ChemPubSoc Europe

DOI: 10.1002/cctc.201501244

CHEMCATCHEM Full Papers

α-Hydroxylation of Carboxylic Acids Catalyzed by Taurine Dioxygenase

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Enzymes still have a limited application scope in synthetic organic chemistry. To expand this, different strategies exist that range from the de novo design of enzymes to the exploitation of the catalytic capabilities of known enzymes by converting different substrates; denoted as substrate promiscuity. We harnessed the synthetic potential offered by the taurine dioxygenase (TauD) from *Escherichia coli* (*E. coli*) by studying its promiscuous catalytic properties in the hydroxylation of carboxylic acid substrates. TauD showed high selectivities in the hydroxylation reaction but reduced levels of activity (26% conversion, >96% *ee*). We enhanced the enzyme substrate scope and improved the conversions for the tested substrates by introducing a point mutation at position 206 (F206Y). The conversions of the improved catalyst increased by at least 140% compared to that of the wild-type enzyme. The number of carboxylic acids that accepted by the enzyme variant doubled from four to eight carboxylic acids.

Introduction

The taurine dioxygenase (TauD) from *Escherichia coli* (*E. coli*) belongs to the class of α-ketoglutarate (α-KG)-dependent enzymes that are known to catalyze a plethora of reactions.[1] Members of this family are involved in DNA repair mechanisms, modifications of amino acids, and the degradation of xenobiotics.[1] TauD is part of the sulfur-starvation response of *E. coli* and, therefore, part of the *tauABCD* gene cluster that is involved in the utilization of aliphatic sulfur compounds such as the natural substrate of TauD, taurine (**1**).[2,3] The mobilization of the sulfonate from the organic compound is performed by an intermediate hydroxylation reaction of the substrate, which leads to an unstable α-hydroxy sulfonate compound (Figure 1) that decomposes spontaneously. This decomposition liberates the sulfite as the sulfur source. Eichhorn et al. have shown that the substrate scope of TauD extends from the natural substrate **1** to longer-chained and sterically more demanding sulfonic acid substrates that include *N*-morpholine propanesulfonic acid (**24**).[4] Furthermore, TauD has been described to accept aliphatic analogues of **1**, which include butanesulfonic acid (**25**).[4] The crystal-structure solution and different mechanistic investigations have helped to clarify the reaction mechanism of this hydroxylation reaction.[5–9] Important residues for substrate anchoring and positioning in the active site were also identified and include the residues H70, F159, F206, and R270.[5,10,11] These studies have also revealed that the hydroxylation of TauD is performed by an intermediate iron(IV) oxo species similar to that known for heme-type oxygenases.[9]

We speculated that the oxidative potential of TauD could also be used for the α-hydroxylation of carboxylic acids by the insertion of oxygen into a C–H bond adjacent to a carboxylic acid functional group. This type of reaction has already been reported for long-chain fatty acids using cytochrome P450 monooxygenases and enzymes involved in the fatty acid metabolism of different plants.[12–16] However, these enzymatic routes are restricted in their application scope to longer-chained fatty acid or arylic carboxylic acid substrates.[12–16] Classical synthetic routes to enantiomerically enriched α-hydroxy carboxylic acids involve the diazotation of α-amino acids, the stereoselective reduction of α-keto acids, the addition of nitriles to aldehydes with the concomitant hydrolysis of the nitrile, or the deracemization of racemic α-hydroxy acids.[17–22] Recently, Kroutil and co-workers established an one-pot bienzymatic approach for the production of enantioenriched α-hydroxy carboxylic acids with aliphatic or arylic side chains.[18] Furthermore, the groups of Schofield and Zaparucha have described the hydroxylations of amino-substituted carboxylic acid substrates by using related dioxygenases. Schofield and coworkers demonstrated that the α-KG-dependent γ-butyrobetaine hydroxylase (BBOX) could be applied in the desymmetrization of achiral *N*,*N*′-dialkyl piperidine-4-carboxylates to the corresponding hydroxylation products to show the versatility of these enzymes to introduce chiral centers.[23] The group of Zaparucha also discovered alternative members of this enzyme family with altered regioselectivities in a genome-mining approach.[24] These current findings in combination with our work indicate the not yet fully explored potential of this class of enzymes as catalysts for synthetic tasks.[23,24]

We studied the TauD-catalyzed enantioselective α-hydroxylation of short-to-medium-chain-length ω-amino carboxylic

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Supporting information for this article can be found under http://dx.doi.org/10.1002/cctc.201501244.

