

Biocatalytic Syntheses of Antiplatelet Metabolites

Subjects: **Medical Informatics**

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Antithrombotic thienopyridines, such as clopidogrel and prasugrel, are prodrugs that undergo a metabolic two-step bioactivation for their pharmacological efficacy. In the first step, a thiolactone is formed, which is then converted by cytochrome P450-dependent oxidation via sulfenic acids to the active thiol metabolites. These metabolites are the active compounds that inhibit the platelet P2Y₁₂ receptor and thereby prevent atherothrombotic events.

thienopyridine

clopidogrel

prasugrel

unspecific peroxxygenase

human drug metabolites

antiplatelet

1. Introduction

Clopidogrel (Plavix®, Iscover®) and prasugrel (Efient®) are antithrombotic prodrugs of the thienopyridine family that ignite their intrinsic effect after metabolic bioactivation and irreversibly inhibit the platelet P2Y₁₂ receptor resulting in prevention of atherothrombotic events ^{[1][2][3]}. The two-step enzymatic bioactivation required for both prodrugs differs in its initial reactions: In the case of clopidogrel, the hydroxylation of the thiophene ring, required for the spontaneous formation of the thiolactone metabolite, 2-oxo-clopidogrel (Scheme 1), is catalyzed by cytochrome P450 (CYP) monooxygenases ^{[4][5]}. In case of prasugrel, the corresponsive thiolactone (R-95913) is formed by hydrolysis of its ester functionality, which seems to be mainly catalyzed by human carboxylesterases hCE2^[6]. The final transformation of these thiolactone derivatives into the respective active thiol metabolites is formally a thioester hydrolysis, since the oxidation state remains unchanged. In fact, thioesterases such as paraoxonase-1 (PON-1) are thought to accomplish this kind of hydrolysis, but the reactions studied led to the biologically inactive endo-isomer, in which the double bond has migrated into the piperidine ring^{[7][8]}. Recent studies disclosed the formation of the active metabolite as a multistep biochemical process that also involves CYP enzymes, while the exact chemical nature of the intermediates is still under discussion. The most plausible metabolic process describes the formation of thiolactone sulfoxides during the oxidative activation, the hydrolytic opening of which leads to sulfenic acids that are efficiently reduced by ascorbate, phosphines, reductases or glutathione to give the active thiol metabolites (Scheme 1) ^{[9][10][11][12]}.

The bioactivation of clopidogrel to form 2-oxo-clopidogrel and the active thiol metabolite can be catalyzed by several CYP enzymes. Kinetic and inhibition studies, however, revealed that just CYP2C19 contributes substantially to both oxidative steps and that CYP3A4 is essential for the formation of the active metabolite. Furthermore, CYP1A2, CYP2B6 and CYP2C9 may be involved in the oxidative cascade^[5]. Several studies revealed that polymorphism in the gene encoding CYP2C19 contributes to the variability of platelet response to

clopidogrel in patients. Individuals who carry a reduced-function CYP2C19 allele had significantly lower levels of the active metabolite of clopidogrel, diminished platelet inhibition and a higher rate of major adverse cardiovascular events [13][14][15][16]. In addition, clopidogrel undergoes an extensive first-pass metabolism in the human liver where most of the prodrug is hydrolyzed to inactive clopidogrel carboxylic acid by carboxylesterase hCE1, followed by glucuronidation and renal excretion [16]. To address these causes for the low plasma level of active metabolites, prasugrel was developed replacing the ester functionality with a metabolically stable ketone and the introduction of an ester group in the thiophene 2-position that switched the first-step activation from CYP2C19 to carboxylesterase hCE2 [17].

In vitro metabolism studies showed that metabolic activation of clopidogrel results in a mixture of four stereoisomers (H1–H4, Figure 1). The H1 and H2 isomers are trans isomers, whereas the exocyclic double bond between C3 and C16 in H3 and H4 appears in cis configuration. Analysis of clinical samples demonstrated that only H3 and H4 are formed in vivo, with only H4 being active. Therefore, the configuration of the thiol group at C4 seems to be crucial for antiplatelet activity. The reactive free thiol group of the active metabolites leads to high instability in plasma samples, which is why they were stabilized by derivatization with 2-bromo-3'-methoxyacetophenone to be subsequently quantified by HPLC-MS [18][19]. For the unambiguous structural elucidation of metabolites H1–H4, syntheses for both the stabilized and free thiol metabolites have been described in the literature with yields below 3% [20][21]. Synthesis of active metabolites using human liver microsomes is hampered by low yield as well and by complicated purification protocols, and hence is not suitable for use on a preparative scale [18]. The active metabolite of prasugrel (PAM, R-138727) found in human plasma samples consists of four stereoisomers, (R, S)-, (R, R)-, (S, S)- and (S, R)-isomers (the first letter stands for the configuration of the thiol group and the second for that at the benzylic position), with 84% found as (R, S)- and (R, R)-, representing the most potent isomers. This underlines the importance of the R-configuration of the thiol group for bioactivity [22][23][24]. A chemical synthesis of PAM (R-138727) has not been reported so far.

