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Enzyme Catalysis

Extended Reaction Scope of Thiamine Diphosphate Dependent Cyclohexane-1,2-dione Hydrolase: From C–C Bond Cleavage to C–C Bond Ligation**

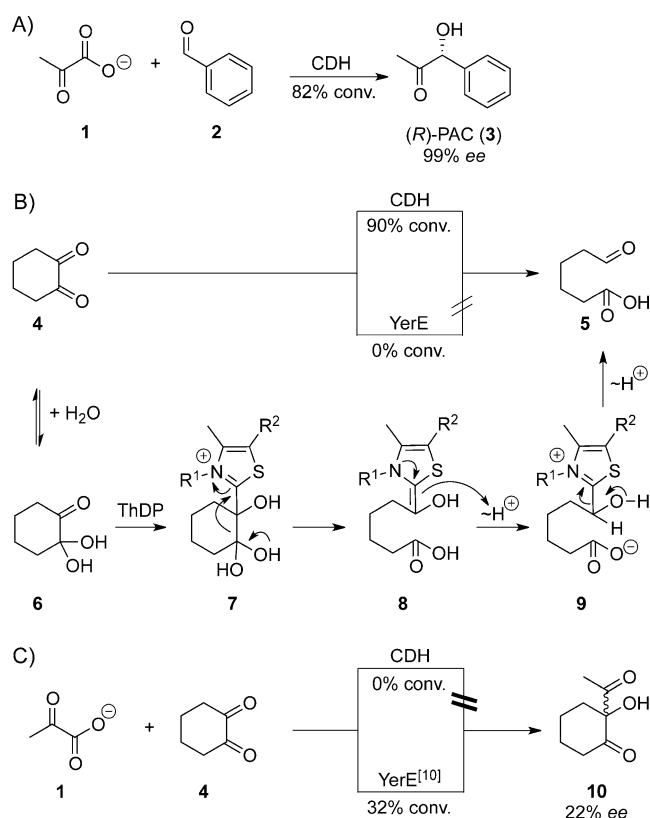
Sabrina Loschonsky, Tobias Wacker, Simon Waltzer, Pier Paolo Giovannini, Michael J. McLeish, Susana L. A. Andrade, and Michael Müller*

Dedicated to Peter M. H. Kroneck on the occasion of his 70th birthday

Abstract: ThDP-dependent cyclohexane-1,2-dione hydrolase (CDH) catalyzes the C–C bond cleavage of cyclohexane-1,2-dione to 6-oxohexanoate, and the asymmetric benzoin condensation between benzaldehyde and pyruvate. One of the two reactivities of CDH was selectively knocked down by mutation experiments. CDH-H28A is much less able to catalyze the C–C bond formation, while the ability for C–C bond cleavage is still intact. The double variant CDH-H28A/N484A shows the opposite behavior and catalyzes the addition of pyruvate to cyclohexane-1,2-dione, resulting in the formation of a tertiary alcohol. Several acyloins of tertiary alcohols are formed with 54–94 % enantiomeric excess. In addition to pyruvate, methyl pyruvate and butane-2,3-dione are alternative donor substrates for C–C bond formation. Thus, the very rare aldehyde–ketone cross-benzoin reaction has been solved by design of an enzyme variant.

Thiamine diphosphate (ThDP) dependent enzymes catalyze a multitude of reactions, including (oxidative) decarboxylations, asymmetric C–C and carbon–heteroatom bond formations, and C–C bond cleavages.^[1] ThDP-dependent cyclohexane-1,2-dione hydrolase (CDH) catalyzes at least two distinct

type of reactions. First, the asymmetric C–C bond-forming reaction of pyruvate (**1**) with benzaldehyde (**2**) results in (*R*)-phenylacetylcarbinol (PAC, **3**; 82 % conversion after 24 h, 99 % *ee*; Scheme 1 A).^[2,3] CDH also converts a variety of substituted benzaldehydes into the corresponding PAC derivatives.^[3] In the absence of aldehydes, CDH catalyzes the decarboxylation and homocoupling of pyruvate to provide (*S*)-acetoin (3-hydroxybutan-2-one) with remarkably high enantioselectivity (up to 93 % *ee*).^[4] In its second reaction, discovered in the denitrifying bacterium *Azoarcus* sp. strain



