Dismutation of aldehydes catalyzed by alcohol dehydrogenases †

Kelly Velonia and Ioulia Smonou *

Department of Chemistry, University of Crete, Iraklio 71 409, Crete, Greece.
E-mail: smonou@chemistry.uch.gr

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The dismutation of aldehydes with the following three alcohol dehydrogenases, the mesophilic *Saccharomyces cerevisiae* ADH; the thermophilic *Thermoanaerobium brockii* ADH; and the recently isolated psychrophilic *Moraxella* sp. TAE123 ADH, was studied with high-resolution ^{1}H NMR spectroscopy. All three ADHs catalyzed the rapid dismutation of aldehydes to the corresponding alcohols and carboxylic acids.

Introduction

Alcohol dehydrogenases (ADHs), obtained from a variety of sources,[1] are among the most extensively studied enzymes. Their ability to catalyze the interconversion of alcohols to the corresponding carbonyl compounds with high enantioselectivity has found numerous applications in asymmetric organic synthesis.[2] While this reaction has been considered to be their physiological function,[3] the oxidation of aldehydes to carboxylic acids has mostly been unrecognized. For many years, the dismutation of aldehydes into equimolar quantities of the corresponding alcohols and carboxylic acids was thought to be a unique, but insignificant, side reaction of horse liver ADH (HLADH).[4–6] This view was reinforced by the fact that the alcohol dehydrogenase from *Saccharomyces cerevisiae* (YADH), which is structurally and mechanistically similar to HLADH, was reported to have no aldehyde dehydrogenase activity.[7] The detailed mechanism of aldehyde oxidation was recently studied and was shown to be more complicated than previously thought,[8–10] involving the rapid dismutation of the aldehyde prior to any production of NADH. Furthermore, members of other major ADH classes, including *Drosophila melanogaster* (DADH)[11] and human liver ADH,[12] have also been reported to display aldehyde dehydrogenase function. However, this reaction is still considered to be an intrinsic characteristic of these enzymes, rather than a general property of alcohol dehydrogenases.

In the present study we investigated systematically the dismutation of aldehydes by using the following three representative alcohol dehydrogenases: the mesophilic YADH,[13] the thermophilic *Thermoanaerobium brockii* ADH (TBADH)[14] and the recently isolated psychrophilic *Moraxella* sp. TAE123 ADH.[15] These enzymes are different in their temperature adaptation as well as in their structural and molecular characteristics.

The mesophilic YADH belongs to the medium ADH family and is a type-A enzyme transferring the pro-*R* hydrogen of C-4 of NADH to the *Re* face of carbonyl substrates and *vice versa*.[16] It reduces a variety of aldehydes and oxidizes mainly primary acyclic alcohols with stoichiometric consumption of NADH or NAD^+ respectively. Despite its *in vivo* role to reduce acetaldehyde to ethanol[17] and its structural and mechanistic similarity to HLADH, it was believed that YADH does not display dismutase activity.[7] Recent[18] results, however, indicate that YADH also catalyzes the dismutation of aldehydes into equimolar quantities of the corresponding alcohols and carboxylic acids. This result has been verified also by our experimental work.

The $NADP^+$-dependent thermophilic alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH) is a medium-chain zinc-containing enzyme,[14,19] remarkably stable at temperatures up to 85 °C. TBADH is also a type-A enzyme,[20] which catalyzes the hydride transfer generally to the *Re* face of carbonyl compounds to afford the corresponding (*S*)-alcohols. TBADH reacts mainly with secondary alcohols, acyclic and cyclic ketones and primary alcohols.

