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Stereospecificity of hydride transfer during the dismutation of aldehydes catalyzed by alcohol dehydrogenases

K. Velonia and I. Smonou*

Department of Chemistry, University of Crete, 71 409 Heraklio, Crete, Greece

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Abstract—The stereochemistry of the oxidation of aldehydes to acids with alcohol dehydrogenases was studied with respect to the selectivity towards the cofactor. © 2002 Published by Elsevier Science Ltd.

1. Introduction

The stereoselectivity in the oxidation of alcohols to the corresponding ketones or aldehydes and the reduction of these carbonyl compounds to the corresponding alcohols with alcohol dehydrogenases (ADHs) has been well established through numerous reports in the literature.[1–5] While it was previously thought that aldehydes are not substrates for oxidative reactions, it has recently been shown that ADHs can also catalyze the dismutation of aldehydes to the corresponding alcohols and carboxylic acids.[6]

One of the main factors which determine the selectivity of ADH-catalyzed transformations is the stereospecificity of hydride transfer to and from C(4) of the nicotinamide ring of the NAD(H) cofactor. With respect to this specificity, ADHs have been classified into two types: Type A enzymes which catalyze hydride transfer to the *re* face of the cofactor and Type B enzymes which do so to the *si* face.

Herein, we report our stereochemical studies on the oxidation of aldehydes to acids with ADHs with respect to the selectivity towards the cofactor and we compare this selectivity to that determined for the oxidation of alcohols by the same enzymes.

2. Results and discussion

The dismutation of aldehydes, according to the mechanism proposed by Oppenheimer[7] (Fig. 1), suggests that the overall reaction consists of two parallel paths: the oxidation of the aldehyde, which releases the corresponding carboxylic acid and the reduction of a second

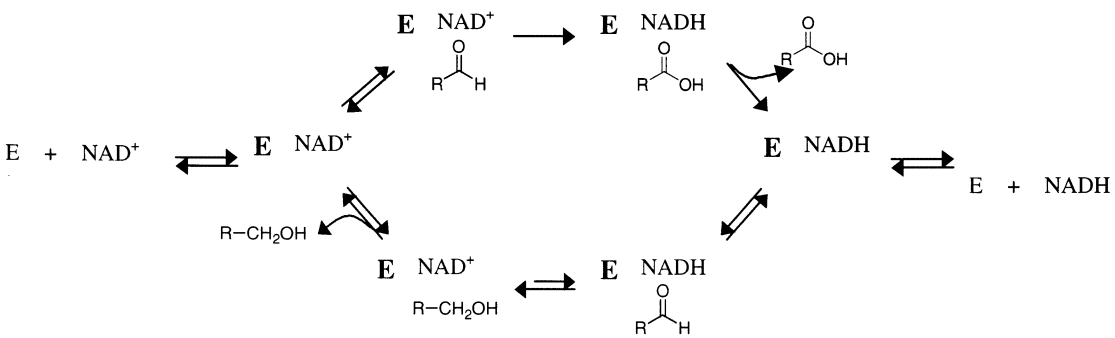

Figure 1. Proposed mechanism for the ADH-catalyzed dismutation of aldehydes.

* Corresponding author. Tel.: +30-81-393610; fax: +30-81-393601; e-mail: smonou@chemistry.uoc.gr

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molecule of the aldehyde to the corresponding alcohol. A lag phase concerning the release of reduced coenzyme has been observed in the enzymes from Horse Liver (HLADH),[8] *Saccharomyces cerevisiae* (YADH)[6,9] and *Thermoanaerobium brockii* (TBADH).[6] In the case of the psychrophilic *Moraxella* sp. TAE 123,[6] oxidation of the alcohol produced in the dismutation step was found to be faster, it being almost concomitant with the dismutation release of the reduced form of the cofactor.

In this study we report on the stereochemistry of the hydride transfer during the dismutation of aldehydes catalyzed by the alcohol dehydrogenases from *S. cerevisiae* (YADH), *T. brockii* (TBADH) and *Moraxella* sp. TAE123. In all the reactions acetaldehyde-1-d_1 was used as the substrate. The reactions were followed by ^{1}H NMR spectroscopy and their stereochemistry was deduced from the structure of the reduced cofactor released from the reaction.