Herein, researchers report biocatalytic syntheses of the bioactive metabolites of clopidogrel and prasugrel by unspecific peroxygenases (UPOs, EC 1.11.2.1) secreted by fungi. UPOs have arisen to 'dream catalysts' for oxyfunctionalization reactions, since they incorporate selectively oxygen into nonactivated hydrocarbons generating hydroxylations and epoxidations that are difficult to attain by chemical methods [25][26][28][27]. These extracellular glycosylated heme-thiolate proteins are activated by hydrogen peroxide and work independently of electron donors, transport proteins and additional cofactors. Their catalytic cycle combines characteristics of heme peroxidases and cytochrome P450s, which is why UPOs and P450 enzymes have partly overlapping reaction portfolios [28,29]. UPOs have already been used for the conversion of various pharmaceuticals, e.g., volixibat (N-dealkylation), cyclophosphamide (aliphatic hydroxylation), propranolol (aromatic hydroxylation), testosterone (epoxidation) and corticosteroids (C–C fission) [30,31,32,33,34]. In this context, it was found that, depending on the catalyzed reactions, the most relevant parameters in respect to reaction optimization were pH, H₂O₂ concentration and dosing rate as well as organic solvents and their content. The effective generation of authentic samples of drug metabolites is important for their structural confirmation and for LC-MS recovery, for evaluation of their potential safety risk, for investigation of drug–drug interactions, for pharmacological testing and for detailed pharmacokinetic and pharmacodynamic analysis [29][30][31].

2. Research

In order to elucidate the feasibility of the oxidation of antithrombotic thienopyridines with UPOs, the studies were first limited to clopidogrel as a substrate because of the two-step oxidation cascade. Incubation of clopidogrel with several UPOs was performed in the presence of an organic co-solvent in a phosphate-buffered system. The oxidant hydrogen peroxide was added intermittently over the reaction period. Ascorbate was added to prevent coupling reactions, which may occur due to the radical-forming peroxidase activity of UPOs [29]. Five homologically produced UPOs (wild-type enzymes) were tested: *Aae*UPO, *Cra*UPO, *Mro*UPO, *Cgl*UPO and *Mwe*UPO (entry 1–7, **Table 1**). With the two UPOs of the genus *Marasmius* (*Mro*UPO, *Mwe*UPO), the reaction was additionally carried out at pH 5.5 (entry 4 and 7, **Table 1**), as this pH corresponds to their reaction optimum [34,37].

Table 1. Screening of different UPOs for their ability to convert thienopyridines into 2-oxo metabolites and active metabolites ¹.

Entry	Substrate	Enzyme(s)	pH	2-Oxo Metabolite (%) ²	Active Metabolite (%) ²
1	CPG	<i>Aae</i> UPO	7.0	29.0 ± 0.5	<2
2	CPG	<i>Cra</i> UPO	7.0	12.7 ± 0.3	<2
3	CPG	<i>Mro</i> UPO	7.0	10.6 ± 0.2	8.6 ± 0.2
4	CPG	<i>Mro</i> UPO	5.5	46.6 ± 0.5	3.6 ± 0.2
5	CPG	<i>Cgl</i> UPO	7.0	3.7 ± 0.1	<2
6	CPG	<i>Mwe</i> UPO	7.0	8.1 ± 0.9	6.4 ± 0.1
7	CPG	<i>Mwe</i> UPO	5.5	31.6 ± 1.2	<2
8	PSG	PLE/ <i>Aae</i> UPO	7.0	87.4 ± 3.0	5.8 ± 0.6
9	PSG	PLE/ <i>Cra</i> UPO	7.0	87.2 ± 0.4	<2
10	PSG	PLE/ <i>Mro</i> UPO	7.0	39.1 ± 2.0	34.2 ± 0.4
11	PSG	PLE/ <i>Cgl</i> UPO	7.0	19.0 ± 0.5	28.2 ± 1.2
12	PSG	PLE/ <i>Mwe</i> UPO	7.0	46.5 ± 2.7	31.0 ± 0.3

HPLC-MS studies of the incubated samples revealed the incipient formation of two pairs of metabolites, each at the same ratio and, to some extent, of partially oxygenated byproducts. The dominant pair was identified as 2-oxo-clopidogrel, as could be deduced from the identical MS² spectra that were obtained for the parent ion (*m/z* = 338.0612, C₁₆H₁₇ClNO₃S⁺) with the associated fragments (*m/z* 183.0208 (100%) and *m/z* 155.0258 (87%), CE20) when using the appropriate reference standard. The second metabolite pair appears to be a stable dimer of

oxygenated clopidogrel, which was not further converted by UPOs (m/z 675.1150, $C_{32}H_{33}Cl_2N_2O_6S_2^+$). Experiments with an increased concentration of radical scavengers did not lead to decreased dimer formation (data not shown). The ratio of 2-oxo-CPG and oxo-CPG-dimer was approx. 2:1 in the case of *Mro*UPO and *Mwe*UPO. In contrast, the ratio obtained with *Aae*UPO was about 1:1 and with *Cra*UPO and *Cg*/UPO even 1:3. A third less dominant pair of metabolites exhibited molecular masses of m/z 356/358 in the isotopic ratio typical for the presence of a chlorine atom. Fragmentation of the parent ion (m/z 356.0712 (^{35}Cl), $C_{16}H_{19}ClNO_4S^+$, CE20) gave clear indication that these are two diastereomers of CAM (**Figure 2**). The highest yields under the conditions described above were obtained with the UPOs from *Marasmius* spp., i.e., *Mro*UPO (8.6%) and *Mwe*UPO (6.4%) at pH 7.0 (entries 3 and 6, **Table 1**). A representative chromatogram with the three metabolite pairs and the MS and MS² spectra of the dimer are given in the Supplementary Material ([Figures S1 and S2](#)).

