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Technical Report**Peroxidase activity of bacterial cytochrome P450 enzymes: Modulation by fatty acids and organic solvents**

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Abstract

The modulation of peroxidase activity by fatty acid additives and organic cosolvents was determined and compared for four bacterial cytochrome P450 enzymes, thermostable P450 CYP119A1, the P450 domain of CYP102A1 (BMP), CYP152A1 (P450_{bsβ}), and CYP101A1 (P450_{cam}). Utilizing a high-throughput microplate assay, we were able to readily screen more than 100 combinations of enzymes, additives and cosolvents in a convenient and highly reproducible assay format. We found that, in general, CYP119A1 and BMP showed an increase in peroxidative activity in the presence of fatty acids, whereas CYP152A1 revealed a decrease in activity and CYP101A1 was only slightly affected. In particular, we observed that the conversion of the fluorogenic peroxidase substrate Amplex Red by CYP119A1 and BMP was increased by a factor of 38 or 11, respectively, when isopropanol and lauric acid were present in the reaction mixture. The activity of CYP119A1 could thus be modulated to reach more than 90% of the activity of CYP152A1 without effectors, which is the system with the highest peroxidative activity. For all P450s investigated we found distinctive reactivity patterns, which suggest similarities in the binding site of CYP119A1 and BMP in contrast with the other two proteins studied. Therefore, this study points towards a role of fatty acids as activators for CYP enzymes in addition to being mere substrates. In general, our detailed description of fatty acid- and organic solvent-effects is of practical interest because it illustrates that optimization of modulators and cosolvents can lead to significantly increased yields in biocatalysis.

Introduction

Cytochrome P450 enzymes (P450s) are a class of enzymes which can catalyze a wide range of reactions including aliphatic and aromatic hydroxylation, epoxidation, oxidative phenolic coupling, heteroatom oxidations, and dealkylations, often in a regio- and stereoselective manner.^[1] This diversity makes them interesting candidates as biocatalysts for industrial applications, however, it also renders the search for suitable substrate-enzyme pairs a tedious task.^[1-6] The amino acid sequences of P450 enzymes can be much more diverse than their reactivities, and still, P450 enzymes, which have distinctively different primary structures, may display similar reactivities. For example, a variety of diverse P450 enzymes can bind and/or convert fatty acid substrates. Besides other bacterial P450 enzymes, fatty acid conversion can also be facilitated by the thermostable P450 CYP119A1 from the thermophilic organism *Sulfolobus acidocaldarius*. This enzyme is considered a potentially powerful biocatalyst, as it displays high stability and activity even under harsh process conditions. Besides the hydroxylation of lauric acid,^[7] it is also able to catalyze the epoxidation of styrene,^[8, 9] the electrochemical reduction of nitrite, nitric oxide, and nitrous oxide, as well as the electrochemical dehalogenation of CCl₄ to yield CH₄.^[10, 11] CYP119A1 has been cloned and overexpressed,^[12] its molecular structure has been determined by X-ray crystallography,^[13-15] its catalytic mechanism has been investigated in detail,^[16-22] and several artificial several electron donor systems have been established.^[23, 24] However, the catalytic turn-over of all substrates known to be converted by CYP119A1 is fairly low, and therefore none of those might represent the endogenous substrate of this enzyme.

Motivated by its potential as biocatalyst and the unknown natural substrate of CYP119A1, we reasoned that comparison of reactivity patterns of bacterial P450 enzymes should contribute to a more fundamental understanding of this class of enzymes. For comparison of CYP119A1 we chose three other bacterial P450 enzymes, the P450 domain of CYP102A1 (BMP),

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3 CYP152A1 (P450_{bsβ}), and CYP101A1 (P450_{cam}), which have been extensively studied as
4 potential biocatalysts.^[25-27] With respect to fatty acid substrates, CYP119A1 is known to
5 hydroxylate fatty acids at the ω -1 to ω -3 position,^[7] thereby showing similar behaviour as
6 CYP102A1.^[7, 28-30] In contrast, CYP152A1 regioselectively hydroxylates fatty acids either at
7 the alpha or beta position,^[31] while CYP101A1 appears to be unable to convert fatty acids at
8 all.^[7, 30, 32] However, it is reported here for the first time that all four enzymes share a
9 peroxidase reactivity against the fluorogenic substrate Amplex Red in the presence of
10 hydrogen peroxide, following the so-called peroxide shunt pathway. In the case of
11 CYP152A1 this observation is in agreement with the expectation because this enzyme
12 utilizes hydrogen peroxide in the physiological reaction,^[26, 32] while the other three P450s
13 usually depend on the presence of molecular oxygen and NAD(P)H to perform reactions.
14 Moreover, it was recently shown that the presence of fatty acid molecules can modulate the
15 peroxidation activity of CYP152A1,^[33, 34] and variations in the length of the fatty acid enabled
16 conclusions on the accessibility of the enzyme's active site. It has also been shown that
17 eucaryotic P450 enzymes may contain a fatty acid binding site^[35] and that their activity can
18 be modulated by phospholipids.^[36] We therefore reasoned that assaying the peroxidase
19 activity of aforementioned bacterial P450 enzymes in the presence of various fatty acids
20 should be readily facilitated in microplates in order to analyze reactivity patterns and gain
21 basic knowledge on the enzymes.