Scheme 1. A) CDH-catalyzed C–C bond formation: the reaction of pyruvate (**1**) and benzaldehyde (**2**) giving (*R*)-phenylacetylcarbinol (PAC, **3**). B) CDH-catalyzed C–C bond cleavage of cyclohexane-1,2-dione (**4**) to 6-oxohexanoic acid (**5**) showing the reaction mechanism proposed by Kroneck et al.^[6b] C) YerE utilizes the same substrate **4** as acceptor together with **1** as donor in a C–C bond formation giving the tertiary alcohol **10**.^[10]

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22Lin,^[5] CDH catalyzes the C–C bond cleavage of cyclohexane-1,2-dione (**4**) to 6-oxohexanoic acid (**5**; Scheme 1B).^[6] The C–C bond cleavage is assumed to be initiated by the attack of the ThDP ylide on the C=O bond of the monohydrate **6**^[7] of 1,2-diketone **4** to form the ThDP adduct **7** (Scheme 1B).^[6b] Compound **7** can also be regarded as a tetrahedral intermediate which breaks down to the carboxylic acid **8**. Protonation of the enamine moiety of **8** results in **9** and the subsequent elimination of ThDP furnishes the oxo acid **5**.^[6b] A seemingly related C–C bond cleavage of 3,4,5-trihydroxycyclohexane-1,2-dione to 5-deoxy-D-glucuronic acid is catalyzed by IolD from *Bacillus subtilis*.^[8] However, CDH and IolD are not homologues and no (significant) sequence homologue of CDH has been identified in the TEED database.^[9] Thus, CDH represents a unique enzyme with respect to reactivity and amino acid sequence.

Although cyclohexane-1,2-dione (**4**) is a substrate of a C–C bond-cleavage reaction catalyzed by CDH (**4**→**5**), CDH is unable to catalyze C–C bond formation (carbologation) using pyruvate (**1**) as acyl anion donor and **4** as the acceptor (Scheme 1C). Conversely, ThDP-dependent YerE from *Yersinia pseudotuberculosis* utilizes **4** as an acceptor in a carbologation reaction with pyruvate (**1**) to form the tertiary alcohol **10** (Scheme 1C).^[10] In fact, YerE was the first recombinant enzyme found to catalyze cross-benzoin condensations with nonactivated ketones as acceptors.^[10] Apart from YerE, very few ThDP-dependent enzymes have been shown to accept ketones as acceptor substrates. Recently, Jiang et al. reported the addition of pyruvate to acetone in a reaction catalyzed by acetoin:2,6-dichlorophenolindophenol oxidoreductase (AcoAB) from *Bacillus subtilis*.^[11] 1,2-Diketones are also substrates of an as yet uncharacterized enzyme from *Bacillus licheniformis*, which catalyzes a cleavage–readdition sequence.^[12]

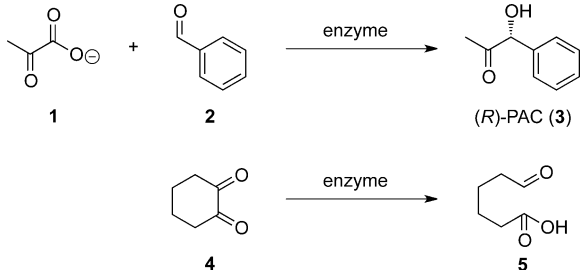
Using both site-directed and saturation mutagenesis, ThDP-dependent enzymes have been engineered to provide finely tuned catalytic properties, with a focus on increased substrate range and enhanced stereospecificity.^[13] Yet, to date, no variants have been generated that are able to use ketones as the acceptor substrates in carbologation reactions. In this report, we present protein engineering experiments designed to selectively knock out either 1) the C–C bond-formation reactivity or 2) the C–C bond-cleavage reactivity of CDH. Furthermore, we present a CDH variant whose catalytic properties have been fundamentally changed such that the 1,2-diketone **4**, which originally served as a substrate for CDH-catalyzed C–C bond cleavage, is now accepted as a substrate for C–C bond formation.