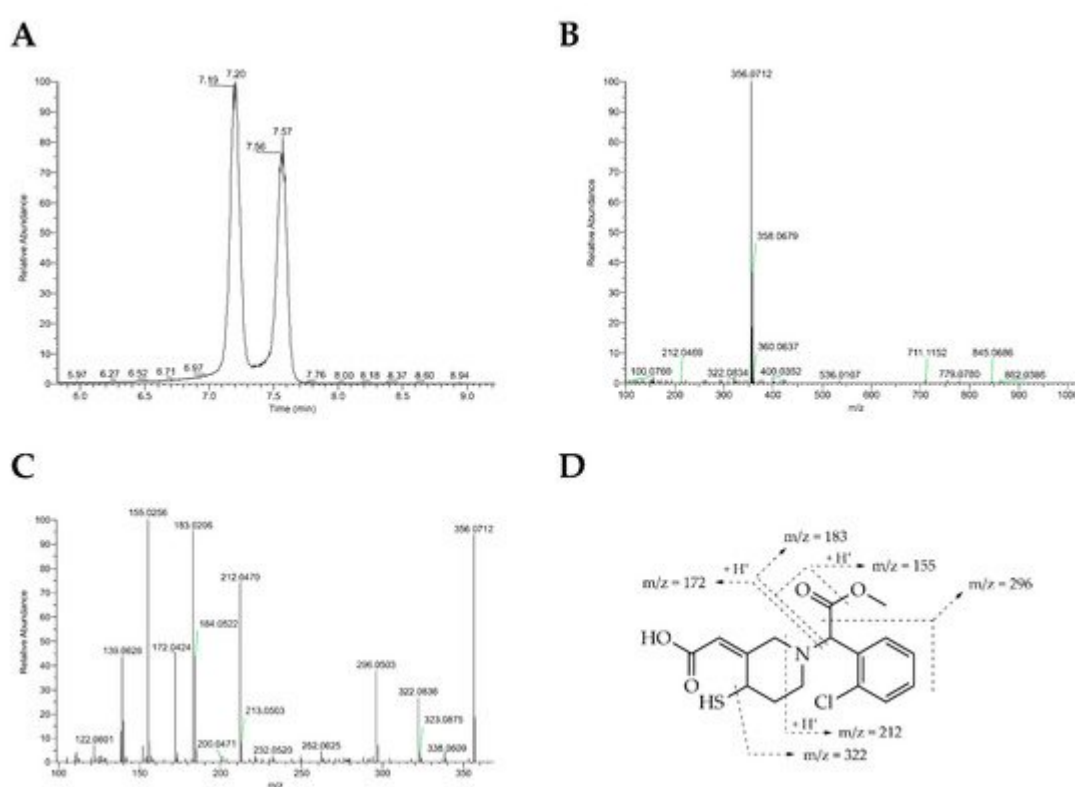


Figure 2. Extracted ion chromatogram and MS and MS² spectra of isomers of active clopidogrel metabolite (CAM) formed during conversion of 1 mM clopidogrel by 2 U mL⁻¹ *Mro*UPO in presence of 5 mM ascorbate, 20 mM KP_i buffer (pH 7.0) and in sum 4 mM hydrogen peroxide. **(A)** Extracted ion chromatogram at m/z 356 (MS); **(B)** MS spectra of the two isomers; **(C)** MS² spectra of the ion at m/z 356 with collision energy of CE20; **(D)** assignment for the fragmentation pattern in **Figure 2C**.

The conversion of prasugrel (PSG) requires hydrolysis of the acetic acid ester in the first step. In order to carry out the entire reaction sequence in one reaction batch, hydrolysis with porcine liver esterase (PLE) was investigated. For this purpose, 1 mM substrate was dissolved in 10% acetone in a phosphate-buffered system (pH 7.0) and incubated with 2 U mL⁻¹ PLE. PSG was completely converted within 30 min to 2-oxo-prasugrel, which was also identified as a double peak (ratio 1:1) of two diastereomers (m/z 332.1115, $C_{18}H_{19}FNO_2S^+$). Subsequently,

ascorbate, UPO and successively hydrogen peroxide was added over a period of 2 h. HPLC-MS analyses of incubated samples revealed that *Mro*UPO (34%, entry 10) and *Mwe*UPO (31%, entry 12) particularly catalyzed the formation of a pair of metabolites, each with m/z 350 (pair of diastereomers in ratio 1:1). Again, MS (m/z 350.1219, $C_{18}H_{21}FNO_3S^+$) and MS² data (from m/z 350.1219, CE25) gave clear hints for the formation of the active prasugrel metabolite PAM (**Figure 3**). Incubation with *Cg*/UPO also resulted in a high yield of PAM, but numerous byproducts emerged (entry 11, **Table 1**).