22 Since especially long chain fatty acids are poorly soluble in aqueous solutions, organic
23 cosolvents are usually employed, and since it is known that cosolvents can significantly
24 influence the activity of P450 enzymes,^[37-39] we investigated their effect as well. Indeed, we
25 found that both fatty acids and cosolvents strongly affected peroxidase activity and the
26 obtained reactivity patterns suggest similarities between CYP119A1 and BMP. The
27 experimental setup presented here allows to screen samples in a high-throughput format, thus
28 enabling us to investigate over 100 different enzyme/additive/solvent combinations.

Materials and Methods

Chemicals. KH_2PO_4 , K_2HPO_4 and hydrogen peroxide were obtained from Sigma. Amplex Red (10-Acetyl-3,7-dihydroxyphenoxazine, sold under the name of Ampliflu Red), resorufin, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid, palmitic acid, DMSO and 2-Propanol (p.a) were from Fluka. Methanol (p.a.) was from Roth and ethanol (p.a.) from Merck. HPLC-grade acetonitrile was purchased at Fischer, IPTG at Gerbu and ampicillin at Applichem.

Recombinant P450_{bsβ} (CYP152A1) was expressed from *Escherichia coli* M15 (pREP4) using the plasmid pQE-30tBSb, which was kindly donated from Dr. Isamu Matsunaga.^[40] The enzyme containing a C-terminal hexahistidine tail was overexpressed in *E. coli* M15 and purified by affinity chromatography, as reported earlier.^[40-42]

Recombinant CYP119A1 was expressed in *E. coli* BL21(DE3) as previously described.^[9] The enzyme contained a C-terminal hexahistidine tail, which was used for purification by affinity chromatography, as reported earlier.^[9]

Cloning of BMP (P450 domain of CYP102A1)

The genomic DNA of *Bacillus megaterium* strain (DSM 32) was isolated using the InstaGene Matrix Kit from Biorad. The DNA sequence encoding for the Cytochrome P450 subunit of CYP102A1 was amplified using the forward primer 5'-
GGGGACAAGTTTGTACAAAAAAGCAGGCTCTA**AGGAGG**ATAGAACC**ATG**ACAATTAAAGAAAT
GCCTCAGC and the reverser primer
GGGGACCACTTTGTACAAGAAAGCTGGGTGGCTAGGTGAAGGAATACCGC. The primers contained *attB* sites (underlined) to introduce the gene into the Gateway® recombinational cloning system (Invitrogen). Additionally the forward primer contained a ribosome binding site (bold) and the start condon (bold, underlined). PCR was carried out with Iproof High Fidelity DNA Polymerase (Biorad), using standard reaction conditions according to supplier's

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3 instructions with an annealing temperature of 56°C. The PCR-product was cloned by
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5 recombination into the pDONR221 vector in a BP reaction, carried out following the
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7 supplier's instructions (Invitrogen). Successful cloning and integrity of the resulting plasmid
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9 pENTR221-CYP102A1 was confirmed by sequence analysis. This entry vector was then
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11 recombined with pET-DEST42 in a LR reaction to yield the expression vector pET-EXP42-
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13 CYP102A1.
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16 17 *Cloning of CYP101A1*