Moraxella sp. TAE123 ADH is a psychrophilic alcohol dehydrogenase. From its molecular characteristics it has been classified in the longer-than-the-classical-type form of ADH families.[15] It has been characterized as a type-A enzyme.[21] *Moraxella* sp. TAE123 ADH exhibits wide substrate specificity, oxidizing mainly primary and secondary alcohols as well as bulky aromatic alcohols (such as benzhydrol). From our previous studies we have found that it reduces butan-2-one to (*S*)-butan-2-ol with high stereoselectivity.[21]

In this paper we demonstrate, by the use of high-resolution ^{1}H NMR spectroscopy, that all the above three ADHs catalyze the rapid dismutation of aldehydes to the corresponding alcohols and carboxylic acids. TBADH and *Moraxella* sp. TAE123 ADH represent the first examples of alcohol dehydrogenases derived from extremophilic bacteria to exhibit dismutase activity. This fact reinforces the view that the dismutation reaction is part of the physiological function of alcohol dehydrogenases.

Results and discussion

The ability of the selected dehydrogenases to catalyze the dismutation of aldehydes was assayed with acetaldehyde and propionaldehyde. According to the mechanism proposed by Oppenheimer,[8] dismutation proceeds without the net production of NADH. The most appropriate method to follow these spectrophotometrically silent reactions was ^{1}H NMR spectroscopy. All reactions were carried out in 5 mm NMR tubes and the spectra were acquired by using solvent (water) suppression with solvent presaturation. For acetaldehyde, the course of the reaction was followed by integrating the methyl hydrogen absorptions of all species present, *i.e.* acetaldehyde (δ 2.20) and the corresponding *gem*-diol (δ 1.29), acetic acid (δ 1.88) and ethanol (δ 1.14).

When we used higher coenzyme concentrations, we were also able to monitor the changes of concentration for the coenzyme $NAD(P)^+$ (NAD^+ C-2 and C-5 protons at δ 9.2 and 9.3 or $NADP^+$ C-2 and C-5 protons at δ 9.07 and 9.25, respectively) and its reduced form NAD(P)H (C-4 and C-3 protons of NADH at δ 2.6–2.7 and 6.9, respectively and C-4 and C-3 protons of NADPH at δ 2.7–2.8 and 6.9, respectively).

We verified the stability of the aldehydes, before any enzymic assay, by incubating them under the enzyme reaction conditions

† ^{1}H NMR spectra and graphs for the dismutation of aldehydes with alcohol dehydrogenases are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p1/b0/b001039l

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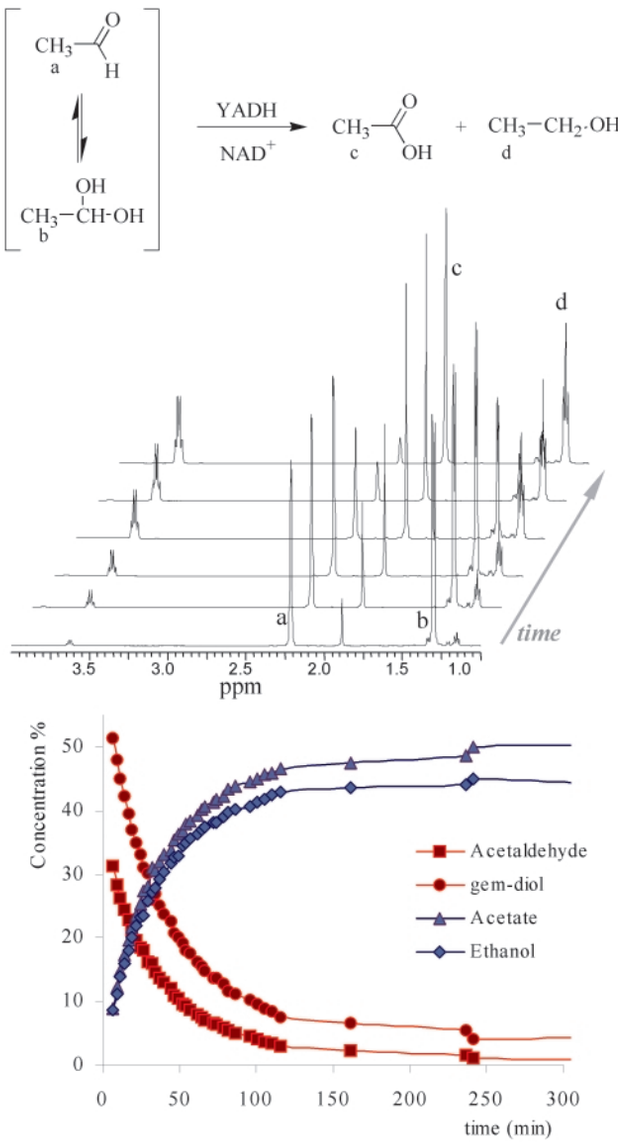