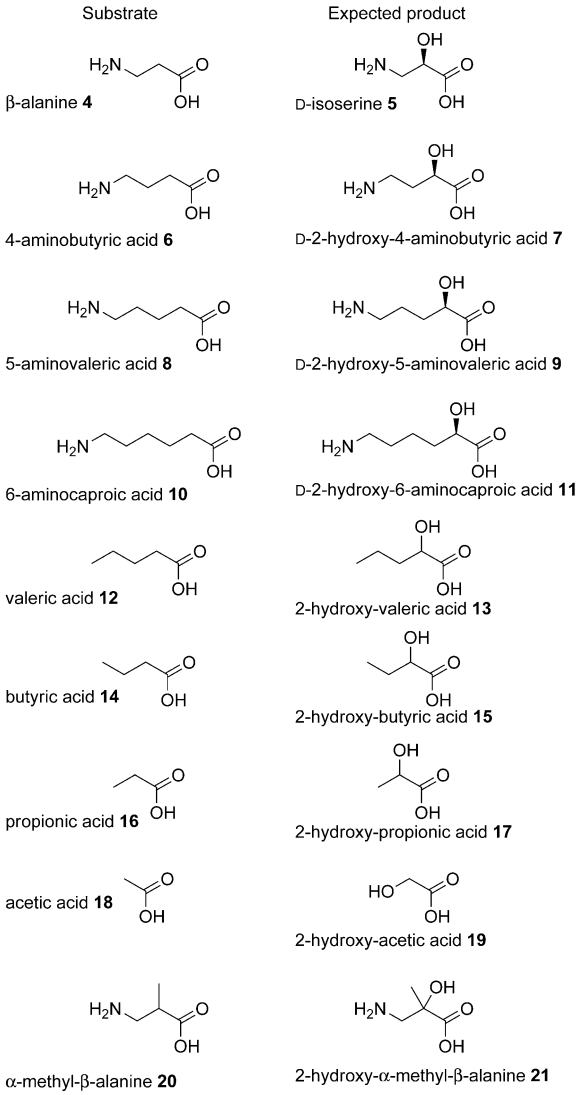


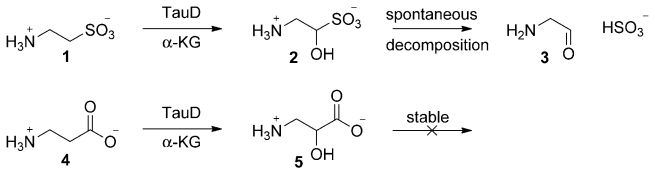
Figure 1. Substrates and expected products of the TauD-catalyzed α-hydroxylation reaction.

acids. These 2-hydroxy-ω-amino carboxylic acids are highly valuable products for different glycoside antibiotics, which include amikacin or arbekacin, and antibiotic peptides such as edeines.[25–27] We created mutants to elucidate the binding mode of the new substrates and to improve the catalytic properties of this promiscuous hydroxylation reaction.

Results and Discussion

Reactivity of TauD from *E. coli*

Many studies were performed to determine the kinetics and the mechanism of the TauD from *E. coli* with its natural substrate **1** and analogues thereof.[3,5–10] However, to date, the catalytic potential of TauD for the introduction of hydroxy functionalities to chemically more distinct but structurally related carboxylic acid substrates has not been exploited. As the TauD is closely related to the 2,4-dichlorophenoxyacetic acid dioxygenase (TfdA) from *Ralstonia eutropha* that catalyzes the hydroxylation of the carboxylic acid substrate 2,4-dichlorophenoxyacetic acid, we assumed that TauD could catalyze the α-hydroxylation of carboxylic acid substrates such as β-alanine (**4**; Scheme 1 and Figure 1).[5,28]



Scheme 1. α-Hydroxylations of **1** and **4**.

Thus, we performed an initial activity test with TauD and **1** as well as the carboxylic acid substrate **4**. We used the assay developed by Eichhorn et al.[4] to determine a specific activity of $(1.47 \pm 0.04)\ U\,mg^{-1}$ for **1** for our enzyme preparation, which is similar to the activity reported in literature.[4] We were pleased to find that TauD converts **4** to D-isoserine (**5**) selectively with an overall conversion of $(22.6 \pm 0.7)\%$ (>96% *ee*, Table 1). Encouraged by these preliminary findings, we ana-

Table 1. Conversions and selectivities of non-natural substrates **4**–**22** with wild-type (WT) TauD and mutant F206Y. The *ee* values are given for the D enantiomer.