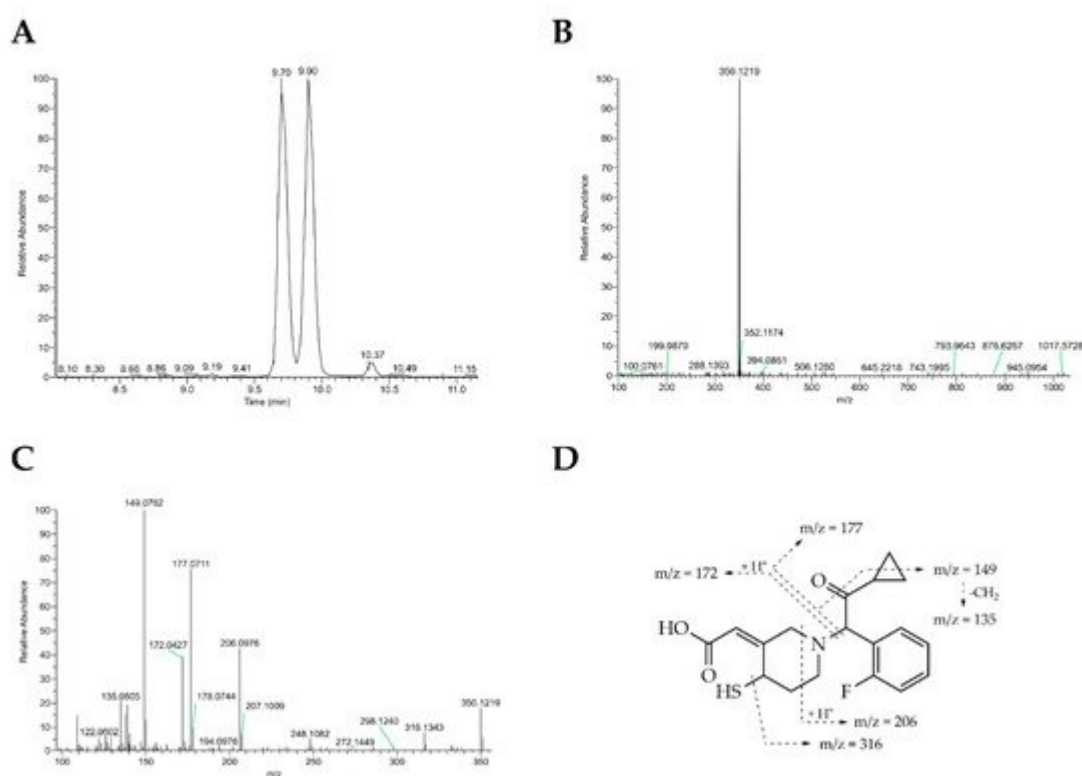


Figure 3. Extracted ion chromatogram and MS and MS² spectra of isomers of active prasugrel metabolite (PAM) formed during conversion of 1 mM prasugrel by 2 U mL⁻¹ PLE for 30 min followed by conversion with 2 U mL⁻¹ *Mro*UPO in the presence of 5 mM ascorbate, 20 mM KPi buffer (pH 7.0) and in sum 4 mM hydrogen peroxide. (A) Extracted ion chromatogram at m/z 350 (MS); (B) MS spectra of the two isomers; (C) MS² spectra of the ion at m/z 350 with collision energy of CE25; (D) assignment for the fragmentation pattern in (C).

The diastereomeric pairs are likely due to the configuration of the thiol group at C4 or the *cis/trans* configuration at C3. To obtain precise insights into the chemical nature of the products, CAM and PAM (presumably identical to human metabolites), NMR studies were performed. For this, the reactions had to be optimized and upscaled in order to isolate the metabolites.

With the aim of optimizing the reaction conditions and increasing the yield of active metabolites, especially in the case of clopidogrel, both reductant and organic solvents as well as their concentration were varied in the approach with *Mro*UPO (**Table 2**). It turned out that the presence of a reductant was particularly necessary. The absence of ascorbate or glutathione resulted in a variety of oxidation and coupling products, with only small amounts of the desired compounds being detected with LC-MS. When glutathione replaced ascorbate, the formation of CAM was

significantly reduced (entry 14, **Table 2**), and a mixture of four isomers with the appropriate molecular mass of m/z 356/358 was identified in traces. Replacing acetone with other water-miscible solvents such as acetonitrile, methanol, dimethyl sulfoxide or dimethyl formamide did not increase the yields of the active metabolite. Interestingly, the reaction with methanol as co-solvent gave a good yield of 2-oxo-CPG (31%) but almost no formation of the active metabolite. Increasing the amount of acetone to 20% increased the CAM yield to about 10%, which was the highest yield that could be achieved (entry 21, **Table 2**). Further extensive attempts such as exchanging buffer systems, varying the substrate–catalyst ratio or the amount and dosing rate of the co-substrate hydrogen peroxide as well as the use of other hydroperoxides or hydrogen peroxide producing systems (e.g., glucose/glucose oxidase, carbamide peroxide) did not significantly improve the yields (data not shown).

Table 2. Optimization of reaction conditions using different solvents and reductants for the conversion of clopidogrel (CPG) by *MroUPO* ¹.