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19 The genomic DNA of *Pseudomonas putida* strain (DSM 4475) was isolated using the
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21 InstaGene Matrix Kit from Biorad. The PCR to amplify the encoding region of CYP101A1
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23 was carried out using the forward primer 5'-
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25 GGGGACAAGTTTGTACAAAAAAGCAGGCTCTAAGGAGGATAGAACCATGACGA
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27 CTGAAACCATACAAAGC and the reverse primer 5'-
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29 GGGGACCACTTTGTACAAGAAAGCTGGGTGTACCGCTTTGGTAGTCGCC. PCR was
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31 carried out with Iproof High Fidelity DNA Polymerase (Biorad) using standard reaction
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33 conditions according to supplier's instructions with an annealing temperature of 58° C. The
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35 PCR-product was used to create the pET-EXP42-CYP101A1 expression vector in the same
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37 manner as described for CYP102A1.
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43 44 *Overexpression and purification of BMP and CYP 101*

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46 For protein expression of BMP and CYP101A1 overnight cultures of the corresponding *E.coli*
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48 BI21 (DE3) cells were started in LB-broth containing 100 µg/ml ampicillin. A part of the
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50 overnight culture was added to a 3 l culture of LB-broth containing 100 µg/ml ampicillin and
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52 0,5 mM aminolevulinic acid. The OD₆₀₀ value at T₀ was adjusted to 0.1. The culture was
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54 incubated at 37° C with 180 rpm shaking. When the culture reached OD₆₀₀ 0.5 – 0.7 IPTG
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56 was added to a final concentration of 0.5 mM and incubation was continued at 25 °C
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58 overnight. Cells were harvested by centrifugation for 10 min at 8000 rpm at 4° C and
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60 subsequently resuspended in 50 mM Tris buffer, 150 mM NaCl, 10 mM Imidazol, pH 8.0,

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sonicated and centrifuged. Since the enzymes were hexa-His-tagged by expression from pET-DEST42, the soluble fraction was passed through a Ni-NTA column and washed with 50 mM Tris buffer, 150 mM NaCl, 20 mM Imidazol, pH 8.0. The protein was eluted from the column using 50 mM Tris buffer, 250 mM Imidazol, pH 8.0. Eluted proteins were subjected to size exclusion chromatography on a Superdex S 200 column (Amersham Pharmacia Biotech). The elution was monitored at A_{417} and A_{280} . Fractions showing absorbance at 417 nm were pooled and concentrated using a concentrating device with a 10-kDa cut-off membrane (Vivaspin). The proteins were judged to be pure by SDS-PAGE. We note that the absorption spectra of all four P450 enzymes used in this study were identical to previously published UV-VIS data, thereby indicating that the active site of all proteins was empty and did not contain any ligands introduced during heterologous expression or purification procedures.

Microtiter plate screening of P450 reactivity. All P450 reactions were carried out in 200 μ l reaction volume, containing 1 mM H_2O_2 , 2 mM of the corresponding fatty acid (obtained from 20 mM stock solutions in either methanol, ethanol, isopropanol or acetonitrile as indicated) or only the solvent, 1 μ M Amplex Red (from a 10 mM DMSO stock, diluted in 50 mM KP_i pH 7.0.) and 1 μ M enzyme in 50 mM KP_i pH 7.0. The reaction mixture therefore always contains 10% (v/v) of organic cosolvent. All components besides the peroxide were premixed in a volume of 100 μ l and the microtiter plate was centrifuged for 30 seconds at 500g to remove bubbles. The reaction was started then by the addition of the peroxide in a volume of 100 μ l. The evolving fluorescence (due to the generation of resorufin) was recorded with a Synergy II microplate reader (BIO-TEK) at 25°C. In all cases, v_0/E_0 , the substrate conversion per enzyme and time (in nmol substrate per nmol enzyme per second), was calculated from the linear phase of product formation, typically recorded in the initial phase of the reaction diagram, where less than 10% of the maximum signal intensities were reached. All reactions were carried out at least as triplicate independent measurements.

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3 Whenever an organic solvent was included, the substrate conversion was corrected for the
4 effect of the solvent on the fluorescence measurement determined by the addition of 10% of
5 the solvent to resorufin solutions of known concentrations (see Fig. S2).
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10 11 12 **Results and Discussion**