Substrate[a]	Conversion [%]		Enantiomeric excess [%]	
	WT	F206Y	WT	F206Y
4	22.6 ± 0.7	54.2 ± 3.8	>96[a]	>96[a]
6	8.1 ± 1.3	37.7 ± 4.9	10 ± 1	>96[a]
8	12.1 ± 0.2	53.5 ± 1.1	>96[a]	>96[a]
10	<3	28.1 ± 2.8	n.d.	>96[a]
12	–	5.2 ± 0.6	–	n.d.
14	–	4.1 ± 0.3	–	n.d.
16	–	3.6 ± 0.2	–	n.d.
18	–	–	–	–
20[b,c]	–	7.1 ± 0.9	–	>96[b]

[a] The other enantiomer was not detected. [b] Conversion determined by substrate consumption. No reference material was available for product quantification. The stereochemistry of the product could not be confirmed. Enantiomeric excesses were calculated from the consumption of the substrate enantiomers as only one enantiomer was consumed in the course of the reaction. n.d.: not determined. [c] The proposed position for hydroxylation of substrate 20 is derived from the position of hydroxylation of substrates 4–18.

lyzed TauD systematically for the conversion of further substrates similar to **4**. We tested a set of substrates (Figure 1) that included longer-chained homologues of **4** such as 4-aminobutyric acid (**6**) and 5-aminovaleric acid (**8**). In addition, we tested valeric acid (**12**) and homologues thereof as well as α-methyl-β-alanine (**20**). These analogues of **4** lack the terminal amino functionality or possess a substitution on the position adjacent to the carboxylic group (Figure 1). The substrate scope of wild-type TauD is distinctly limited to **4** and its higher homologues that range from ω-amino carboxylic acid sub-

strates of chain lengths C_3–C_6. The results of this screening are summarized in Table 1. We found that substrates with even-numbered chain lengths showed significantly lower conversions than the substrates with odd-numbered chain lengths. Aliphatic carboxylic acids of chain lengths C_2–C_5 (**14**–**20**) and the substituted β-alanine analogue α-methyl-β-alanine (**22**) did not show any detectable conversion. Biotransformations were further subjected to chiral analyses. The enzyme could convert nearly all accepted substrates almost exclusively to the D enantiomeric product. Compound **6** was the only substrate to yield a hydroxylation product with a poor enantiomeric excess of only 10% *ee* (Table 1).

Binding mode of β-alanine to TauD

Previous work by Elkins and co-workers revealed that the residues that form the substrate binding motif are partially conserved within a defined group of members of the α-KG-dependent dioxygenases.[5] An arginine at position 270 (R270) found within different members of this family is considered to be an ion-pairing residue to the natural substrate. However, the histidine at position 70 (H70) of TauD is only found in family members that metabolize sulfonate or sulfate substrates and is, thus, supposed to be involved in the discrimination of tetrahedral substrates against planar ones through hydrogen bridges.[5,11]

To study the role of these amino acids in the conversion of the planar carboxylic acid analogue **4**, we replaced R270 and H70 with alanine or serine amino acid residues. We created single-point mutants H70A, H70S, R270A, and R270S and the corresponding double mutants H70A_R270A, H70A_R270S, H70S_R270A, and H70S_R270S. We hypothesized that these substitutions would impair hydrogen bond formation to **4.** Based on results from previous work, we expected the mutants with substitutions at position R270 to show a significantly reduced activity towards **4**.[5,11] In contrast, the mutations at position H70 were expected to have minor effects on the activity. As expected, mutants R270A and R270S and the double mutants showed no detectable conversion of **4** to **5.** This is most probably because of the loss of the ion-pairing ability of the substrate binding pocket of TauD towards the tested substrate. The mutation H70A also showed almost no conversion of **4** (conversion 1.9±0.2%), whereas the H70S mutant displayed a dramatically decreased formation of product **5** of (4.8±1.7)%.