Entry	Substrate	Solvent	Reductant	2-Oxo Metabolite (%) ²	Active Metabolite (%) ²
13 (3)	CPG	Buffer/acetone 90:10	Ascorbate	10.6 ± 0.2	8.6 ± 0.2
14	CPG	Buffer/acetone 90:10	Glutathione	26.2 ± 1.9	<2
15	CPG	Buffer/acetone 90:10	None	<2	<2
16	CPG	Buffer/MeCN ³ 90:10	Ascorbate	3.2 ± 0.1	4.0 ± 0.1
17	CPG	Buffer/MeOH ³ 90:10	Ascorbate	31.1 ± 0.3	<2
18	CPG	Buffer/DMSO ³ 90:10	Ascorbate	16.9 ± 1.8	<2
19	CPG	Buffer/DMF ³ 90:10	Ascorbate	25.5 ± 0.5	4.5 ± 0.1
20	CPG	Buffer	Ascorbate	11.5 ± 0.2	<2
21	CPG	Buffer/acetone 80:20	Ascorbate	8.7 ± 0.6	9.8 ± 0.5
22	CPG	Buffer/acetone 60:40	Ascorbate	6.3 ± 0.4	<2

The optimal reaction conditions (entry 21, **Table 2**) were chosen to perform the syntheses of CAM and PAM at a 100 mg scale. The hydrogen peroxide feed was changed from intermittent addition to continuous feed by a syringe pump to ensure a permanent low hydrogen peroxide concentration and thus an optimal UPO performance. During the reaction, the concentrations of substrate, 2-oxo metabolite and active metabolite as well as the residual activity of the catalyst were monitored. Clopidogrel was almost completely converted within one hour, with the formation of 2-oxo-CPG predominantly at the beginning and, to a somewhat minor extent, the previously mentioned dimerization product. After about 45 min, the concentration of 2-oxo-CPG reached a maximum of about 500 μM and declined afterwards as the thiolactone was further converted mostly to the active metabolite (**Figure 4A**). Here, an isomer of 2-oxo-CPG was preferentially transformed by *MroUPO* (**Figure S1B**). After 2 h, the residual activity of *MroUPO* was below 5%, and 2-oxo-CPG was almost completely converted. The final concentration of active metabolite (pair of stereoisomers) was about 250 μM , and both isomers were present in equal amounts. The metabolite pair was successfully isolated and purified by preparative HPLC. NMR spectra were difficult to interpret because of the presence of isomers. Therefore, spin–spin coupling constants could not be determined in some cases due to the overlapping of the signals of both compounds. The ratio of isomers in the isolate was fortunately about 2:3 so that the individual signals could be assigned to the metabolites with the use of the correlation spectra (COSY, HMBC, HSQC). The singlets of the ^1H NMR spectra at 5.56 ppm (60%) and 5.45 (40%) were allocated to H-16 and confirmed the *cis* configuration at C3–C16 (CAM-H3 and CAM-H4), which were in agreement with those in the literature [20]. The metabolite, which was initially formed and preferably by *MroUPO*, eluted earlier from the HPLC column and was identified as CAM-H3. In principle, the isolation of 2-oxo-CPG would also be possible by a kinetically controlled approach. However, the isolation of the metabolites will be challenging due to the presence of the oxo-CPG dimer.