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14 Since only little is known about the modulation of peroxidase activity of bacterial
15 cytochromes by fatty acids,^[34] we compared the enzymes CYP119A1, BMP, CYP152A1 and
16 CYP101A1, all of which are considered as potential candidates for biocatalytic
17 applications.^[24, 26, 27, 43] The four enzymes reveal low sequence homology (Table 1), despite
18 the fact that some similarities in terms of fatty acid hydroxylation are evident. Both
19 CYP119A1 and BMP oxidize lauric acid mainly at the ω -1 position,^[7, 30] CYP152A1
20 hydroxylates fatty acids at either alpha or beta position,^[31] while CYP101A1 seems unable to
21 convert fatty acids. Thus, it was interesting to investigate whether the peroxidation reaction
22 depicted in Fig. 1 is influenced by the presence of fatty acids and/or organic cosolvents. In
23 this reaction the fluorogenic substrate Amplex Red is oxidized to the highly fluorescent
24 product resorufin by the P450 enzyme in the presence of H₂O₂. Since Amplex Red offers
25 greater sensitivity in fluorescence detection than other substrates, such as guaiacol, the reaction
26 can be easily monitored in a multiwell plate reader, thus offering the opportunity of high-
27 throughput analysis of a variety of reaction conditions.
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48 We initially investigated the peroxidase activity of the four enzymes in the absence of fatty
49 acid modulators or cosolvents. To investigate all four enzymes under comparable conditions,
50 we used the artificial oxygen donor hydrogen peroxide, although P450 BM-3 and CYP101A1
51 might as well be activated by their natural NADPH-dependent redox partners. This approach
52 also enabled us to keep the complexity of the reaction mixture as low as possible.
53 Employment of an NADPH regeneration system would render the comparison of the data
54 rather difficult if not impossible since influences of cosolvents and the fatty acids on the
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3 activity of the regeneration system would have to be separately tested as well. Although
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5 CYP119A1 is more active at elevated temperatures, all reactions were performed at 25°C, in
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7 order to allow for convenient use of a multiplate reader and thus screen conditions for the four
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9 enzymes under comparable conditions in a high-throughput format. As shown in Fig. 2, all
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11 four enzymes reveal a significant activity with Amplex Red as the substrate. CYP152A1
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13 displayed by far the highest peroxidase activity, which might reflect the fact that the other
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15 three enzymes usually require NAD(P)H-dependent electron donor systems to work
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17 efficiently.^[7, 44, 45] Nonetheless, all four enzymes under investigation were sufficiently active
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19 to be reliably analyzed by the Amplex Red microplate assay.
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25 Since long chain fatty acids have a low solubility in aqueous solutions, we carefully studied
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27 the literature on the conversion of fatty acids by P450 enzymes to identify a suitable
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29 cosolvent. The survey revealed that a large variety of cosolvents has been described, including
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31 acetonitrile,^[46] acetone,^[47] DMSO,^[48] or ethanol,^[40] in addition to a variety of pure buffers,
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33 such as TRIS-HCl,^[49] MOPS,^[50] potassium carbonate,^[51] or alkaline solution^[52] (for a detailed
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35 survey, see table S1 in the supporting information). Notably, Schwaneberg et al. have recently
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37 shown that cosolvents can also enter the active site and bind to the iron, which in turn
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39 modulates the enzymatic activity.^[53] We therefore decided to also include a systematic
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41 variation of cosolvents in our study, by using either methanol, ethanol, isopropanol or
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43 acetonitrile.
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49 It is shown in Fig. 3 that the peroxidase activity of the four enzymes is affected to a different
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51 extent by the presence of the cosolvent. CYP119A1 showed a general increase in activity due
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53 to the presence of cosolvents, while CYP152A1 was inhibited by cosolvents. CYP119A1 and
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55 BMP revealed highest activities in the presence of isopropanol. BMP and CYP101A1
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57 revealed either inhibition or activation, depending on the cosolvent. As such, both enzymes
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59 were inhibited by methanol and ethanol, whereas they displayed the highest activity in the
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presence of isopropanol and acetonitrile, respectively. Since no general trend is obvious in

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3 Fig. 3, the data suggest that the cosolvent indeed influences the mechanism of catalysis, rather
4 than simply affecting, for instance, solubility of reaction components. We also confirmed that
5 the fatty acids do not interfere with the fluorescence read-out of our assay (see Fig. S2). These
6 results also emphasize the importance of cosolvents, thereby indicating that experimental
7 details on preparation of stock solutions and dilution steps should be carefully documented in
8 published protocols. Regrettably, this has not always been the case in previous work (Table
9 S1).