Optimization of the substrate binding site for non-natural substrates

To improve the activity of TauD towards new non-natural substrates, we first aimed to gain a deeper understanding of the binding mode of these planar carboxylic acid substrates. We expected them to be similar to those of the natural sulfonate substrates. Having shown that the model substrate **4** utilizes the same anchoring mechanism in the active site as the natural substrate **1**, we modeled the docking of **4** to TauD (PDB ID: 1OS7) by using Rosetta Design.

Residues that surround the catalytic side chains and transition state were repacked and redesigned to optimize steric, Coulombic, and hydrogen-bonding interactions with the transition state and associated catalytic residues by using Rosetta Design as described previously.[29,30] We intended to analyze amino acids at the substrate binding site that hinder the binding of new substrates and to target amino acids that could possibly be mutated to introduce additional interactions to improve the binding of **4**.[31–33] The docking of the model substrate **4** indicated a positioning of the new substrate at the same substrate binding site as for the natural substrate **1** (Figure 2). The docking also revealed that the carboxylic acid substrate seems to have a distinct orientation that exposes one hydrogen atom, namely, the pro-D-hydrogen atom, almost exclusively towards the reactive Fe center. These results resemble the enantiomeric excess values found experimentally for the products of the tested conversion for the new substrates as shown in Table 1.

Furthermore, the docking study indicated that residues F159 and F206 in the active site of TauD play a key role in the substrate orientation and positioning to bring the substrate in

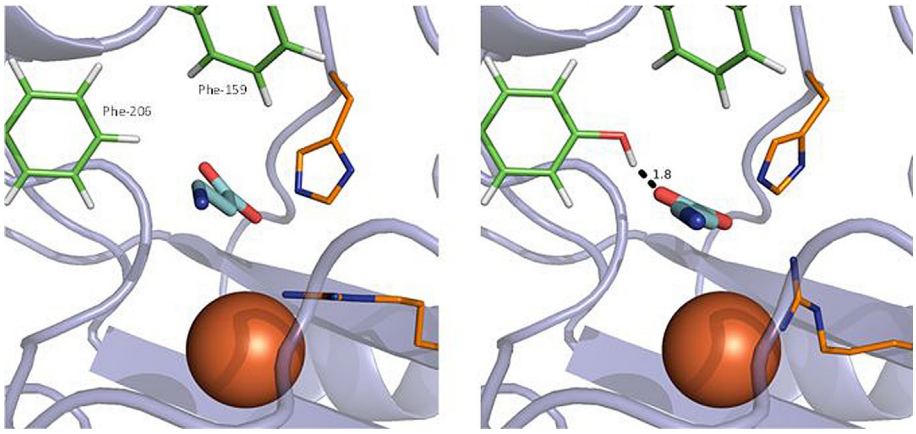


Figure 2. Docking of **4** (blue sticks) into the wild-type TauD (on the left) and the mutant F206Y (on the right). The active site Fe is shown as an orange sphere, and the active site residues F159 and F206/Y206 are depicted as green sticks. Acid-coordinating residues H70 and R270 are shown as orange sticks.

close proximity to the reactive Fe center as already demonstrated for the natural substrate **1**.[10] McKusker and Klinman showed that the amino acid F159 is involved in the coupling of the oxygen activation and hydroxylation of **1**.[10] The authors demonstrated that the exchange of F159 with a smaller amino acid led to a significant uncoupling of the oxygen activation and the actual hydroxylation of **1** as well as the release of sulfite. The exact role of F206 is not known yet. Based on our docking results with **4** using wild-type TauD, we assumed that the exchange of F206 by a tyrosine will affect substrate binding through the introduction of an additional hydrogen bond. We anticipated an increase of the enzyme affinity and the activity towards this new substrate as binding of the substrate constitutes a bottleneck for hydroxylation. We replaced both residues F159 and F206 against leucine as well as tyrosine. Both leucine variants showed no detectable conversion of **4**, which may be because of the unproductive binding of this substrate in the active site.[34] In the case of the two tyrosine variants, the mutant F159Y showed a dramatically reduced activity with conversions that decreased to 3.9%, whereas the mutant F206Y displayed an increased conversion of **4** of ≈140% compared to that of the wild-type. Encouraged by these findings, we re-assayed the initial substrate set (Figure 2). Additionally, we performed a second docking study with **4** using TauD variant F206Y (Figure 2, right). All results for the tested conversions of the improved TauD variant F206Y are summarized in Table 1. The docking study showed that the hydroxyl group of the new Y206 residue lies within a distance of 1.8 Å to the carboxylic acid functionality, which is in the typical range of a hydrogen bridging interaction. The TauD mutant F206Y displayed a twofold decreased activity towards **1**. Specific activities of $(0.71 \pm 0.04)\ U\,mg^{-1}$ for the TauD F206Y mutant and $(1.47 \pm 0.04)\ U\,mg^{-1}$ for the wild-type were determined (literature data from 1.64 to $14.0 \pm 1.5\ U\,mg^{-1}$).[4,11] This indicates that the introduction of the F206Y mutation enhanced the activity towards new substrates and induced selectivity, which started to switch the overall substrate preference of the enzyme from **1** to the new substrate **4**.