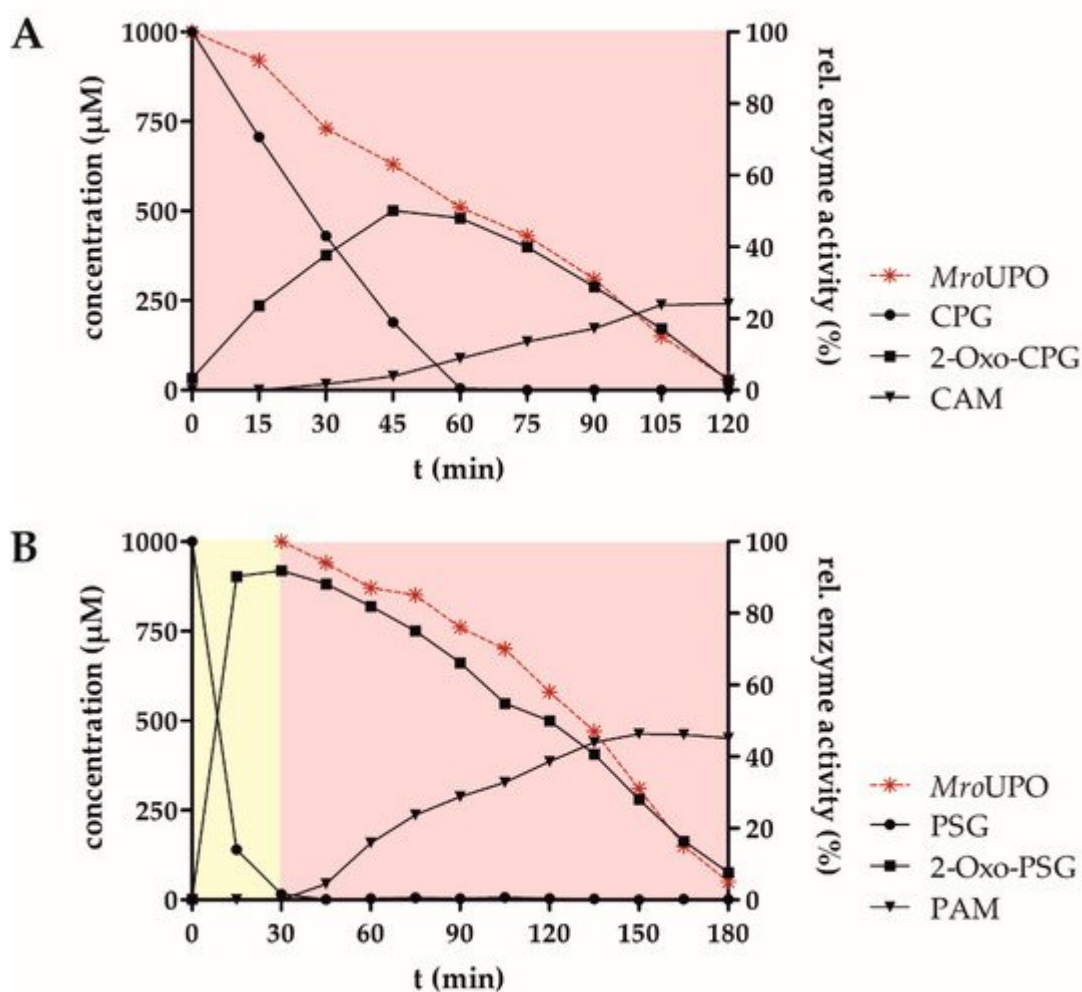


Figure 4. Kinetics of clopidogrel (**A**) and prasugrel (**B**) conversion (0.2 mmol each) by 2 U mL^{-1} *MroUPO* in presence of 5 mM ascorbate, 20 mM KPi buffer ($\text{pH } 7.0$) and continuous supply of hydrogen peroxide (2 mM h^{-1}). Prasugrel was preincubated with 2 U mL^{-1} PLE for 30 min . Black circles are the thienopyridine substrates; black squares represent the 2-oxo metabolites and black triangles are the active metabolites. Isomeric double peaks were merged. The red stars reflect the residual UPO activity. Yellow background represents the PLE reaction; red background indicates the UPO reaction. Ordinates are the concentrations of the metabolites recalculated after calibration with isolated compounds.

Incubation of 150 mg prasugrel with PLE at 25°C and $\text{pH } 7$ resulted in complete conversion after 60 min as verified by TLC. MS and MS^2 data confirmed the exclusive formation of 2-oxo-clopidogrel. For structural confirmation, the metabolite pair was isolated by preparative HPLC. In a second batch, 75 mg prasugrel was incubated analogously, with a complete turnover after 30 min as analyzed by TLC and HPLC. After subsequent addition of *MroUPO*, reductant and feeding of hydrogen peroxide, 2-oxo-prasugrel was continuously converted in an almost linear way to the stereoisomer pair PAM (**Figure 4B**). In this case, no preferential conversion of an isomer by UPO was observed. After 180 min total reaction time, the yield of PAM was about 44% . NMR analyses confirmed the formation of the active metabolites in *cis* configuration analogously to CAM. HPLC-ELSD elution profiles, ^1H and ^{13}C NMR spectra as well as two-dimensional correlation spectra (COSY, HMBC, HSQC) of isolated CAM, PAM and 2-oxo-PSG as well as the assignment of the signals are given in [Supplementary Material](#).

3. Conclusions

It has been demonstrated a biocatalytic method for the syntheses of the active metabolites CAM (25% yield) and PAM (44% yield) starting from the parental drugs clopidogrel and prasugrel by using fungal unspecific peroxygenases (UPOs). Both metabolites were isolated and their structure elucidated by mass spectrometry and NMR spectroscopy. This provides easy access to these compounds to be used, e.g., in pharmacological assays or as reference material. Moreover, this UPO-based reaction sequence, starting with the formation of a thiolactone and followed by the cleavage of the thieno ring via a thiolactone sulfoxide intermediate, has been described for the first time and can be adapted to other chemical problems. In general, UPOs could contribute to the elucidation of biotransformation mechanisms catalyzed by hepatic cytochrome P450 enzymes, as there are numerous examples of mimicking intrinsic P450 activity. On the other hand, UPOs can become valuable catalysts for the synthesis of active metabolites of prodrugs for those cases where polymorphism or other deficiencies do not allow a targeted in vivo transformation and personalized therapeutic solutions are required.

References

1. Jarvis, B.; Simpson, K. Clopidogrel. *Drugs* 2000, 60, 347–377. [Google Scholar] [CrossRef]
2. Wiviott, S.D.; Braunwald, E.; McCabe, C.H.; Montalescot, G.; Ruzyllo, W.; Gottlieb, S.; Neumann, F.-J.; Ardissino, D.; De Servi, S.; Murphy, S. Prasugrel Versus Clopidogrel in Patients with Acute Coronary Syndromes. *N. Engl. J. Med.* 2007, 357, 2001–2015. [Google Scholar] [CrossRef]
3. Farid, N.A.; Kurihara, A.; Wrighton, S.A. Metabolism and Disposition of the Thienopyridine Antiplatelet Drugs Ticlopidine, Clopidogrel, and Prasugrel in Humans. *J. Clin. Pharmacol.* 2010, 50, 126–142. [Google Scholar] [CrossRef]
4. Pereillo, J.-M.; Maftouh, M.; Andrieu, A.; Uzabiaga, M.-F.; Fedeli, O.; Savi, P.; Pascal, M.; Herbert, J.-M.; Maffrand, J.-P.; Picard, C. Structure and Stereochemistry of the Active Metabolite of Clopidogrel. *Drug Metab. Dispos.* 2002, 30, 1288–1295. [Google Scholar] [CrossRef]
5. Kazui, M.; Nishiya, Y.; Ishizuka, T.; Hagihara, K.; Farid, N.A.; Okazaki, O.; Ikeda, T.; Kurihara, A. Identification of the Human Cytochrome P450 Enzymes Involved in the Two Oxidative Steps in the Bioactivation of Clopidogrel to Its Pharmacologically Active Metabolite. *Drug Metab. Dispos.* 2010, 38, 92–99. [Google Scholar] [CrossRef]
6. Williams, E.T.; Jones, K.O.; Ponsler, G.D.; Lowery, S.M.; Perkins, E.J.; Wrighton, S.A.; Ruterbories, K.J.; Kazui, M.; Farid, N.A. The Biotransformation of Prasugrel, a New Thienopyridine Prodrug, by the Human Carboxylesterases 1 and 2. *Drug Metab. Dispos.* 2008, 36, 1227–1232. [Google Scholar] [CrossRef]
7. Dansette, P.M.; Rosi, J.; Bertho, G.; Mansuy, D. Paraoxonase-1 and Clopidogrel Efficacy. *Nat. Med.* 2011, 17, 1040–1041. [Google Scholar] [CrossRef] [PubMed]