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11 Realizing that the cosolvent can largely impact the peroxidase activity, we decided to test all
12 four enzymes against 28 possible combinations, obtained from seven fatty acids with chain
13 lengths varying from C₁₀ to C₁₆ and the four cosolvents (Fig. 4). For CYP119A1 (Fig. 4a), we
14 observed that the presence of longer fatty acid chains led to an increased rate of resorufin
15 generation, revealing a peak activity at lauric acid (C₁₂). In this case, the cosolvent had only
16 little effect. However, the presence of ethanol increased the stimulatory effect of fatty acids
17 longer than 11 carbon atoms. Moreover, the optimal chain length in the presence of
18 acetonitrile was C₁₄. The conversion of Amplex Red by CYP119A1 was enhanced by a factor
19 of ≥ 38 , when lauric acid and isopropanol were added to the reaction mixture. We further
20 confirmed that the effect is concentration dependent (Fig. S3). It should be noted that
21 concentration exceeding 2 mM can not be realized because the fatty acids are not soluble
22 above this concentration. These results suggest that the different size and nature of cosolvent
23 molecules plays an important role in the fatty acid modulation, which can be explained by the
24 fact that the cosolvent is either situated in the enzyme's active site or that it generally
25 enhances the enzymatic activity by effecting its tertiary structure.

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27 BMP (Fig. 4b) also revealed an increase in Amplex Red conversion in the presence of fatty
28 acids. Similar to CYP119A1, the highest peroxidase activity was observed in the presence of
29 lauric acid, however, the peak activity was much sharper than that observed for CYP119A1.
30 Modulation of BMP was always highest with lauric acid, regardless of the cosolvent, and in
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3 combination with the addition of isopropanol, lauric acid increased the peroxidation activity
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5 about 11-fold.
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8 In case of CYP152A1 (Fig. 4c), we observed a decrease in peroxidase activity with increasing
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10 chain length of the fatty acid. This result is in agreement with the previous study of Watanabe
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12 and coworkers, who found that fatty acids with chain lengths of more than seven carbon
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14 atoms, inhibited the oxidation of guaiacol.^[34] They explained that competitive inhibition
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16 occurs due to increased blocking of the active site, which sterically hinders the access of the
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18 peroxidase substrate.^[33, 34] Consequently, hydroxylation of the fatty acid takes place instead of
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20 the peroxidation reaction when the fatty acid chain is sufficiently long.^[33] Furthermore the
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22 data in Fig. 4 also indicate a decrease in peroxidase activity from methanol to ethanol to
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24 isopropanol. This effect can not be explained by simple denaturation of the enzyme, because
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26 in this case, it should be observed for all fatty acids. Instead, the data clearly indicate different
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28 effects. For example the addition of methanol alone led to almost no change in the activity of
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30 CYP152A1, while the activity dropped by almost 70% when both methanol and lauric acid
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32 were present. The addition of isopropanol alone led to a decrease of 50%, but in this case the
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34 addition of lauric acid only leads to a minor decrease in activity (Fig. 4c).
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41 The enzyme CYP101A1 revealed only small changes in its activity in the presence of the
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43 various fatty acids in the four cosolvents. Maximum relative changes were only of about a
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45 factor of 3 (e.g., C₁₂ in acetonitrile as compared to the reaction mixture lacking fatty acid or
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47 cosolvent), while they were up to 38-fold the other enzymes (see above). Yet, a low general
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49 tendency of increased activity in the presence of acetonitrile was evident.
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53 Comparison of the reactivity patterns shown in Fig. 4, suggests some similarity between
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55 CYP119A1 and BMP in their response towards the addition of fatty acids. Both patterns
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57 reveal a distinctive increase in reactivity with increasing chain length, and a general peak
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59 activity at C₁₂. Given the fact that these two enzymes both catalyze hydroxylation of lauric
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acid at the ω-1 position, the similarity in their reactivity patterns suggests that in both cases

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3 the ω -end of the fatty acid is dipping into the active center, while the carboxyl group is either
4 located somewhere inside the substrate entry channel or on the enzyme's surface. The
5 resulting increase in hydrophobicity at the active site obviously gives rise to the altered
6 reactivity against Amplex Red. To support this hypothetical mode of action, we generated
7 data by comparing CYP119A1 peroxidase activity in the presence of either lauric acid,
8 dodecane, dodecanol or 12-hydroxylauric acid (Fig. 5). These data clearly indicate that the
9 hydrophobic ω -end is important for the activation of the enzyme, because neither dodecanol
10 nor 12-hydroxylauric acid led to a comparable increase in peroxidase activity as obtained with
11 lauric acid as modulator. The lack of increased activity in the presence of dodecane and
12 dodecanol also supports the presence of a binding site to stabilize the lauric acid ligand by its
13 carboxyl headgroup. Therefore, the data provide a hint that CYP119A1 has a binding site for
14 the carboxyl group, similar as it is in the case for BMP. This assumption is supported by
15 differences in UV/vis spectra obtained from the ligand-free CYP119A1 and the enzyme after
16 incubation with the four aforementioned compounds (Fig. S4). Only in case of lauric acid the
17 typical low-spin to high-spin conversion was observed, thus suggesting that lauric acid indeed
18 enters the binding site of CYP119A1.
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43 Conclusions

