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Solar Biomanufacturing: Engineering photosynthetic electron transfer for P450 biocatalysis in Chlamydomonas reinhardtii

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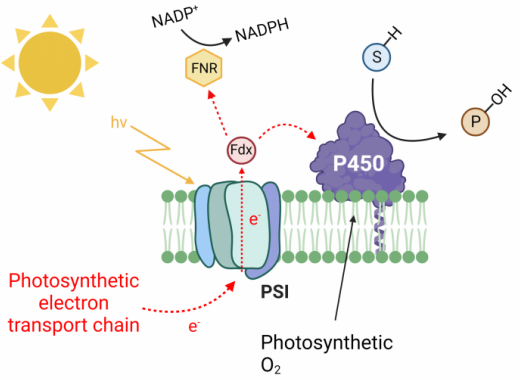
PURPOSE OF THE ABSTRACT
Biocatalysis is increasingly of interest in the pharmaceutical and fine chemical industries for sustainable production of high-value and structurally complex chemicals. Cytochrome P450 monooxygenases (P450s) are attractive biocatalysts for industrial applications due to their ability to transform a wide range of substrates in a stereo- and regiospecific manner. However, the industrial application of P450s is limited by their dependence on costly NADPH and an auxiliary redox partner protein, cytochrome P450 reductase (CPR). The aim of the present study was to couple P450s with the photosynthetic machinery of Chlamydomonas reinhardtii, to take advantage of photosynthetically generated electrons for driving catalysis and eliminate the dependence on a reducing cofactor.
Highly thermostable ancestral P450s have been shown previously to be active towards a wide range of drug substrates. To explore the potential of these P450s for light-driven biocatalysis, an in vitro system was developed initially using the P450 ancestor, CYP2ABGSFTCEH_N13, thylakoid membranes from C. reinhardtii, and a soluble C. reinhardtii ferredoxin (PetF) as a redox partner. This system was assessed for the ability to catalyse the hydroxylation of the small molecule substrates, diclofenac, in the presence and absence of light and PetF. The reaction was found to be light and PetF-dependent with an apparent reaction rate 2.6 times higher than catalysed by the same P450 supported by NADPH and human CPR. The ability of the ancestral P450s to couple to the photosynthetic machinery was then assessed in vivo, using C. reinhardtii mutants expressing CYP2ABGSFTCEH_N13 in the chloroplast. Western blot analysis demonstrated that the P450 was expressed and bound to the chloroplast of C. reinhardtii. Furthermore, using diclofenac as a substrate, C. reinhardtii mutants expressing CYP2ABGSFTCEH_N13 showed approximately three-fold higher diclofenac 5-hydroxylation compared to the wildtype C. reinhardtii. These results show that the photosynthetic machinery of C. reinhardtii can act as an alternative electron delivery system to CYP2ABGSFTCEH_N13 via ferredoxin in the presence of light. These findings highlight the potential of C. reinhardtii as a chassis for light-driven P450 biocatalysis to produce fine chemicals and pharmaceuticals.

FIGURES



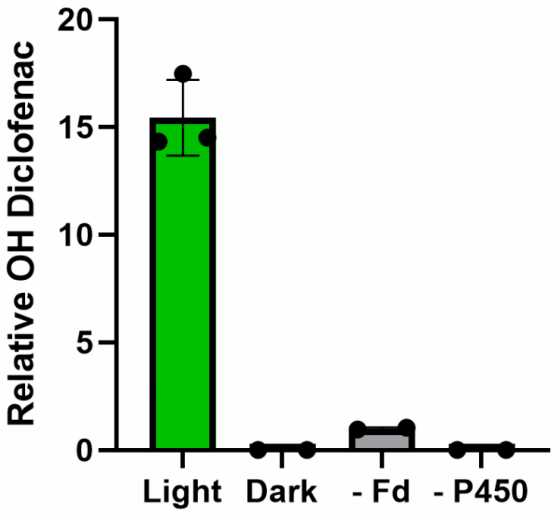


FIGURE 1

Fig. 1 P450 biocatalysis powered using photosynthetic reducing power.

The red dashed arrows indicate photosynthetic electron flow. Fd, ferredoxin; P450, cytochrome P450 monooxygenase, S-H, substrate; P-OH, product

FIGURE 2

Fig. 2 relative hydroxy (OH) metabolite of diclofenac in in vitro light-driven system using P450 ancestor, CYP2ABGSFTCEH_N13, C reinhardtii thylakoid membrane and ferredoxin

Light and dark, reactions in the presence and absence of light, respectively. -Fd and -P450 control reactions without ferredoxin or P450, respectively

KEYWORDS

Cytochrome P450 | photosynthesis | light-driven | biocatalysis

BIBLIOGRAPHY