in the form of a metal catalyst inside a protein environment has enabled researchers to develop hybrid enzymes that take advantage of the features of both organometallic catalysts and enzymes. Pioneering work in the development of hybrid enzyme Diels-Alderases and ATHases has shown that effective tuning of not only the catalyst but also the protein in which the catalyst is harbored is essential for high reactivity and enantioselectivity. Hybrid enzymes, shown to be compatible with native enzymes, will greatly expand the repertoire of reactions available for conducting synthetic cascades in biologically relevant conditions.

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