We demonstrated that our improved TauD variant F206Y increased the conversion of the model substrate **4** and improved the activity towards all of our substrates tested.

The increase in the conversion ranged from 140% for **4** to up to >800% for **10** relative to that of the wild type. The F206Y variant also displayed improved stereoselectivities for substrates that had formerly showed not very pronounced enantiomeric excesses in biotransformations with wild-type enzyme. Remarkably, mutant F206Y not only displayed improved catalytic properties towards the substrates already converted by the wild-type enzyme but also displayed a wider substrate scope as it accepted homologues of the model substrate **4** as well as aliphatic carboxylic acids that were not accepted by TauD wild-type. Conversions of the aliphatic carboxylic acid substrates **12**, **14**, and **16** were between (5.2 ± 0.6) and (3.6 ± 0.2)%. In the second screening, we tested further analogues of **4**, namely, 3-hydroxypropionic acid and 3-aminopropionic amide in which the amine functionality was replaced by a hydroxyl group and the acid by an amide, respectively. Surprisingly, no conversion of these analogues of **4** under the tested conditions could be detected (data not shown).

The reason for the discrimination of substrates with odd-numbered chain lengths versus those with even-numbered chain lengths still remains unclear. Additionally, the enzyme selectivity for substrates that possess an amino substituent distal to the carboxylic acid is not yet fully understood. We assume that the protonated amine group facilitates the binding of the substrates in the active site, whereas substrates that lack this feature are, therefore, disfavored. This hypothesis is underlined by significant differences in the Michaelis constants (K_M) defined experimentally between aliphatic and amino-substituted sulfonic acid substrates. The K_M values of the amino-substituted substrates are one order of magnitude lower than those of the aliphatic substrates.[4] However, further experiments are needed to clarify the exact role of substrate substituents on their acceptance. The fact that the enzyme does not accept substrates that bear an amide instead of a carboxylic acid can be explained by the acid-coordinating residues H70 and R270 in the active site of TauD. This might lead to a repulsion of the amide group and, therefore, a nonproductive positioning in the active site.

Conclusions

We studied the promiscuous activity of taurine dioxygenase (TauD) from *Escherichia coli* in the hydroxylation of ω-amino carboxylic acids. We demonstrated that the native reaction mechanism of the enzyme can be used for the direct α-hydroxylation of β-alanine (**4**) and its higher homologues. We further improved the catalytic activity of TauD by the introduction of rational mutations in the active site. The TauD variant F206Y showed improved catalytic activities as well as a broadened substrate scope.

Experimental Section

General remarks: All commercially available chemicals used in this study were purchased from Sigma Aldrich or Alfa Aesar at the highest purity level available and used without any further purification. Restriction enzymes were purchased from Fermentas, and the components used for culture media from Carl Roth company.

DNA modification and sequencing: Wild-type TauD was amplified from genomic DNA from *E. coli* JM109 by a standard polymerase chain reaction (PCR) technique using the following primers (restriction sites are highlighted in bold) TauD_f: GGGATTTC**CATATG**AGTGAACGTCTGAGCATTACC with the *Nde*I restriction site and TauD_r: CG**GAATTC**TTACCCCGCCCG ATAAAACGG with the *EcoR*I restriction site. The resulting PCR fragment was purified by agarose gel electrophoresis, double digested using the corresponding restriction enzymes, and subsequently cloned into a digested pET22b(+) vector system using T4 ligase (pITB_1022). The stop codon of the TauD was removed using site-directed mutagenesis to result in a C-terminally His-tagged TauD construct (pITB_988) that was used throughout this study.