8. Dansette, P.M.; Rosi, J.; Bertho, G.; Mansuy, D. Cytochromes P450 Catalyze Both Steps of the Major Pathway of Clopidogrel Bioactivation, Whereas Paraoxonase Catalyzes the Formation of a Minor Thiol Metabolite Isomer. *Chem. Res. Toxicol.* 2012, 25, 348–356. [Google Scholar] [CrossRef] [PubMed]
9. Dansette, P.M.; Libraire, J.; Bertho, G.; Mansuy, D. Metabolic Oxidative Cleavage of Thioesters: Evidence for the Formation of Sulfenic Acid Intermediates in the Bioactivation of the Antithrombotic Prodrugs Ticlopidine and Clopidogrel. *Chem. Res. Toxicol.* 2009, 22, 369–373. [Google Scholar] [CrossRef]
10. Dansette, P.M.; Thébault, S.; Bertho, G.; Mansuy, D. Formation and Fate of a Sulfenic Acid Intermediate in the Metabolic Activation of the Antithrombotic Prodrug Prasugrel. *Chem. Res. Toxicol.* 2010, 23, 1268–1274. [Google Scholar] [CrossRef]
11. Hagihara, K.; Kazui, M.; Kurihara, A.; Iwabuchi, H.; Ishikawa, M.; Kobayashi, H.; Tanaka, N.; Okazaki, O.; Farid, N.A.; Ikeda, T. Biotransformation of Prasugrel, a Novel Thienopyridine Antiplatelet Agent, to the Pharmacologically Active Metabolite. *Drug Metab. Dispos.* 2010, 38, 898–904. [Google Scholar] [CrossRef]
12. Dansette, P.M.; Levent, D.; Hessani, A.; Bertho, G.; Mansuy, D. Thiolactone Sulfoxides as New Reactive Metabolites Acting as Bis-Electrophiles: Implication in Clopidogrel and Prasugrel Bioactivation. *Chem. Res. Toxicol.* 2013, 26, 794–802. [Google Scholar] [CrossRef]
13. Mega, J.L.; Close, S.L.; Wiviott, S.D.; Shen, L.; Hockett, R.D.; Brandt, J.T.; Walker, J.R.; Antman, E.M.; Macias, W.; Braunwald, E. Cytochrome P-450 Polymorphisms and Response to Clopidogrel. *N. Engl. J. Med.* 2009, 360, 354–362. [Google Scholar] [CrossRef]
14. Shuldiner, A.R.; O'Connell, J.R.; Bliden, K.P.; Gandhi, A.; Ryan, K.; Horenstein, R.B.; Damcott, C.M.; Pakyz, R.; Tantry, U.; Gibson, Q. Association of Cytochrome P450 2c19 Genotype with the Antiplatelet Effect and Clinical Efficacy of Clopidogrel Therapy. *JAMA* 2009, 302, 849–857. [Google Scholar] [CrossRef]
15. Gong, I.Y.; Crown, N.; Suen, C.M.; Schwarz, U.I.; Dresser, G.K.; Knauer, M.J.; Sugiyama, D.; DeGorter, M.K.; Woolsey, S.; Tirona, R.G. Clarifying the Importance of Cyp2c19 and Pon1 in the Mechanism of Clopidogrel Bioactivation and in Vivo Antiplatelet Response. *Eur. Heart J.* 2012, 33, 2856–2864. [Google Scholar] [CrossRef] [PubMed]
16. Zhu, H.-J.; Wang, X.; Gawronski, B.E.; Brinda, B.J.; Angiolillo, D.J.; Markowitz, J.S. Carboxylesterase 1 as a Determinant of Clopidogrel Metabolism and Activation. *J. Pharmacol. Exp. Ther.* 2013, 344, 665–672. [Google Scholar] [CrossRef] [PubMed]
17. Zetterberg, F.; Svensson, P. State of Affairs: Design and Structure–Activity Relationships of Reversible P2y12 Receptor Antagonists. *Bioorg. Med. Chem. Lett.* 2016, 26, 2739–2754. [Google Scholar] [CrossRef]