Steinbach et al. reported the crystal structure of native CDH, showing it to be a homotetrameric protein with one FAD and one ThDP per monomer.^[6c,d] Crystals soaked with cyclohexane-1,2-dione (**4**) showed that, in addition to ThDP, four amino acids (N484, from one monomer, and H28, H76, and Q116 from a second monomer) directly interact with the carbonyl groups of **4** at the active site.^[6c,d] To investigate the role of these residues, we prepared four single variants (H28A, H76A, Q116A, and N484A), as well as two double variants, H28A/N484A and H76A/Q116A. In CDH-H28A/N484A, the active-site residues coordinating one oxygen

atom of **4** in the active site were replaced, while in CDH-H76A/Q116A, the amino acids coordinating the other oxygen atom of **4** in the active site were changed. The variants were prepared with a C-terminal hexahistidine tag, expressed in *E. coli* BL21(DE3) cells and purified by affinity chromatography (see the Supporting Information).

Wild-type (wt) CDH^[3] and the purified variants were screened towards 1) the formation of PAC (**3**) and 2) the 1,2-diketone cleavage of **4** (Table 1). Three variants (H76A,

Table 1: C–C bond-formation^[a] and C–C bond-cleavage^[b] reactivity of wt-CDH and six variants.



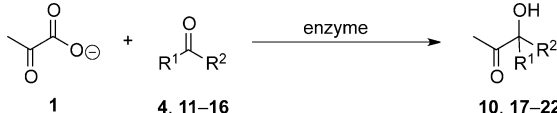







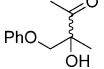

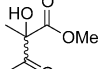
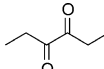
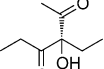


CDH variant	Conversion [%] ^[c] (<i>ee</i> [%]) ^[d]	
	1 + 2 → 3	4 → 5
wt	98 (99, <i>R</i>)	90
N484A	18 (99, <i>R</i>)	48
H28A	12 (> 99, <i>R</i>)	78
H76A	3 (n.d.) ^[e]	3
Q116A	0 (n.d.) ^[e]	3
H28A/N484A	73 (> 99, <i>R</i>)	17
H76A/Q116A	0 (n.d.) ^[e]	9

[a] Conditions: pyruvate (25 mM), benzaldehyde (10 mM), CDH variant (1 mg mL^{−1}), buffer (50 mM MES, 1 mM MgSO₄, 0.5 mM ThDP, pH 6.5), 30 °C, 48 h. [b] Conditions: cyclohexane-1,2-dione (25 mM), CDH variant (1 mg mL^{−1}), buffer (50 mM MES, 1 mM MgSO₄, 0.5 mM ThDP, pH 6.5), 30 °C, 48 h. [c] Determined by ¹H NMR spectroscopy (400 MHz, CDCl₃). [d] Determined by HPLC on a chiral stationary phase. [e] Not determined. MES = 2-(*N*-morpholino)ethanesulfonic acid.

Q116A, and H76A/Q116A) proved to be essentially inactive, while two other variants showed a selective reduction of one of the two activities. The H28A variant showed an eightfold decrease in the formation of PAC (12 %), but 1,2-diketone cleavage was nearly unaffected (78 % conversion). The double variant H28A/N484A showed acceptable formation of PAC (73 %), but conversion toward the cleavage product was decreased by a factor of five (17 % conversion). Regardless of the mutation, the PAC product (**3**) of every active variant had (*R*)-configuration (≥ 99 % *ee*).