Site-directed mutagenesis: Mutants were created by using the standard QuikChange® PCR technique. The following primers were

used for the construction of the mutants mentioned in this work with modified sequences highlighted in bold italic letters. The forward primers are always given, and the reverse primers were constructed by inverting the sequence. The resulting plasmid constructs are given in brackets behind the primer sequence. TauD-His_6: CCGTTTTATCGGGCGGGG***AATT***CGAGCTCCGTCGACAAG (pITB 988); F159 L: GCGGAGCATGATTTCCGTAAATCG***TTG***CCGGAATACAAATACCGCAAAAC (pITB_998); F159Y: GCGGAGCATGATTTCCGTAAATCG***TAC***CCGGAATACAAATACCGCAAAAC (pITB_997); F206L: CAGGCGCTGTTTGTGAATGAAGGC***TTG***ACTACGCGAATTGTTGATG (pITB_1000); F206Y: CAGGCGCTGTTTGTGAATGAAGGC***TAC***ACTACGCGAATTGTTGATG (pITB_999); H70 A: CCCAGCGTTTTGGCGAATTGCATATT***GCG***CCTGTTTACCCGCATGCC (pITB_989); H70S: CCCAGCGTTTTGGCGAATTGCATATT***TCC***CCTGTTTACCCGCATGCC (pITB_990); R270 A: GCCACAGCGACGGATAATGCAT***GCG***GCGACGATCCTTGGGGG (pITB_991); R270S: GCCACAGCGACGGATAATGCAT***TCC***GCGACGATCCTTGGGGG (pITB_992).

Protein expression and purification: Expression and purification of the His_6-tagged wild-type TauD and the corresponding TauD mutants were performed in *E. coli* BL21(*DE3*).Cells were grown at 37 °C in LB-medium (low salt) until the OD_{600} reached 0.4–0.6 and isopropyl β-thiogalactoside (IPTG) was added to a final concentration of 0.5 mM. After induction, the cells were grown at 30 °C for another 5 h and harvested by centrifugation. Cells were resuspended in 50 mM BisTris buffer pH 7 (5 mL per g cell wet weight) and disrupted by sonification (Branson sonifier W250, Danbury, USA). The resulting solution was clarified by centrifugation to remove cell debris. The supernatant was filtered through a 0.2 μm syringe filter. In the next step, the protein was purified by using an ÄKTA-purifier system (GE Healthcare, Chalfont St. Giles, UK) through a 5 mL HiTrap Chelating HP column (GE Healthcare, Chalfont St. Giles, UK). After the sample was applied, the column was washed with two column volumes of binding buffer (20 mM phosphate buffer pH 7, 150 mM NaCl, and 20 mM imidazole). In the second step a linear gradient over eight column volumes was applied and the buffer concentration was varied from 0 to 80% elution buffer (20 mM phosphate buffer pH 7, 150 mM NaCl, and 500 mM imidazole) to elute the target protein. The protein preparation was >90% pure as judged by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After the elution of the protein, the concentration of the elution buffer was increased to 100% for two column volumes to wash the column. After purification, the resulting enzyme preparation was dialyzed twice against 50 mM BisTris buffer pH 7 to remove salts from the purification and then concentrated with VivaSpin concentrator columns until the targeted protein concentration was reached. The protein concentration was determined by using a Pierce BCA protein kit (Thermo Scientific) following the manufacturer's instructions. The activity of the enzyme preparations were determined as described elsewhere.[4]

Biotransformations of alternative substrates: All biotransformations were performed in 1.5 mL Eppendorf tubes. For the biotransformations, a reaction setup adapted from the standard reaction setup reported by Eichhorn et al. for taurine was used.[4] The reaction mixture contained the corresponding purified protein in a final concentration of 1.2 mg mL^{-1} and 10 mM BisTris buffer pH 7. The target substrate and the cosubstrate α-ketoglutarate were both added in a final concentration of 10 mM. Ascorbic acid was added to the reaction mixture at a final concentration of 0.4 mM. The reaction was started by adding iron(II) sulfate to a final concentration of 0.1 mM. The iron solution was prepared freshly before addition to prevent the iron from oxidation. Ascorbic acid solutions were prepared fresh weekly. All reagents besides the iron(II) solution were adjusted to pH 7 before use. The reaction mixture was incubated at 30 °C for 18 h by using an Eppendorf ThermoMixer at a shaking speed of 850 rpm.