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45 In conclusion, we employed a high-throughput microplate assay to demonstrate that the
46 peroxidase activity of CYP119A1, BMP, CYP152A1 and CYP101A1 can be modulated to a
47 different extent by the presence of fatty acids of different chain lengths and organic
48 cosolvents. In particular, the results indicated that lauric acid and isopropanol increase the
49 reactivity of BMP and CYP119A1 by a factor of 11 or 38, respectively. Although CYP152A1
50 without any effectors showed the highest reactivity of all combinations tested, the optimized
51 reactivity of CYP119A1 reaches 90% of the value of CYP152A1. In contrast to BMP and
52 CYP119A1, CYP152A1 is largely inhibited by fatty acids and cosolvents, while these
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3 additives affect only weakly the activity of CYP101A1. The strong effects of the cosolvent
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5 observed here are of general importance for P450 biocatalysis because the often hydrophobic
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7 substrates are usually added as solution in organic solvents. It is obvious that the influence of
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9 particular cosolvents has to be specifically tested and a proper choice might increase the
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11 enzymatic activity and thereby reaction yields in biocatalysis. Moreover, comparison of
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13 reactivity patterns observed here suggested similarities in the binding site of CYP119A1 and
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15 BMP, and additional experiments provided evidence that the fatty acid penetrates the active
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17 site of CYP119A1 with its hydrophobic end in close proximity to the heme center. The results
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19 of this study may also hint towards a role of fatty acids as activators for CYP enzymes^[36] in
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21 addition to being mere substrates.
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46 enzymes.
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Figure legends

Figure 1. Conversion of Amplex Red to Resorufin by a P450 enzyme using hydrogen peroxide as the oxygen donor.

Figure 2. Initial rate of peroxidation of Amplex Red in the presence of CYP119A1, BMP, CYP152A1, CYP101A1. Error bars represent the standard error of three independent experiments.

Figure 3. Effect of different cosolvents on the initial rate of peroxidation of Amplex Red in the presence of CYP119A1, BMP, CYP152A1, CYP101A1. Error bars represent the standard error of three independent experiments.

Figure 4. Titration of CYP119A1 (a), BMP (b), CYP152A1 (c) and CYP101A1 (d) with fatty acids of different chain length. Each reaction was performed with four different cosolvents. The first, black bar in each set represents v_0/E_0 for the conversion of Amplex Red, measured in the absence cosolvent or fatty acid. Error bars represent the standard error of three independent experiments.

Figure 5. Effect of lauric acid, dodecane, dodecanol and 12-hydroxylauric acid on the rate of Amplex Red conversion by CYP119A1 with different cosolvents. Error bars represent the standard error of three independent experiments.

Table 1: Similarity and identity (in brackets) of the P450 enzymes compared in this study.

The values were calculated using the EMBOSS Pairwise Alignment Algorithms, applying the Needleman-Wunsch algorithm with a gap open penalty of 10.0, a gap extension penalty of 0.5 and Blosum62 as matrix.

Similarity (Identity)	CYP119A1	BMP	CYP152A1	CYP101A1
CYP119A1	-	32,8 (19,7)	35,4 (16,6)	36,9 (22,2)
BMP	32,8 (19,7)	-	32,4 (20,6)	34,3 (19,7)
CYP152A1	35,4 (16,6)	32,4 (20,6)	-	30,6 (18,7)
CYP101A1	36,9 (22,2)	34,3 (19,7)	30,6 (18,7)	-

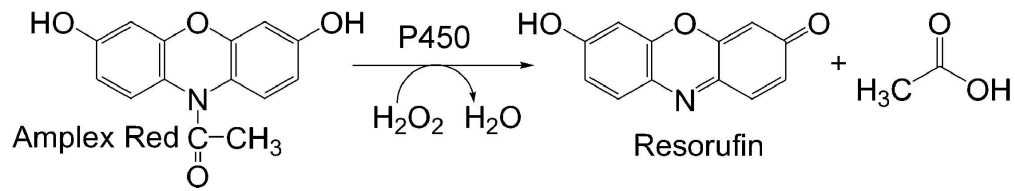


Figure 1. Conversion of Amplex Red to Resorufin by a P450 enzyme using hydrogen peroxide as the oxygen donor.