Overall, the H28A/N484A variant showed considerably reduced relative cleavage reactivity with its physiological substrate (**4**→**5**, 17 % conversion), but still catalyzed C–C bond formation using pyruvate as a donor (**1** + **2**→**3**). Therefore, we speculated that this variant might catalyze a C–C bond-forming reaction, again with pyruvate (**1**) as a donor, but now using **4** as the acceptor. To date, the only known enzyme to accept **4** in such a reaction is YerE (32 % conversion, 22 % *ee*; Scheme 1).^[10] We were pleased to see that the H28A/N484A variant of CDH showed a comparable

Table 2: Substrate range of the CDH variant, H28A/N484A,^[a] and comparison with YerE.^[10]

					
Acceptor		Conversion [%]	<i>ee</i> [%]		Product
CDH-H28A/N484A			YerE ^[10]		
	4	25 ^[b] (88 ^[d])	32 ^[b] (22 ^[d])		10^[e]
	11	18 ^[b] (—)	55 ^[c] (—)		17
	12	14 ^[b] (n.d. ^[f])	97 ^[c] (84 ^[d])		18
	13	55 ^[b] (94 ^[g])	48 ^[c] (91 ^[g])		19^[h]
	14	89 ^[c] (n.d. ^[f]) (see Table 3)	> 99 ^[c] (30 ^[c])		20
	15	24 ^[b] (54, <i>S</i>)	58 ^[b] (37, <i>S</i>) ^[i]		(<i>S</i>)-21
	16	31 ^[c] (—) (see Table 4)	94 ^[c] (—)		22

[a] Conditions: pyruvate (50 mM), acceptor (20 mM), CDH variant (1 mg mL⁻¹), buffer (50 mM MES, 1 mM MgSO₄, 0.5 mM ThDP, pH 6.5), 25 °C, 24 h. [b] Determined by GC-MS. [c] Determined by ¹H NMR spectroscopy. [d] Determined by GC on a chiral stationary phase. [e] CDH-H28A/N484A and YerE give the same enantiomer of **10**. [f] Not determined. [g] Determined by HPLC on a chiral stationary phase. [h] Different chiral stationary phases had been applied to determine the *ee* value of **19** from CDH-H28A/N484A and YerE. Accordingly, it can not be concluded whether the same enantiomer was obtained. [i] This work.

conversion of **4** + **1** → **10** (25 %), together with a considerably improved *ee* value (88 %, Table 2).

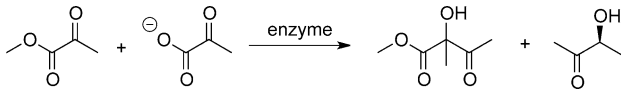
Control experiments also showed 2 % conversion of **4** + **1** → **10** by the N484A variant; however, wt-CDH and the other CDH variants from Table 1 were not able to use **4** as an acceptor substrate. This observation implies that the mutation of two active site residues not only reduces the physiological C–C bond-cleavage ability of the H28A/N484A variant, but also enables the utilization of the same substrate **4** as acceptor in a carboligation reaction.

In addition to 1,2-diketone **4**, a number of monoketones were also tested as acceptor substrates with the H28A/N484A variant (Table 2). The cyclic monoketones **11** and **12** were converted in 18 % and 14 %, respectively, while the acyclic monoketone **13** showed 55 % conversion (94 % *ee*). Control experiments showed 9 % conversion of **11** + **1** → **17** for the single variant, N484A, but wt-CDH and all other variants from Table 1 did not utilize **11**. With the H28A/N484A variant, the acyclic 1,2-diketones **15** and **16** were transformed

