BIOTRANS
LA ROCHELLE 2023

N°403 / PC
TOPIC(s) : Synthetic biology, metabolic engineering / Biocatalytic cascade reactions

Expanding the one-carbon chemistry of thiamine diphosphate-dependent enzymes

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PURPOSE OF THE ABSTRACT

The synthesis of complex molecules from simple, renewable carbon units (and eventually from CO2) is essential to achieve a circular economy. A significant challenge in this regard is the direct condensation of one-carbon molecules, for which only highly complex enzymes are known so far. Thiamine diphosphate-dependent (ThDP) enzymes offer a promising solution because they are versatile C-C bond forming catalysts and can be readily engineered. In this work, we explored and expanded the biocatalytic potential of the oxalyl-CoA decarboxylase (OXC)/2-hydroxyacyl-CoA lyase (HACL) superfamily, which catalyzes the shortening of acyl-CoA thioester substrates through the release of the one-carbon unit formyl-CoA. We demonstrated that members of this superfamily can operate in reverse to extend various aldehydes with one-carbon units, yielding the corresponding 2-hydroxyacyl-CoA thioesters.

We improved the catalytic properties of OXC by rational enzyme engineering and combined it with two newly described enzymes to create an enzymatic cascade that enabled the continuous conversion of oxalate and aromatic aldehydes into valuable (S)-α-hydroxy acids with up to 99% enantiomeric excess. Furthermore, we employed directed evolution to convert OXC into a glycolyl-CoA synthase (GCS) that condenses the two one-carbon units formyl-CoA and formaldehyde. The quadruple variant MeOXC4 showed a 100,000-fold switch between OXC and GCS activities, a 200-fold increase in GCS activity compared to the wild type, and formaldehyde affinity comparable to natural formaldehyde-converting enzymes.

Our work highlights the potential of ThDP-dependent enzymes in biocatalytic one-carbon conversion and offers a promising strategy for the sustainable production of valuable products from renewable feedstocks.

FIGURES

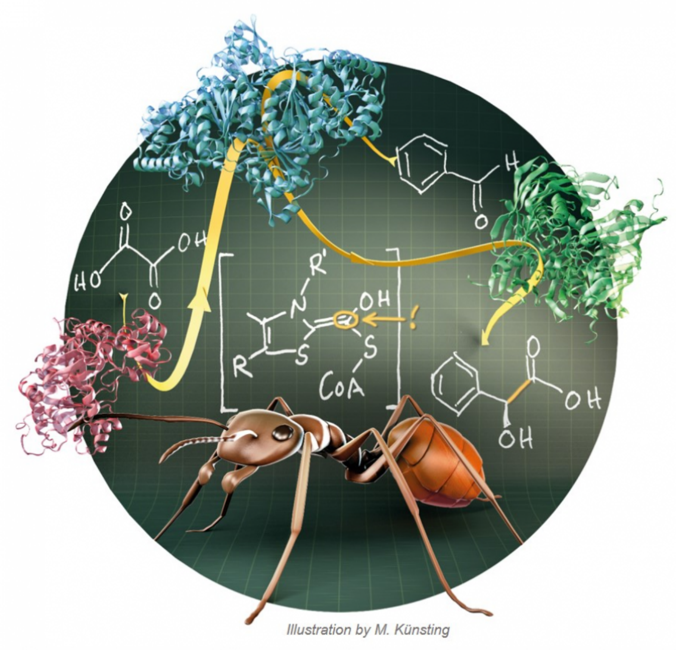

Illustration by M. Künsting

FIGURE 1

Oxalyl-CoA Decarboxylase Enables Nucleophilic One-Carbon Extension of Aldehydes to Chiral 2-Hydroxy Acids

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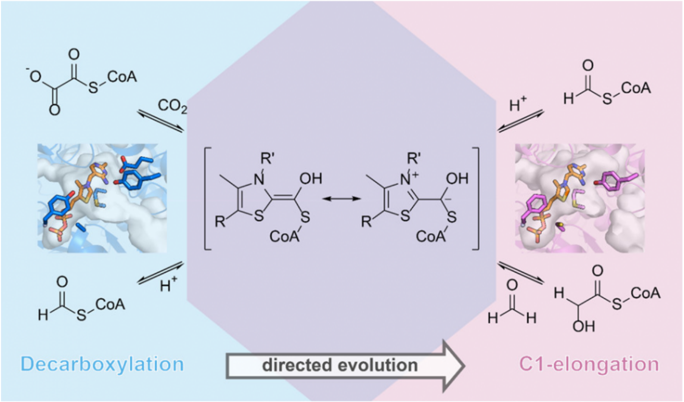

FIGURE 2

Engineering a Highly Efficient Carboligase for Synthetic One-Carbon Metabolism

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KEYWORDS

One-carbon chemistry | Thiamine diphosphate-dependent enzymes | Enzyme engineering | Synthetic biochemistry

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