According to the mechanism proposed by Oppenheimer (Fig. 1), in the first step of the dismutation one molecule of aldehyde is oxidized to give the corresponding carboxylic acid. If this reaction is stereoselective, (4*R*)-[4D]-NADD would be expected to be formed from attack of NAD^+ to the *re*-face, while (4*S*)-[4D]-NADD would be formed from *si*-facial attack (Fig. 2a). Since the enzymes used in these experiments were type A alcohol dehydrogenases, we expected that the second step of the dismutation of acetaldehyde-1-d_1 (reduction to ethanol) would proceed according to this stereoselectivity, i.e. transfer of the (4*R*)-H/D to the *re* face of acetaldehyde (Fig. 2b).

In the case of the YADH-catalyzed dismutation of acetaldehyde-1-d_1 we followed the release of the reduced form of the cofactor (NADD) by ^{1}H NMR spectroscopy (Fig. 3A). In order to deduce the stereochemistry of the reaction, we compared the chemical shifts of the characteristic peak of the proton at C(4) of NADD produced in this reaction with the corresponding proton of NADH and (4*R*)-[4D]-NADD produced by the HLADH-catalyzed oxidation of ethanol-d_6. The NADD released in the YADH-catalyzed dismutation of acetaldehyde-1-d_1 was found to contain only one proton at C(4) of the nicotinamide ring with a chemical shift identical to that of the (4*S*) proton at C(4) of the (4*R*)-[4D]-NADD produced by the HLADH-catalyzed oxidation of ethanol-d_6. This result clearly shows that in the oxidative step of the dismutation of aldehydes, YADH follows the same stereospecificity as in the oxidation of alcohols.

Similar results were obtained during the TBADH-catalyzed dismutation of acetaldehyde-1-d_1 (Fig. 3B). In this case, we monitored the release of NADPD that contained only one proton at C(4) of the nicotinamide ring with chemical shift (s, 6.9 ppm) identical to that of the corresponding proton of (4*R*)-[4D]-NADD. We therefore concluded that the hydride transfer during TBADH-catalyzed dismutation reactions follows the same stereochemistry with those of the YADH reactions.

During the catalyzed dismutation of acetaldehyde-1-d_1 by the psychrophilic *Moraxella* sp. TAE123 we observed the release of both NADH and NADD, even in very low reaction yields, in comparable amounts. (Fig. 3C,D). By integrating these peaks and comparing the integrations with the corresponding ones of the unlabelled NADH, we concluded that the released coenzyme is actually a mixture of (4*R*)-[4D]-NADD, (4*S*)-[4D]-NADD and NADH. This stereochemical outcome can only be explained by a non-selective oxi-