in 24 % and 31 % conversion, respectively. The absolute configuration of the tertiary alcohol (*S*)-**21** was established by comparison with an authentic sample of (*R*)-**21**, which was formed from butane-2,3-dione (**16**) and hexane-3,4-dione (**15**) by “acetylaceton synthase” from *Bacillus licheniformis*.^[12] Reinvestigation of the same reaction catalyzed by YerE provided (*S*)-**21** (37 % *ee*). In all cases CDH-H28A/N484A provided higher enantioselectivity than YerE,^[10] but lower conversion of ketones and pyruvate into tertiary alcohols. Interestingly, methyl pyruvate (**14**) was converted into methyl acetolactate (**20**) in 89 %, suggesting that the H28A/N484A variant, as well as YerE (>99 %),^[10] efficiently accepts activated ketones.

For comparison, we examined the wt-CDH-catalyzed reaction of pyruvate (**1**) and methyl pyruvate (**14**). In this instance, (*S*)-acetoin (**23**, 91 % *ee*) was formed in addition to **20** (Table 3). (*S*)-**23** is likely the product of the homocoupling

Table 3: Formation of methyl acetolactate (**20**) from methyl pyruvate (**14**) in the presence and absence of pyruvate (**1**).^[a]

					
CDH variant	14 [mM]	1 [mM]	14 :	20 :	23
wt	20	50	21	32	47 [91 % (<i>S</i>)]
wt	25	0	70	12	18 [94 % (<i>S</i>)]
H28A/N484A ^[b]	20	50	11	89	0
H28A/N484A	25	0	11	89	0

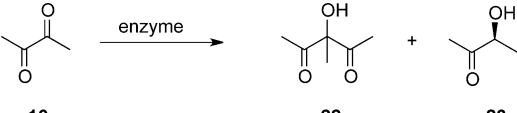
[a] Reaction conditions as in Table 2. [b] Result from Table 2. [c] Determined by ¹H NMR spectroscopy.

of (deprotonated) hydroxyethyl-ThDP and its protolytic derivative, that is, acetaldehyde. Recently, we have shown that wt-CDH catalyzes a similar reaction starting from pyruvate.^[4] However, in this case, hydroxyethyl-ThDP might also stem from the CDH-catalyzed cleavage of methyl pyruvate (**14**). To explore this possibility, we subjected **14** to wt-CDH in the absence of pyruvate (Table 3). Again, both the tertiary alcohol **20** and (*S*)-acetoin (**23**, 94 % *ee*) were formed, albeit to a much lesser extent. It would appear that there must have been an initial cleavage of **14** to form hydroxyethyl-ThDP, followed by addition to a second molecule of **14** (→**20**) or by homocoupling (→**23**).

Following this hypothesis, we examined the CDH-H28A/N484A-catalyzed reaction of methyl pyruvate (**14**) in the absence of pyruvate (**1**; Table 3). Methyl acetolactate (**20**) was again formed, to the same extent as in the presence of pyruvate (**1**); however, the formation of acetoin (**23**) and, therefore, homocoupling, was not observed for this variant.

We also postulated that acetylaceton (**22**), which was formed by CDH-H28A/N484A from a mixture of butane-2,3-dione (**16**) and pyruvate (**1**) as detailed in Table 2, might be the product of the homocoupling of butane-2,3-dione (**16**): such a reaction could be rationalized if **16** was cleaved to

Table 4: Formation of acetylacetoïn (22) and acetoin (23) from the homocoupling of butane-2,3-dione (16).^[a]



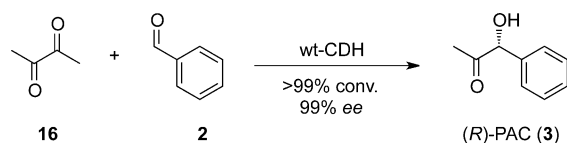
CDH variant	16	:	22	:	23
wt	5	:	41	:	54 [92% (S)]
H28A/N484A	97	:	3	:	0

[a] Conditions: **16** (25 mM), CDH variant (1 mg mL⁻¹), buffer (50 mM MES, 1 mM MgSO₄, 0.5 mM ThDP, pH 6.5), 25 °C, 24 h. [b] Determined by ¹H NMR spectroscopy.

hydroxyethyl-ThDP, which was then transferred to a second molecule of **16**, forming acetylacetoïn (**22**).