Fig. 1 Part of the ^{1}H NMR time-dependent spectrum showing the methyl proton absorptions in the dismutation of acetaldehyde catalyzed by *Saccharomyces cerevisiae* ADH.

(sodium phosphate buffer pH 6.0 to pH 9.0) without the presence of enzyme and/or coenzyme. Both acetaldehyde and propionaldehyde were shown to be stable at all conditions assayed for time periods longer that those of the enzymic reaction. The control experiments confirm beyond doubt that the observed chemical transformations of various substrates are due to an enzymic reaction and not to a chemical transformation independent of the enzyme.

All three ADHs were shown to act as aldehyde dismutases.

Fig. 1 shows the time-dependent ^{1}H NMR methyl hydrogen absorptions recorded for the dismutation of acetaldehyde with YADH. It is clear that the concentrations of acetaldehyde and the corresponding *gem*-diol decrease rapidly with the concomitant production of ethanol and acetic acid. The direct ^{1}H NMR determination of acetic acid concentration present in the enzymic reaction could not have been observed previously by UV spectroscopy.[18] These results indicate convincingly the stoichiometry of the dismutation reaction. By integrating the proper ^{1}H NMR signals, acetic acid and ethanol are produced in almost equimolar quantities (1.08/1.00) up to 90% conversion (115 min), while this ratio gradually reaches 1.14/1.00 at 95.5% conversion (320 min). These results are in agreement with the mechanism proposed by Oppenheimer.[8,11] The ability of YADH to catalyze the dismutation of acetaldehyde was also tested at different pH-values. We conclude (see Table 1) that the

Table 1 Acetic acid/ethanol quotient during the dismutation of acetaldehyde catalyzed by *Saccharomyces cerevisiae* ADH (YADH), *Thermoanaerobium brockii* ADH (TBADH) or *Moraxella* sp. TAE123 ADH (MADH)

ADH	pH	Conversion[a] (%)	[Acetic acid]/ [ethanol]
YADH	8.8	90	1.08
	7.4	75	1.1
TBADH	8.8	78.5	1.1
	7.4	40	1.23
MADH	8.8	63	1.6
	7.4	70	2.1
	6.0	55	1.9

[a] In the case of acetaldehyde the conversion was calculated from the sum of the methyl hydrogen absorptions of acetic acid and ethanol, *versus* the sum of the methyl hydrogen absorptions of reagents and products, *i.e.* acetaldehyde, *gem*-diol, acetic acid and ethanol.

mesophilic enzyme catalyzes the dismutation of acetaldehyde in a pH range from 7.4 to 9.0. The fact that YADH displays dismutase activity is in complete agreement with previous reports that indicated the *in vivo* participation of yeast ADH in the metabolism of the ethanol-derived acetaldehyde.[17]

The thermophilic *Thermoanaerobium brockii* ADH and the psychrophilic *Moraxella* sp. TAE123 ADH were also found to act as aldehyde dismutases when they reacted with acetaldehyde. This is the first example, as far as we know, of ADHs, derived from extremophilic bacteria, displaying aldehyde dismutase activity. As shown in Table 1, TBADH follows the same pattern with YADH, producing thus equimolar quantities of acetic acid and ethanol during the dismutation of acetaldehyde.