Synthesis of reference substances for HPLC analysis: Substrate **13** was not commercially available and was, therefore, synthesized as a reference for analysis according to Ref. [35]. The purification procedure of the product, however, was altered from that described in the literature. After the reaction, the pH of the reaction mixture was adjusted to 1 by adding concentrated hydrochloric acid, and the mixture was extracted by adding a Dowex SCX strong cation exchanger. The Dowex pearls were then collected by filtration, and the bound products were eluted by adding aqueous NH_3 (25%). The aqueous phase was evaporated to dryness, and the resulting oil was re-dissolved in water. In the next step, the resulting aqueous product mixture was separated by using HPLC and a Reprosil Chiral AA column (250×4.6 mm; 8 μm; Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) and the method described below for the analysis of amino carboxylic substrates.

Chiral HPLC analysis of ω-amino carboxylic substrates: The analysis of **4** and its higher homologues was performed by using an Agilent HPLC system (1200 series) equipped with an Agilent Infinity 1260 evaporative light scattering detector (ELSD) and Reprosil Chiral AA column (250×4.6 mm; 8 μm; Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). The mobile phase was an isocratic mixture of methanol and water (50:50), the column temperature was set to 30 °C, and the flow was set to 0.8 mL min^{-1}. The retention times of the reagents were proven by the injection of standard references.

HPLC analysis of carboxylic substrates: The analysis of the aliphatic carboxylic acids such as acetic acid and its higher homologues was performed by using an Agilent HPLC system (1200 series) equipped with an Agilent Infinity 1200 refractive index detector (RID) and an Aminex HPX-87H column (7.8×300 mm; 9 μm; BioRad, Hercules, CA). The mobile phase was 5 mM aqueous sulfuric acid, the column temperature was set to 50 °C, and the flow was set to 0.5 mL min^{-1}.

Acknowledgements

The authors thank Dr. Rainer Stürmer from Stürmer Scientific as well as Dr. Alexander Seiffert for thoughtful comments and Dr. Juliane Stahmer for help with the docking. Funding by the European Union's Seventh Framework Programme FP7/2007–2013 under grant agreement no. 266025 is gratefully acknowledged.

Keywords: biocatalysis · biotransformations · carboxylic acids · enzymes · hydroxylation

[1] R. P. Hausinger, *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39*, 21–68.
[2] J. R. van der Ploeg, M. A. Weiss, E. Saller, H. Nashimoto, N. Saito, M. A. Kertesz, T. Leisinger, *J. Bacteriol.* **1996**, *178*, 5438–5446.
[3] M. R. Uria-Nickelsen, E. R. Leadbetter, W. G. Iii, *Arch. Microbiol.* **1994**, *161*, 434–438.
[4] E. Eichhorn, J. R. van der Ploeg, M. A. Kertesz, T. Leisinger, *J. Biol. Chem.* **1997**, *272*, 23031–23036.
[5] J. M. Elkins, M. J. Ryle, I. J. Clifton, J. C. D. Hotopp, J. S. Lloyd, N. I. Burzlaff, J. E. Baldwin, R. P. Hausinger, P. L. Roach, *Biochemistry* **2002**, *41*, 5185–5192.

