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Bringing dead proteins to life; development of an artificial Stetterase.

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PURPOSE OF THE ABSTRACT
Thiamine diphosphate (ThDP, vitamin B1) is one of nature's versatile organo-catalysts. ThDP-dependent enzymes catalyse a broad range of reactions, the most useful of which are the C-C bond forming biocatalysts that can be used to create chiral products from simple achiral building blocks. Several ThDP-dependent biocatalysts have been used to form α-hydroxyketones in high enantomeric excesses, including benzaldehyde lyases (BAL) that catalyses an enantioselective benzoin condensation. In contrast, only a limited number of ThDP-dependent biocatalysts can catalyse the irreversible formation of 1,4-dicarbonyl compounds from aldehydes and □,□ unsaturated carbonyl containing substrates. These so-called Stetterases have great synthetic potential but are limited in their substrate scope. Here, we aim to expand this pool of Stetterases by creating an artificial biocatalyst using a catalytically-inactive protein scaffold that contains a covalently attached thiamine analogue.
Our work utilises a small inactive protein scaffold, the recombinant human steroid carrier protein 2L (SCP-2L). The SCP-2L protein contains a hydrophobic tunnel and has a single cysteine residue engineered via mutagenesis. A thiazolium salt was incorporated into the SCP-2L scaffold by a site-specific, thiol-maleimide linkage to this cysteine residue. This artificial ‘active site' within the protein successfully catalyses an intramolecular Stetter reaction, in effect bringing a dead protein to life. Moving from the initial human construct to a thermophilic, bacterial version of the SCP scaffold (TT_SCP) we have been able to achieve 12 turnovers with modest e.e. (5%) without further optimisation. We also describe plans for engineering the artificial Stetterase active site, firstly to create an environment which aids deprotonation of the TT_SCP-bound thiazolium moiety. Secondly, we aim to increase the enantioselectivity of our ‘artificial biocatalyst' as well as broaden its substrate scope.
Work on Genetic Code Expansion (GCE) to increase the yield of the functionalised protein is in progress. The chemical synthesis of a thiazolium based unnatural amino acid (UAA) has been achieved and development of a method to incorporate this into a protein scaffold is underway. This proof of concept study paves the way for the in vivo synthesis and incorporation of a thiazolium-based UAAs into any protein scaffold. By combining organo-catalysis with directed evolution, our work provides a platform to design, select and engineer novel biocatalysts.

FIGURES

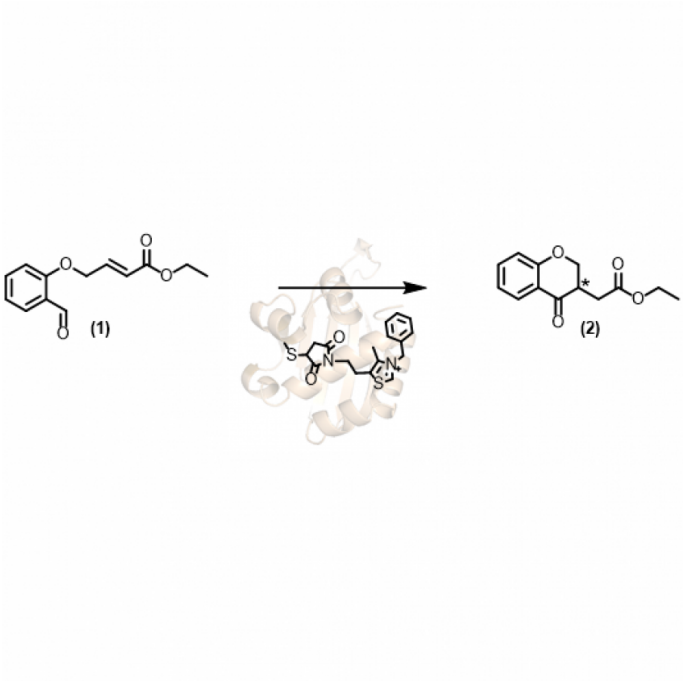

FIGURE 1

Figure 1

An intramolecular Stetter reaction catalysed by a thiazolium functionalised protein scaffold

FIGURE 2

KEYWORDS

Stetter | Genetic code expansion | Artificial enzyme | Biocatalysis

BIBLIOGRAPHY

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