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Extended Biocatalytic Halogenation Cascades Enabled by a Single-Polypeptide Regeneration System for Diffusible FADH2

AUTHORS

Nicolai MONTUA / BIELEFELD UNIVERSITY, UNIVERSITÄTSSTRASSE 25, BIELEFELD
Norbert SEWALD / BIELEFELD UNIVERSITY, UNIVERSITÄTSSTRASSE 25, BIELEFELD

PURPOSE OF THE ABSTRACT

In recent years flavin dependent halogenases (FHals) have increasingly emerged as attractive biocatalysts for the enzymatic introduction of halogen substituents to unactivated aromatic compounds, even at electronically unfavoured positions. Compared to conventional halogenation procedures, halogenases offer an environmentally friendly and highly regiospecific alternative, requiring only an aqueous buffer, molecular oxygen, and halide salt.
While activity and stability of wild type FHals leave much to be desired[1], enzyme engineering and directed evolution campaigns are underway to improve these shortcomings. Tryptophan halogenases in particular have been thoroughly characterized and engineered regarding thermostability[2], regioselectivity[3], and substrate spectrum[4,5].
As most flavin dependent halogenases do not exhibit flavin reductase activity, their application as biocatalysts requires a two-component cascade for the regeneration of diffusible FADH2. Enzymatic cofactor regeneration cascades typically comprising a dehydrogenase enzyme and a separate flavin reductase are most commonly used. This requires multiple cultivations and purification procedures severely limiting scalability. Therefore, we constructed a bifunctional fusion protein for the regeneration of diffusible FADH2 from inexpensive phosphonate as a sacrificial substrate. This fusion protein proved amenable to coexpression with various flavin dependent halogenases and even allowed for the coexpression of an additional dioxygenase enabling the synthesis of L-4-Cl-Kynurenine, a promising prodrug candidate currently undergoing clinical evaluation, at preparative scale in a one-pot reaction directly from inexpensive L-Trp.

FIGURES

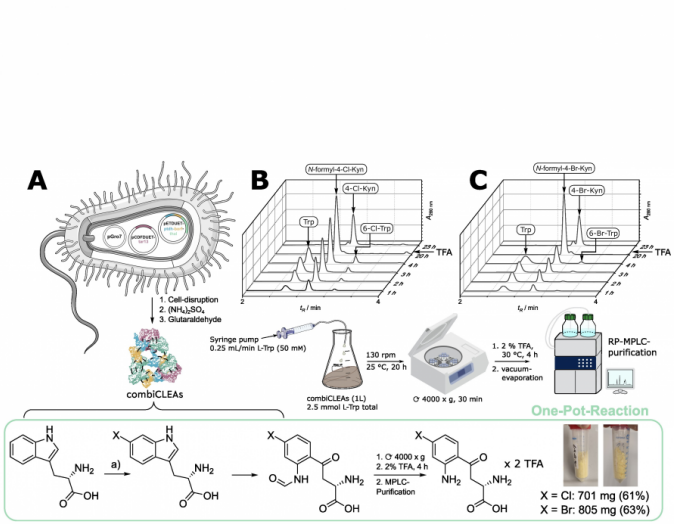

FIGURE 1

Figure 1

Direct one-pot synthesis of L-4-halo-kynurenine from inexprensive L-Tryptophan using combiCLEAs from lysates of an E. coli BL21 (DE3) derived coexpression strain halogenase and FADH2-regeneration cascade.

FIGURE 2

KEYWORDS

One-Pot cascade | Halogenase | Prodrug | Immobilization

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