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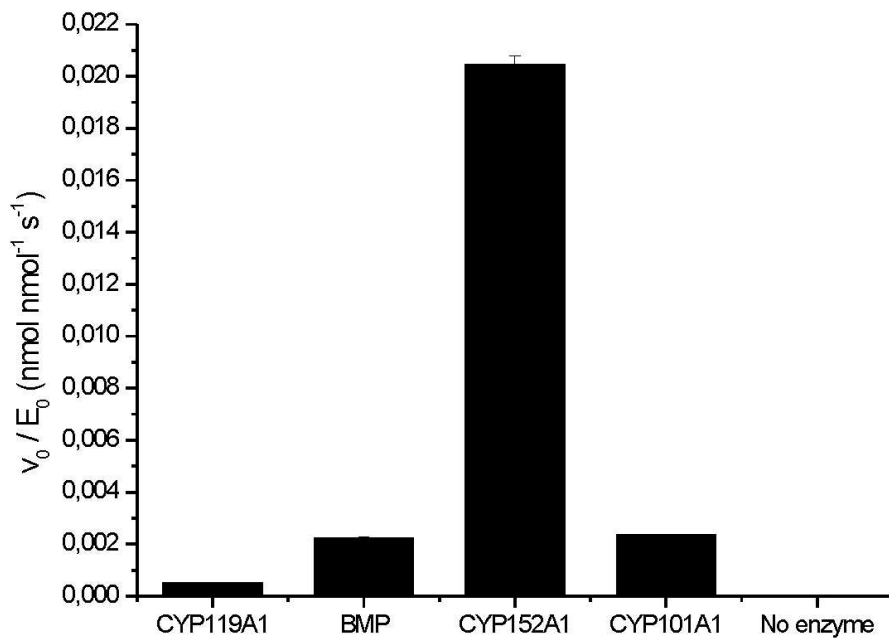


Figure 2. Initial rate of peroxidation of Amplex Red in the presence of CYP119A1, BMP, CYP152A1, CYP101A1. Error bars represent the standard error of three independent experiments.

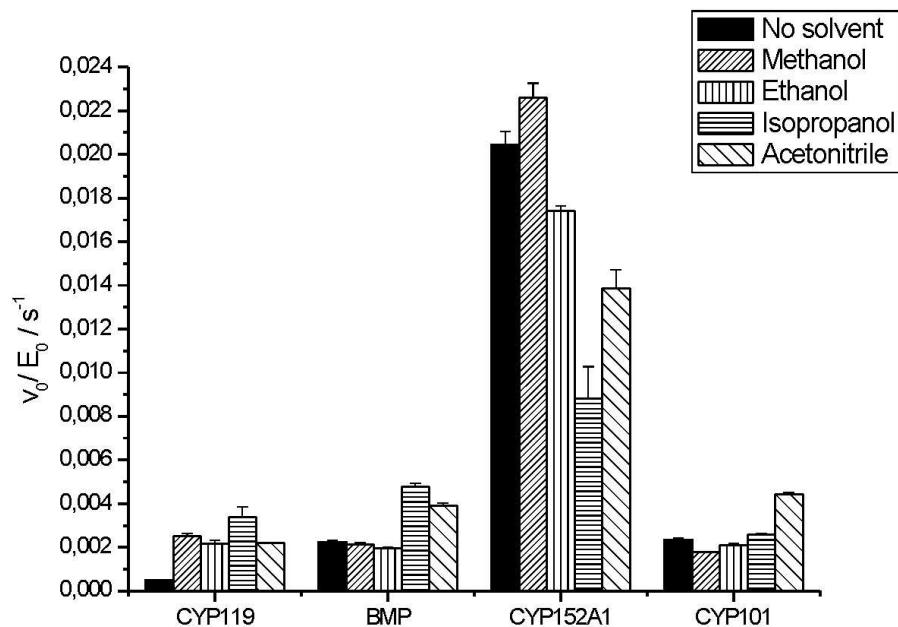


Figure 3. Effect of different cosolvents on the initial rate of peroxidation of Amplex Red in the presence of CYP119A1, BMP, CYP152A1, CYP101A1. Error bars represent the standard error of three independent experiments.