We tested this hypothesis by subjecting butane-2,3-dione (**16**) both to wt-CDH and the H28A/N484A variant (Table 4). Surprisingly, wt-CDH catalyzed the formation of both acetylacetoïn (**22**) and (*S*)-acetoin (**23**, 92% *ee*). The latter must have been the product of the homocoupling of hydroxyethyl-ThDP. By contrast, CDH-H28A/N484A catalyzed only the formation of traces of acetylacetoïn (**22**). No acetoin (**23**) was observed for this variant.

Finally, we tested whether the hydroxyethyl-ThDP generated by the wt-CDH-catalyzed C–C bond cleavage of butane-2,3-dione (**16**) could be used in a subsequent carbonylation reaction with benzaldehyde (**2**; Scheme 2). Indeed,



Scheme 2. Formation of (*R*)-PAC (**3**) from benzaldehyde (**2**) and butane-2,3-dione (**16**). Conditions: **2** (10 mM), **16** (25 mM), wt-CDH (1 mg mL⁻¹), buffer (50 mM MES, 1 mM MgSO₄, 0.5 mM ThDP, pH 6.5), 25 °C, 24 h.

after 24 hours, (*R*)-PAC [(*R*)-**3**] was obtained in more than 99% conversion (based on **2**) and a high *ee* value (99%).

Thus, the two activated ketones, methyl pyruvate (**14**) and butane-2,3-dione (**16**), turned out to be substrates for both wt-CDH and its H28A/N484A variant. Further, in addition to acting as acceptors in the presence of pyruvate (**1**), both **14** and **16** could be utilized as donors. The H28A/N484A variant obtains hydroxyethyl-ThDP from methyl pyruvate (**14**; Table 3), whereas wt-CDH is able to obtain the same intermediate from butane-2,3-dione (**16**; Table 4).

To gain a better understanding of these differences in catalytic reactivity, X-ray structures were obtained for recombinant wt-CDH (PDB ID 4D4E) and the H28A/N484A variant (PDB ID 4D5G). The structure of the wt-enzyme was identical to that of the published structure (PDB ID 2PGN)^[6c,d] but with an additional hexahistidine tag. Figure 1 shows that the mutations do not affect the overall

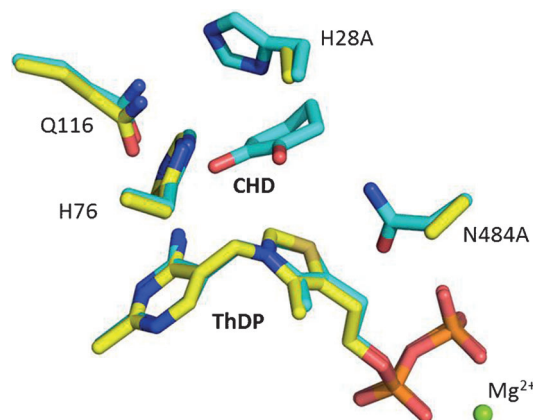


Figure 1. The active sites of CDH (PDB 2PGN, cyan) and its H28A/N484A variant (PDB ID 4D5G, yellow) superimpose with an r.m.s.d. of 0.29 Å over all atoms. The H28A/N484A variant loses a hydrogen-bond interaction with one carbonyl group of the substrate, cyclohexane-1,2-dione (**4**, CHD). In addition, the space above the binding site of CHD is significantly increased. For clarity, backbone atoms have been removed. r.m.s.d. = root-mean-square deviation.