18. Tuffal, G.; Roy, S.; Lavis, M.; Brasseur, D.; Schofield, J.; Touchard, N.D.; Savi, P.; Bremond, N.; Rouchon, M.-C.; Hurbin, F. An Improved Method for Specific and Quantitative Determination of the Clopidogrel Active Metabolite Isomers in Human Plasma. *Thromb. Haemost.* 2011, 105, 696–705. [Google Scholar] [PubMed]
19. Karażniewicz-Łada, M.; Danielak, D.; Teżyk, A.; Żaba, C.; Tuffal, G.; Głowska, F. HPLC–MS/MS Method for the Simultaneous Determination of Clopidogrel, Its Carboxylic Acid Metabolite and Derivatized Isomers of Thiol Metabolite in Clinical Samples. *J. Chromatogr. B* 2012, 911, 105–112. [Google Scholar] [CrossRef]
20. Shaw, S.A.; Balasubramanian, B.; Bonacorsi, S.; Cortes, J.C.; Cao, K.; Chen, B.-C.; Dai, J.; Decicco, C.; Goswami, A.; Guo, Z. Synthesis of Biologically Active Piperidine Metabolites of Clopidogrel: Determination of Structure and Analyte Development. *J. Org. Chem.* 2015, 80, 7019–7032. [Google Scholar] [CrossRef]
21. Bluet, G.; Blankenstein, J.; Brohan, E.; Prevost, C.; Cheve, M.; Schofield, J.; Roy, S. Synthesis of the Stabilized Active Metabolite of Clopidogrel. *Tetrahedron* 2014, 70, 3893–3900. [Google Scholar] [CrossRef]
22. Wickremsinhe, E.R.; Tian, Y.; Ruterbories, K.J.; Verburg, E.M.; Weerakkody, G.J.; Kurihara, A.; Farid, N.A. Stereoselective Metabolism of Prasugrel in Humans Using a Novel Chiral Liquid Chromatography-Tandem Mass Spectrometry Method. *Drug Metab. Dispos.* 2007, 35, 917–921. [Google Scholar] [CrossRef]
23. Hasegawa, M.; Sugidachi, A.; Ogawa, T.; Isobe, T.; Jakubowski, J.A.; Asai, F. Stereoselective Inhibition of Human Platelet Aggregation by R-138727, the Active Metabolite of Cs-747 (Prasugrel, Ly640315), a Novel P2y₁₂ Receptor Inhibitor. *Thromb. Haemost.* 2005, 94, 593–598. [Google Scholar] [CrossRef] [PubMed]
24. Farid, N.A.; Smith, R.L.; Gillespie, T.A.; Rash, T.J.; Blair, P.E.; Kurihara, A.; Goldberg, M.J. The Disposition of Prasugrel, a Novel Thienopyridine, in Humans. *Drug Metab. Dispos.* 2007, 35, 1096–1104. [Google Scholar] [CrossRef] [PubMed]
25. Sigmund, M.-C.; Poelarends, G.J. Current State and Future Perspectives of Engineered and Artificial Peroxygenases for the Oxyfunctionalization of Organic Molecules. *Nat. Catal.* 2020, 3, 690–702. [Google Scholar] [CrossRef]
26. Kiebitz, J.; Hofrichter, M.; Zuhse, R.; Scheibner, K. Oxyfunctionalization of Pharmaceuticals by Fungal Peroxygenases. In *Pharmaceutical Biocatalysis*; Jenny Stanford Publishing: Singapore, 2019; Volume 5, pp. 643–680. [Google Scholar]
27. Hobisch, M.; Holtmann, D.; Gomez de Santos, P.; Alcalde, M.; Hollmann, F.; Kara, S. Recent Developments in the Use of Peroxygenases—Exploring Their High Potential in Selective Oxyfunctionalisations. *Biotechnol. Adv.* 2020, 51, 107615. [Google Scholar] [CrossRef]

28. Hofrichter, M.; Kellner, H.; Herzog, R.; Karich, A.; Liers, C.; Scheibner, K.; Kimani, V.W.; Ullrich, R. Fungal Peroxygenases: A Phylogenetically Old Superfamily of Heme Enzymes with Promiscuity for Oxygen Transfer Reactions. In *Grand Challenges in Fungal Biotechnology*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 369–403. [Google Scholar]
29. Hofrichter, M.; Kellner, H.; Pecyna, M.J.; Ullrich, R. Fungal Unspecific Peroxygenases: Heme-Thiolate Proteins That Combine Peroxidase and Cytochrome P450 Properties. *Adv. Exp. Med. Biol.* 2015, 851, 341–368. [Google Scholar]
30. Kiebist, J.; Holla, W.; Heidrich, J.; Poraj-Kobielska, M.; Sandvoss, M.; Gröbe, G.; Atzrodt, J.; Hofrichter, M.; Scheibner, K. One-Pot Synthesis of Human Metabolites of SAR548304 by Fungal Peroxygenases. *Bioorg. Med. Chem.* 2015, 23, 4324–4332. [Google Scholar] [CrossRef]
31. Steinbrecht, S.; Kiebist, J.; König, R.; Thiessen, M.; Schmidtke, K.-U.; Kammerer, S.; Küpper, J.-H.; Scheibner, K. Synthesis of Cyclophosphamide Metabolites by a Peroxygenase from *Marasmius Rotula* for Toxicological Studies on Human Cancer Cells. *AMB Express* 2020, 10, 1–13. [Google Scholar] [CrossRef] [PubMed]

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