[6] P. K. Grzyska, E. H. Appelman, R. P. Hausinger, D. A. Proshlyakov, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 3982 – 3987.
[7] P. K. Grzyska, M. J. Ryle, G. R. Monterosso, J. Liu, D. P. Ballou, R. P. Hausinger, *Biochemistry* **2005**, *44*, 3845 – 3855.
[8] J. C. Price, E. W. Barr, T. E. Glass, C. Krebs, J. M. Bollinger, *J. Am. Chem. Soc.* **2003**, *125*, 13008 – 13009.
[9] J. C. Price, E. W. Barr, B. Tirupati, J. M. Bollinger, C. Krebs, *Biochemistry* **2003**, *42*, 7497 – 7508.
[10] K. P. McCusker, J. P. Klinman, *J. Am. Chem. Soc.* **2010**, *132*, 5114 – 5120.
[11] J. R. O'Brien, D. J. Schuller, V. S. Yang, B. D. Dillard, W. N. Lanzilotta, *Biochemistry* **2003**, *42*, 5547 – 5554.
[12] M. Girhard, S. Schuster, M. Dietrich, P. Dürre, V. B. Urlacher, *Biochem. Biophys. Res. Commun.* **2007**, *362*, 114 – 119.
[13] M. Hamberg, I. Ponce de Leon, M. J. Rodriguez, C. Castresana, *Biochem. Biophys. Res. Commun.* **2005**, *338*, 169 – 174.
[14] M. Hamberg, I. Ponce de Leon, A. Sanz, C. Castresana, *Prostaglandins Other Lipid Mediators* **2002**, *68 – 69*, 363 – 374.
[15] M. Landwehr, L. Hochrein, C. R. Otey, A. Kasrayan, J.-E. Bäckvall, F. H. Arnold, *J. Am. Chem. Soc.* **2006**, *128*, 6058 – 6059.
[16] W. Adam, W. Boland, J. Hartmann-Schreier, H. Humpf, M. Lazarus, A. Saffert, C. R. Saha-Möller, R. V. April, *J. Am. Chem. Soc.* **1998**, *120*, 11044 – 11048.
[17] S. Lüttenberg, T. D. Ta, J. von der Heyden, J. Scherkenbeck, *Eur. J. Org. Chem.* **2013**, 1824 – 1830.
[18] E. Busto, N. Richter, B. Grischek, W. Kroutil, *Chem. Eur. J.* **2014**, *20*, 11225 – 11228.
[19] W. Adam, M. Lazarus, C. R. Saha-Moller, P. Schreier, *Acc. Chem. Res.* **1999**, *32*, 837 – 845.
[20] Y.-P. Xue, Y.-G. Zheng, Y.-Q. Zhang, J.-L. Sun, Z.-Q. Liu, Y.-C. Shen, *Chem. Commun.* **2013**, *49*, 10706 – 10708.
[21] K. Faber, *Biotransformations in Organic Chemistry*, Springer Verlag, Berlin, **2011**.
[22] P. Brewster, E. D. Hughes, C. K. Ingold, P. A. D. S. Rao, *Nature* **1950**, *166*, 179 – 180.
[23] A. M. Rydzik, I. K. H. Leung, G. T. Kochan, M. A. McDonough, T. D. W. Claridge, C. J. Schofield, *Angew. Chem.* **2014**, *126*, 11105 – 11107.
[24] D. Baud, P.-L. Saaidi, A. Monfleur, M. Harari, J. Cuccaro, A. Fossey, M. Besnard, A. Debard, A. Mariage, V. Pellouin, J.-L. Petit, M. Salanoubat, J. Weissenbach, V. de Berardinis, A. Zaparucha, *ChemCatChem* **2014**, *6*, 3012 – 3017.
[25] G. Dinos, D. N. Wilson, Y. Teraoka, W. Szaflarski, P. Fucini, D. Kalpaxis, K. H. Nierhaus, *Mol. Cell* **2004**, *13*, 113 – 124.
[26] M. K. Kharel, B. Subba, D. B. Basnet, J. S. Woo, H. C. Lee, K. Liou, J. K. Sohng, *Arch. Biochem. Biophys.* **2004**, *429*, 204 – 214.
[27] A. Czerwinski, H. Wojciechowska, A. R. Andruszkiewicz, J. Grzybowska, J. Gumeniak, E. Borowski, *J. Antibiot.* **1983**, *36*, 1001 – 1006.
[28] J. C. Dunning Hotopp, R. P. Hausinger, *Biochemistry* **2002**, *41*, 9787 – 9794.
[29] S. A. Combs, S. L. DeLuca, S. H. DeLuca, G. H. Lemmon, D. P. Nannemann, E. D. Nguyen, J. R. Willis, J. H. Sheenan, J. Meiler, *Nat. Protoc.* **2013**, *8*, 1277 – 1298.
[30] J. Meiler, D. Baker, *Proteins Struct. Funct. Bioinf.* **2006**, *65*, 538 – 548.
[31] J. Kirchmair, G. Wolber, C. Laggner, T. Langer, *J. Chem. Inf. Model.* **2006**, *46*, 1848 – 1861.
[32] J. Boström, J. R. Greenwood, J. Gottfries, *J. Mol. Graphics Modell.* **2003**, *21*, 449 – 462.
[33] D. Kutlán, P. Presits, I. Molnár-Perl, *J. Chromatogr. A* **2002**, *949*, 235 – 248.
[34] K. P. McCusker, J. P. Klinman, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 19791 – 19795.
[35] J.-P. Gouesnard, *Bull. Soc. Chim. Fr.* **1989**, 88 – 94.

Received: November 12, 2015
Revised: January 18, 2016
Published online on February 23, 2016