arrangement of the active site, but create significantly more space around the site where the substrate 1,2-diketone **4** binds. The loss of the hydrogen-bonding interaction to N484 likely results in incorrect positioning of the substrate and contributes to the reduced ability to cleave C–C bonds (Table 1). Conversely, the more open binding site increases the range of possible acceptors.^[14]

In summary, we have designed a variant of CDH, H28A/N484A, that is able to use ketones as acceptor substrates in cross-benzoin reactions, resulting in the formation of tertiary α-hydroxy ketones. Further, while wt-CDH catalyzes the cleavage of cyclohexane-1,2-dione (**4**), the H28A/N484A variant efficiently uses **4** for C–C bond ligation. In doing this, we have broadened the concept of engineering ThDP-dependent enzymes from improving the stereoselectivity and enhancing the substrate range^[13] to altering the spectrum of catalytic reactivity. Given that the X-ray structure of ketone-accepting YerE is yet to be reported, the structure of CDH-H28A/N484A gives rise to the hope that it will be possible to develop an enhanced understanding of this reactivity. Our concept of engineering two distinct amino acids in the active center of CDH might be applicable to other ThDP-dependent enzymes, also enabling them to utilize ketones as acceptors. Finally, cyclohexane-1,2-dione-accepting YerE might be engineered accordingly so that a putative YerE variant is able to utilize 1,2-diketone **4** as the substrate of a C–C bond cleavage.

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Keywords: asymmetric synthesis · benzoin condensation · enzyme catalysis · protein engineering · tertiary alcohols

- [1] a) R. Kluger, K. Tittmann, *Chem. Rev.* **2008**, *108*, 1797–1833; b) R. A. W. Frank, F. J. Leeper, B. F. Luisi, *Cell. Mol. Life Sci.*