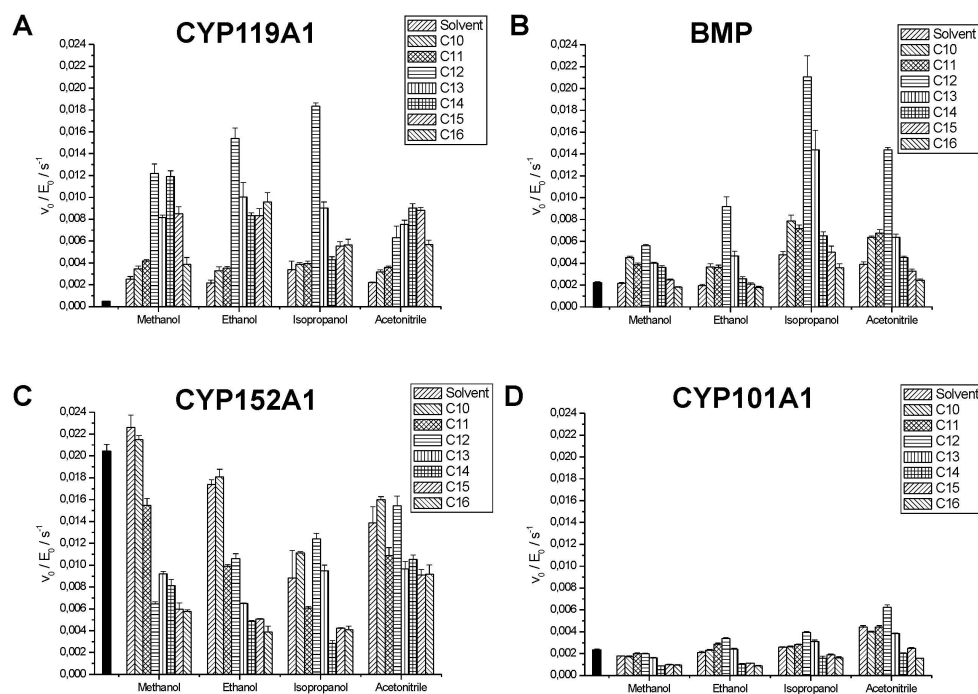


Figure 4. Titration of CYP119A1 (a), BMP (b), CYP152A1 (c) and CYP101A1 (d) with fatty acids of different chain length. Each reaction was performed with four different cosolvents. The first, black bar in each set represents v_0/E_0 for the conversion of Amplex Red, measured in the absence of cosolvent or fatty acid. Error bars represent the standard error of three independent experiments.

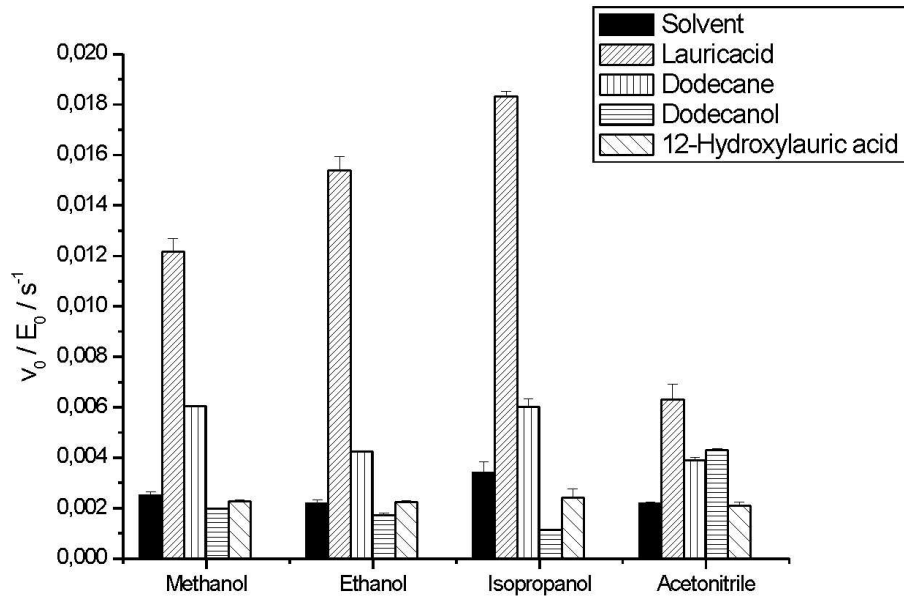


Figure 5. Effect of lauric acid, dodecane, dodecanol and 12-hydroxy lauric acid on the rate of Amplex Red conversion by CYP119A1 with different cosolvents. Error bars represent the standard error of three independent experiments.