In the reaction catalyzed by *Moraxella* sp. TAE123 ADH, the ratio of acetic acid and ethanol favors acetic acid formation (Table 1). The dismutation of acetaldehyde with the psychrophilic enzyme was also performed at different pH and temperature conditions, resulting always in larger quantities of acetic acid (Table 1). The excessive formation of acetic acid is attributed to a second reaction, namely the oxidation of ethanol. To confirm this assumption, we studied the relative rates of the reactions of the three ADHs with ethanol and acetaldehyde by using the same experimental conditions. Both TBADH and YADH showed a faster turnover number V for the dismutation of acetaldehyde. The same pattern in kinetic behavior was previously reported for the dismutation of butyraldehyde compared to the oxidation of butanol with HLADH.[5,9] This fact was attributed to the coupled *ping-pong* mechanism, which bypasses the slow steps of enzyme dissociation. It is constructive to emphasize here that, unlike the other ADHs, the psychrophilic *Moraxella* sp. TAE123 ADH catalyzes both reactions, production of acetic acid and ethanol, with comparable rates.

Furthermore, we have also studied the reaction after the depletion of substrates. The overincubation of the ADHs mentioned above with ethanol and acetic acid was studied using stoichiometric coenzyme concentrations and resulted in the complete conversion of ethanol to acetic acid. Therefore, it is evident that these ADHs can catalyze the sequential oxidation of ethanol to acetic acid. These results provide a further confirmation of the mechanism proposed by Oppenheimer and coworkers.[8,11] According to this mechanism aldehyde dismutation and aldehyde oxidation are part of the same catalytic process and can be viewed at different times along the assay-progress curve.

With the use of higher coenzyme concentrations we were also able to monitor the NADH production. Although in the mesophilic and thermophilic ADH the duration of the lag phase was found to be dependent on both acetaldehyde and enzyme concentration, in the case of the psychrophilic ADH no lag phase was observed.