- 2007, 64, 892–905; c) F. Jordan, *Nat. Prod. Rep.* **2003**, 20, 184–201.
- [2] S. Fraas, Ph.D. thesis, Universität Konstanz (Germany), **2009**.
- [3] S. Loschonsky, S. Waltzer, S. Fraas, T. Wacker, S. L. A. Andrade, P. M. H. Kroneck, M. Müller, *ChemBioChem* **2014**, 15, 389–392.
- [4] S. Loschonsky, S. Waltzer, V. Brecht, M. Müller, *ChemCatChem* **2014**, 6, 969–972.
- [5] J. Harder, *Arch. Microbiol.* **1997**, 168, 199–204.
- [6] a) S. Fraas, A. K. Steinbach, A. Tabbert, J. Harder, U. Ermler, K. Tittmann, A. Meyer, P. M. H. Kroneck, *J. Mol. Catal. B* **2009**, 61, 47–49; b) A. K. Steinbach, S. Fraas, J. Harder, A. Tabbert, H. Brinkmann, A. Meyer, U. Ermler, P. M. Kroneck, *J. Bacteriol.* **2011**, 193, 6760–6769; c) A. K. Steinbach, S. Fraas, J. Harder, E. Warkentin, P. M. H. Kroneck, U. Ermler, *FEBS J.* **2012**, 279, 1209–1219; d) S. Fraas, A. K. Steinbach, E. Warkentin, P. M. H. Kroneck, U. Ermler, <http://www.rcsb.org/pdb/explore/explore.do?structureId=2PGN>.
- [7] a) R. Bakule, F. A. Long, *J. Am. Chem. Soc.* **1963**, 85, 2309–2312; b) R. Bakule, F. A. Long, *J. Am. Chem. Soc.* **1963**, 85, 2313–2318.
- [8] a) K. Yoshida, D. Aoyama, I. Ishio, T. Shibayama, Y. Fujita, *J. Bacteriol.* **1997**, 179, 4591–4598; b) K. Yoshida, M. Yamaguchi, T. Morinaga, M. Kinehara, M. Ikeuchi, H. Ashida, Y. Fujita, *J. Biol. Chem.* **2008**, 283, 10415–10424. c) The enzyme encoded by *IolD* showed the highest relationship to the ThDP-dependent acetolactate synthase enzymes from *Spirulina platensis* and *Synechococcus* sp. (26% sequence identity).
- [9] a) M. Widmann, R. Radloff, J. Pleiss, *BMC Biochem.* **2010**, 11, 9; b) <http://www.teed.uni-stuttgart.de>; c) C. Vogel, J. Pleiss, *Proteins* **2014**, 82, 2523–2537.
- [10] P. Lehwald, M. Richter, C. Röhr, H.-w. Liu, M. Müller, *Angew. Chem. Int. Ed.* **2010**, 49, 2389–2392; *Angew. Chem.* **2010**, 122, 2439–2442.
- [11] X. Jiang, H. Zhang, J. Yang, Y. Zheng, D. Feng, W. Liu, X. Xu, Y. Cao, H. Zou, R. Zhang, T. Cheng, F. Jiao, M. Xian, *Sci. Rep.* **2013**, 3, 2445, DOI: 10.1038/srep02445.
- [12] a) P. P. Giovannini, P. Pedrini, V. Venturi, G. Fantin, A. Medici, *J. Mol. Catal. B* **2010**, 64, 113–117; b) P. P. Giovannini, G. Fantin, A. Massi, V. Venturi, P. Pedrini, *Org. Biomol. Chem.* **2011**, 9, 8038–8045.
- [13] a) H. C. Hailes, D. Rother, M. Müller, R. Westphal, J. M. Ward, J. Pleiss, C. Vogel, M. Pohl, *FEBS J.* **2013**, 280, 6374–6394; b) R. Westphal, C. Vogel, C. Schmitz, J. Pleiss, M. Müller, M. Pohl, D. Rother, *Angew. Chem. Int. Ed.* **2014**, 53, 9376–9379; *Angew. Chem.* **2014**, 126, 9530–9533; c) D. Meyer, L. Walter, G. Kolter, M. Pohl, M. Müller, K. Tittmann, *J. Am. Chem. Soc.* **2011**, 133, 3609–3616; d) R. Westphal, S. Waltzer, U. Mackfeld, M. Widmann, D. Rother, J. Pleiss, M. Beigi, M. Müller, M. Pohl, *Chem. Commun.* **2013**, 49, 2061–2063; e) R. Westphal, S. Jansen, C. Vogel, J. Pleiss, M. Müller, D. Rother, M. Pohl, *ChemCatChem* **2014**, 6, 1082–1088.
- [14] During the peer review process of this manuscript, one referee suggested a second explanation: The modification of the active site might also result in the correct positioning of the substrate to react reversibly with the ThDP ylide, however, the subsequent C–C bond cleavage might be impaired as a result of missing and/or incorrectly positioned catalytic groups. The authors thank the referee for the valuable input.

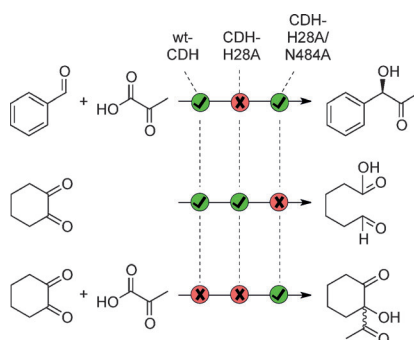
Communications



Enzyme Catalysis

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Extended Reaction Scope of Thiamine
Diphosphate Dependent Cyclohexane-
1,2-dione Hydrolase: From C–C Bond
Cleavage to C–C Bond Ligation



The ThDP-dependent enzyme CDH catalyzes an asymmetric benzoin condensation and the C–C bond cleavage of cyclohexane-1,2-dione. Mutation experiments resulted in the selective knock-down of one of these reactivities; CDH-H28A/N484A catalyzes the addition of pyruvate to cyclohexane-1,2-dione. This variant is one of the few ThDP-dependent enzymes that uses ketones as substrates in a C–C bond-forming reaction.