Enhanced Fatty Acid Decarboxylase Activity using a Thioredoxin-mediated Electron Relay

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Long chain α-olefins are useful synthetic intermediates for the production of industrial lubricants. These terminal alkenes can be made enzymatically by the oxidative decarboxylation of their corresponding fatty acids using the Cytochrome P450 - OleT. This transformation has been demonstrated extensively using $OleT_{JE}$ (*Jeotgalicoccus sp.* ATCC 8456). $OleT_{JE}$ activity has been improved with the redox partner proteins CamA and CamB (*Pseudomonas putida*) although 1-alkene titres remained moderate.[1] These include the canonical Fe_2-S_2 ferredoxin (CamA) which serves as the electron-shuttle protein, and the partnering ferredoxin reductase (CamB). Coexpression of the native ferredoxin has enhanced activity for other Cytochrome P450s, although this strategy has yet to be applied to the decarboxylation of fatty acids using OleT.[2]

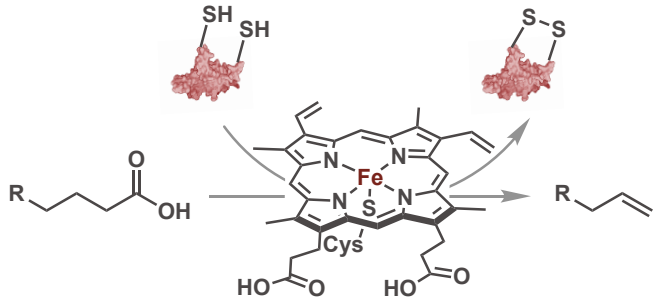

Figure 1: Aliphatic alkene production using a Cytochrome P450 supported by a thioredoxin.

In this work, several decarboxylases were screened which identified the highly active homologue $OleT_{MC}$ (*Macrococcus caseolyticum*). Coexpression of $OleT_{MC}$ with suitable redox partners led to the highest reported titre for aliphatic primary alkenes (>450 mg/L). Interrogation of the genome could not identify a ferredoxin or ferredoxin reductase, instead a distal thioredoxin and partnering reductase were located. Coexpression of these non-canonical redox proteins with $OleT_{MC}$ was shown to enhance alkene production. Our investigation presents a robust whole-cell biocatalytic route to terminal alkenes, along with the first example of a bacterial Cytochrome P450 which utilises this class of redox partners.

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[1] A. Dennig, *et al*., *Angew. Chem. Int. Ed.,* **2015**, *30*, 8819-8822

[2] H. Hussain, J. Ward, *Appl. Environ. Microbiol.*, **2003**, *69*, 373-382.