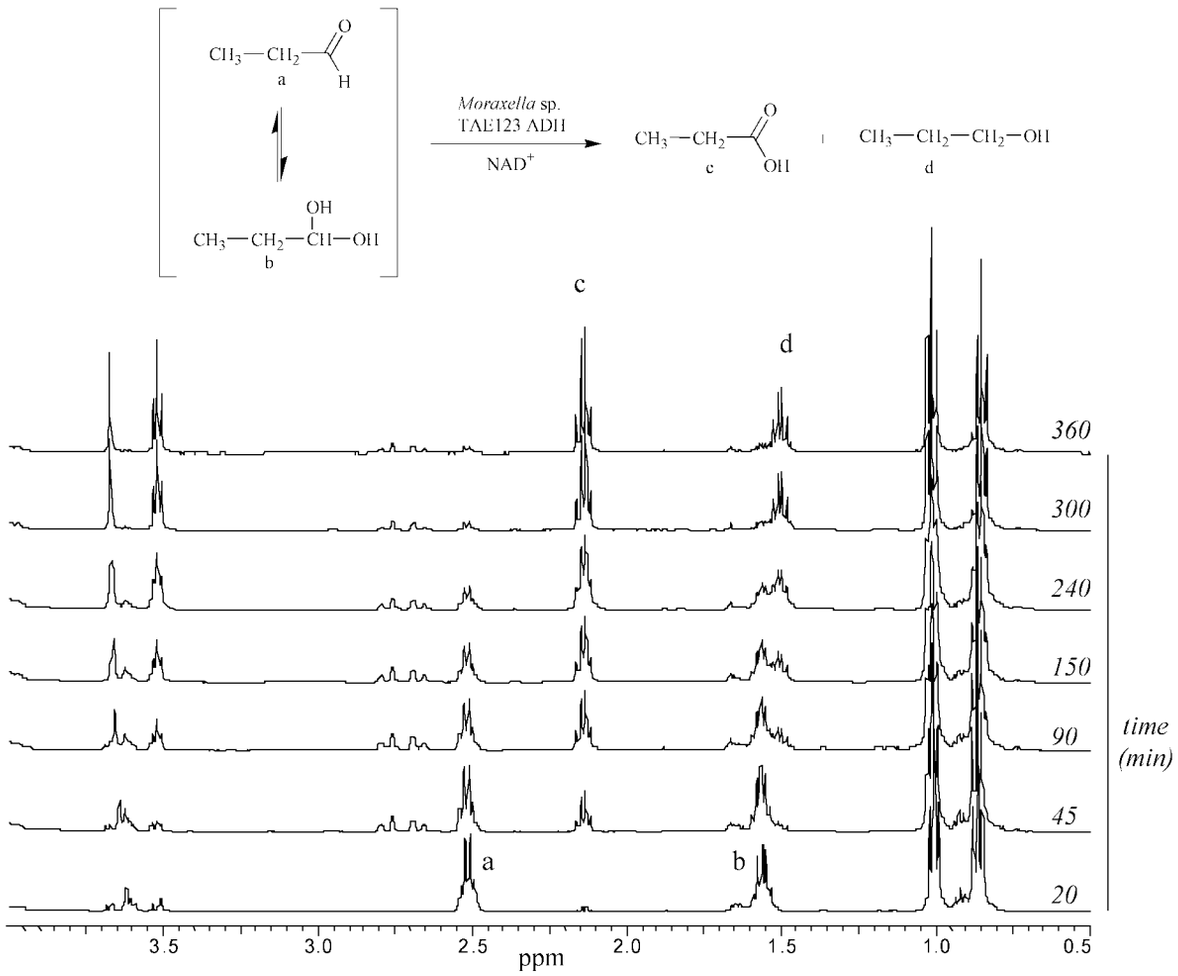

Fig. 2 Part of the ^{1}H NMR time-dependent spectrum showing the methylene proton absorption in the dismutation of propionaldehyde catalyzed by the psychrophilic *Moraxella* sp. TAE123 ADH.

The dismutation of aldehydes with the above ADHs was also studied using propionaldehyde and benzaldehyde as substrates. In the case of propionaldehyde, the reaction was followed by ^{1}H NMR integration of the methylene hydrogen absorptions of all species present in the reaction mixture; namely propionaldehyde (δ 2.52) and the corresponding *gem*-diol (δ 1.55–1.6), propionic acid (δ 2.12) and propan-1-ol (δ 1.5). All ADHs studied here were able to catalyze the dismutation of propionaldehyde with the same catalytic behavior as that in the dismutation of acetaldehyde. It is clear from the stacked portions of the ^{1}H NMR spectra, presented in Fig. 2, that the psychrophilic ADH produces larger quantities of propionic acid than propan-1-ol. These results were again attributed to the parallel oxidation of propan-1-ol, which is produced during the dismutation.

In the case of benzaldehyde the direct detection of products was not possible by using ^{1}H NMR spectroscopy. The reaction profile was concluded by the detection of the NADH burst, which accompanies dismutation reactions. For this purpose higher enzyme concentrations were used. When benzaldehyde was incubated under standard dismutation conditions in the absence of enzyme no net production of NADH was detected. YADH and *Moraxella* sp. TAE123 ADH were found to catalyze the dismutation of benzaldehyde (while with the thermophilic TBADH no net NADH production was detected).

Conclusion

Our results on the dismutation of aldehydes catalyzed by three different ADHs conclusively establish that they display aldehyde dismutase activity. Since these ADHs belong to different living organisms, which exhibit different temperature adaptations, this type of reaction may be considered as a general property of alcohol dehydrogenases.

Experimental

Materials and methods

YADH and TBADH were purchased from Sigma Chemical Co. The psychrophilic *Moraxella* sp. TAE123 ADH was purified according to the literature method.[15] All other chemicals, including the oxidized and reduced form of the coenzyme NAD(P)H, were purchased from Sigma and Aldrich in the highest available purity.

^{1}H NMR spectra were recorded in a 500 MHz Bruker AMX spectrometer. All spectra were acquired in 10% D_2O, using solvent suppression, with solvent presaturation. *J*-Values are given in Hz.

Standard assay for the dismutation reaction

The standard assay mixture (0.6 ml) for the dismutation reaction consisted of 50 mM sodium phosphate buffer, 15 mM aldehyde, 1.5 mM NAD(P)$^+$ and 10% D_2O. The reaction was initiated by addition of the corresponding alcohol dehydrogenase and was followed by ^{1}H NMR spectroscopy (500 MHz) using solvent suppression with solvent presaturation. For each alcohol dehydrogenase the quantities of the reagents present in the reaction mixture were modified in order to achieve completion of the reaction in a maximum time period of approximately 12 h.

In the case of acetaldehyde the course of the reaction was followed by integrating the methyl proton absorptions of all

organic species present in the reaction mixture. Acetaldehyde: δ_H 9.64 (1H, q, *J* 2.9, C**H**=O), 2.20 (3H, d, *J* 2.9, C**H**$_3$); *gem*-diol: δ_H 5.21 [1H, q, *J* 5.2, C**H**(OH)$_2$], 1.29 (3H, d, *J* 5.2, C**H**$_3$); acetate: δ_H 1.88 (3H, s, C**H**$_3$); ethanol: δ_H 3.62 (2H, q, *J* 7.1, C**H**$_2$), 1.14 (3H, t, *J* 7.1, C**H**$_3$). In this reaction the conversion was calculated from the sum of the methyl hydrogen absorptions of acetate and ethanol, *versus* the sum of the methyl hydrogen absorptions of reagents and products, *i.e.* acetaldehyde, *gem*-diol, acetate and ethanol.

In the case of propionaldehyde the reaction was followed by integrating the methylene proton absorptions of all organic species present in the reaction mixture. Propionaldehyde: δ_H 9.64 (1H, t, *J* 1.0, C**H**=O), 2.52 (2H, q, *J* 7.3, C**H**$_2$), 1.01 (3H, t, *J* 7.3, C**H**$_3$); *gem*-diol: δ_H 5.4 [1H, q, *J* 5.5, C**H**(OH)$_2$], 1.55 (2H, m, *J* 7.4, C**H**$_2$), 0.87 (3H, t, *J* 7.5, C**H**$_3$); propionate: δ_H 2.12 (2H, q, *J* 7.6, C**H**$_2$), 0.97 (3H, t, *J* 7.6, C**H**$_3$); propan-1-ol: δ_H 3.52 (2H, t, *J* 6.6, C**H**$_2$OH), 1.50 (2H, m, *J* 7.1 C**H**$_2$), 0.84 (3H, t, *J* 7.4, C**H**$_3$). In this reaction the conversion was calculated from the sum of the methylene hydrogen absorptions of propionate and propan-1-ol, *versus* the sum of the methylene hydrogen absorptions of reagents and products, *i.e.* propionaldehyde, *gem*-diol, propionate and propan-1-ol.

Standard assay for the overincubation experiments during the dismutation of acetaldehyde

During the overincubation experiments the standard assay for the dismutation reaction was followed, using stoichiometric coenzyme concentrations. Thus the assay mixture (0.6 ml) consisted of 50 mM sodium phosphate buffer, 15 mM acetaldehyde, 15 mM NAD(P)$^+$ and 10% D_2O. The reaction was initiated by addition of the corresponding alcohol dehydrogenase and was followed by ^{1}H NMR spectroscopy (500 MHz) using solvent suppression with solvent presaturation. The reactions were followed until the depletion of ethanol.

Dismutation reactions catalyzed by YADH

Dismutation of acetaldehyde. The dismutation of acetaldehyde catalyzed by YADH was performed under the standard assay conditions for dismutation reactions, using 0.1 U ml^{-1} of *Saccharomyces cerevisiae* ADH at pH 8.8, 7.4 and 6.0. The reactions were performed at room temperature, and were followed by ^{1}H NMR spectroscopy. For the dismutation of acetaldehyde at pH 8.8 ^{1}H NMR spectra were acquired every 2.5 min for the first 2 h of the reaction, and then every hour until the completion of the reaction.

Dismutation of propionaldehyde. The dismutation of propionaldehyde catalyzed by YADH was performed under the standard assay conditions for dismutation reactions, using 0.1 U ml^{-1} of *Saccharomyces cerevisiae* ADH at pH 8.8, 7.4 and 6.0. The reactions were performed at room temperature, and were followed by ^{1}H NMR spectroscopy (500 MHz). For the dismutation of propionaldehyde at pH 8.8 ^{1}H NMR spectra were acquired every 16 min for the first 4 h of the reaction, and then every hour until the completion of the reaction.

Dismutation of benzaldehyde. The dismutation of benzaldehyde catalyzed by YADH was performed under the standard assay conditions for dismutation reactions, using 1.0 U ml^{-1} of *Saccharomyces cerevisiae* ADH at pH 8.8 and stoichiometric NAD$^+$ concentration (15 mM). The reactions were performed at room temperature, and were followed by ^{1}H NMR spectroscopy. The reaction's outcome was deduced from the production of NADH (C-4 proton, dd, δ 2.6). For this reaction, ^{1}H NMR spectra were acquired every hour for a time period of 12 h.

Dismutation reactions catalyzed by *Moraxella sp.* TAE123 ADH

Dismutation of acetaldehyde. The dismutation of acetaldehyde catalyzed by *Moraxella* sp. TAE123 ADH was performed under the standard assay conditions for dismutation reactions, using 0.01 U ml^{-1} of *Moraxella* sp. TAE123 ADH at pH 8.8, 7.4 and 6.0. The reactions were performed at 30 °C and 0 °C and were followed by ^{1}H NMR spectroscopy (500 MHz). For the dismutation of acetaldehyde at pH 8.8 ^{1}H NMR spectra were acquired every 2 min until 50% conversion, and then every 30 min until completion of the reaction (6–8 h).

Dismutation of propionaldehyde. The dismutation of propionaldehyde catalyzed by *Moraxella* sp. TAE123 ADH was performed under the standard assay conditions for dismutation reactions, using 0.01 U ml^{-1} of *Moraxella* sp. TAE123 ADH at pH 8.8. The reactions were performed at 30 °C and 0 °C and were followed by ^{1}H NMR spectroscopy (500 MHz). For the dismutation of propionaldehyde at pH 8.8 ^{1}H NMR spectra were acquired every 4 min until 50% conversion, and then every hour until completion of the reaction.

Dismutation of benzaldehyde. The dismutation of benzaldehyde catalyzed by *Moraxella* sp. TAE123 ADH was performed under the standard assay conditions for dismutation reactions, using 0.1 U ml^{-1} of *Moraxella* sp. TAE123 ADH at pH 8.8 and stoichiometric NAD$^+$ concentration (15 mM). The reaction was performed at room temperature, and followed by ^{1}H NMR spectroscopy. The reaction's outcome was deduced from the production of NADH (C-4 proton, dd, δ 2.67). For this reaction, ^{1}H NMR spectra were acquired every hour for a time period of 12 h.

Dismutation reactions catalyzed by TBADH

Dismutation of acetaldehyde. The dismutation of acetaldehyde catalyzed by TBADH was performed under the standard assay conditions for dismutation reactions, using 0.1 U ml^{-1} of *Thermoanaerobium brockii* ADH at pH 8.8. The reactions were performed at 30 °C and were followed by ^{1}H NMR spectroscopy (500 MHz). For the dismutation of acetaldehyde at pH 8.8 ^{1}H NMR spectra were acquired every 4 min for 3 h, and then every hour until completion of the reaction.

Dismutation of propionaldehyde. The dismutation of propionaldehyde catalyzed by TBADH was performed under the standard assay conditions for dismutation reactions, using 0.1 U ml^{-1} of *Thermoanaerobium brockii* ADH at pH 8.8. The reactions were performed at 30 °C and were followed by ^{1}H NMR spectroscopy (500 MHz). For the dismutation of propionaldehyde at pH 8.8 ^{1}H NMR spectra were acquired every 16 min for the first 4 h, and then every hour until completion of the reaction.

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References

1 K. Faber, *Biotransformations in Organic Chemistry*, Springer-Verlag, Berlin, 1997, pp. 160–206.
2 (*a*) J. B. Jones, *Tetrahedron*, 1986, **42**, 3351; (*b*) S. Servi, *Synthesis*, 1990, 1; (*c*) W. Hummel and M.-R. Kula, *Eur. J. Biochem.*, 1989, **184**, 1.
3 H. Sund and M. Theorell, *Alcohol Dehydrogenase*, in The *Enzymes*, ed. P. D. Boyer, H. Lardy and K. Myrback, Academic Press, New York, 2nd edn., 1960, vol. VII, pp. 25–83.
4 R. H. Abeles and H. A. Lee, *J. Biol. Chem.*, 1960, **235**, 1499.
5 K. Dalziel and F. M. Dickinson, *Nature*, 1965, **206**, 255.
6 N. K. Gupta and W. G. Robinson, *Biochim. Biophys. Acta*, 1966, **118**, 429.
7 F. M. Dickinson and G. P. Monger, *Biochem. J.*, 1973, **131**, 261.

8 G. T. M. Henehan and N. J. Oppenheimer, *Biochemistry*, 1993, **32**, 735.
9 G. L. Shearer, K. Kim, K. M. Lee, K. Wang and B. V. Plapp, *Biochemistry*, 1993, **32**, 11186.
10 L. P. Olson, J. Luo, Ö. Almarsson and T. C. Bruice, *Biochemistry*, 1996, **35**, 9782.
11 G. T. M. Henehan, S. H. Chang and N. J. Oppenheimer, *Biochemistry*, 1995, **34**, 12294.
12 S. Svensson, A. Lundsjö, T. Cronholm and J.-O. Höög, *FEBS Lett.*, 1996, **394**, 217.
13 K. Nakamura, T. Miyai, J. Kawai, N. Nakajima and A. Ohno, *Tetrahedron Lett.*, 1990, **31**, 1159.
14 R. Lamed and J. G. Zeikus, *Biochem. J.*, 1981, **195**, 183.
15 I. Tsigos, K. Velonia, I. Smonou and V. Bouriotis, *Eur. J. Biochem.*, 1998, **254**, 356.
16 (*a*) V. Prelog, *Pure Appl. Chem.*, 1964, **9**, 119; (*b*) R. MacLeod, H. Prosser, L. Fikentscher, J. Lanyi and H. S. Mosher, *Biochemistry*, 1964, **3**, 838.
17 J. Thielen and M. Ciriacy, *J. Bacteriol.*, 1991, **173**, 7012.
18 S. Trivić, V. Leskova and G. W. Winston, *Biotechnol. Lett.*, 1999, **21**, 231.
19 R. Lamed and J. G. Zeikus, *J. Bacteriol.*, 1980, **141**, 1251.
20 M. Peretz, O. Bogin, E. Keinan and Y. Burstein, *Int. J. Pept. Protein Res.*, 1993, **42**, 490.
21 K. Velonia, I. Tsigos, V. Bouriotis and I. Smonou, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 65.