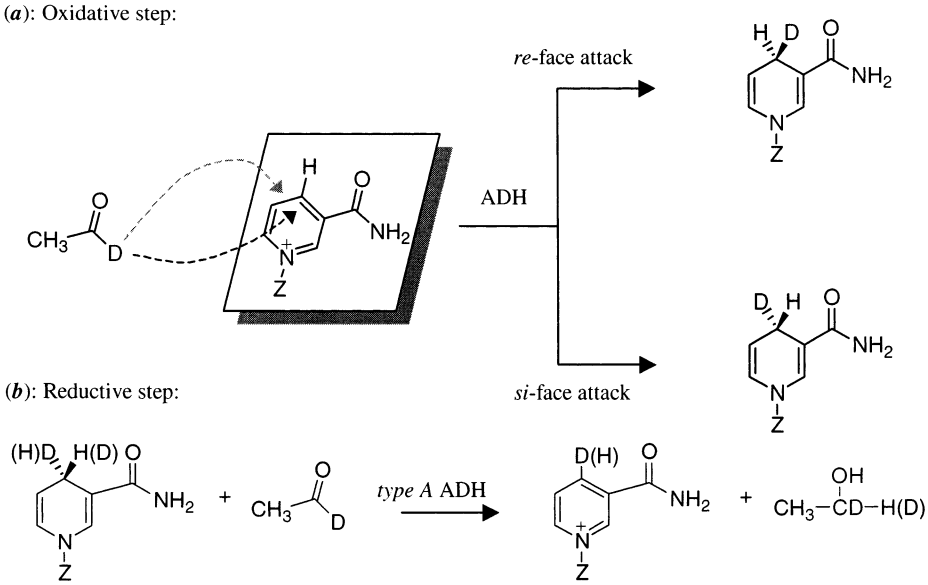

Figure 2. (a) Possible stereochemical outcome of hydride transfer to the *re*- or *si*-face of NAD^+ during the oxidative step of the ADH-catalyzed dismutation of acetaldehyde-1-d_1. (b) Type A ADH stereoselectivity expected on the reductive step of the dismutation.

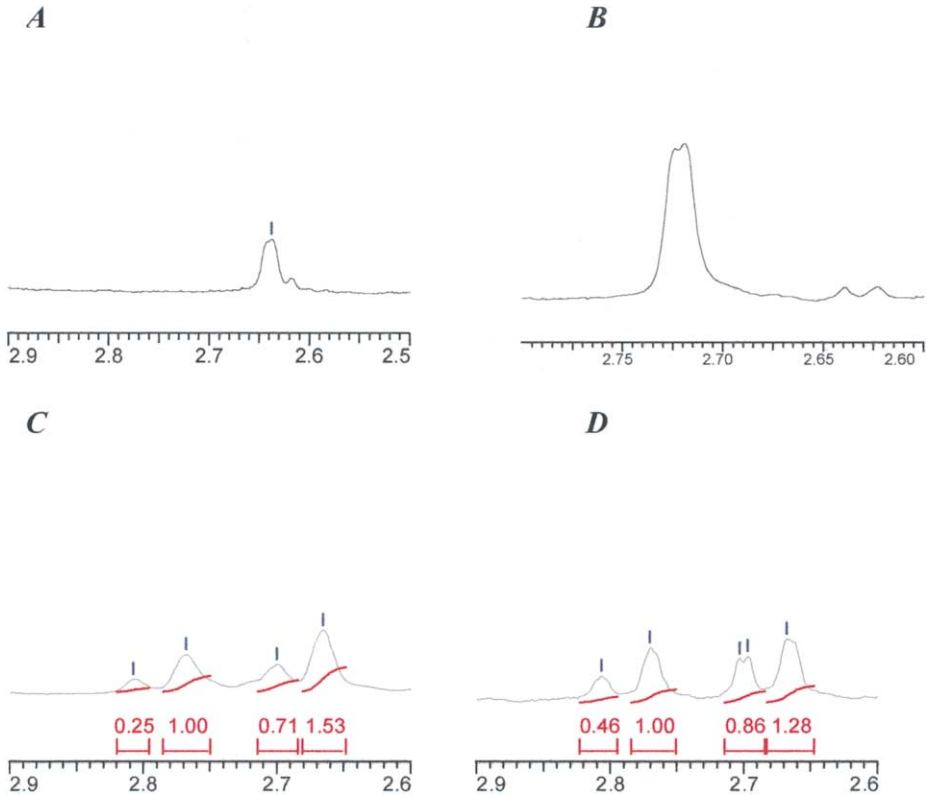

Figure 3. ^{1}H NMR spectra of the region of C(4) NAD(P)D produced during the dismutation of acetaldehyde-1-d_1 catalyzed by (A) YADH, (B) TBADH, (C) *Moraxella* sp. TAE 123 ADH at ~10% conversion and (D) *Moraxella* sp. TAE 123 ADH at ~40% conversion.

dative step during the dismutation of acetaldehyde-1-d_1 which leads to the formation of both (4*R*)- and (4*S*)-[4D]-NADD (Fig. 2a). Since the psychrophilic ADH is a type A enzyme it is expected to transfer the pro-*R* hydrogen of (4*S*)-[4D]-NADD and the pro-*R* deuterium of (4*R*)-[4D]-NADD to acetaldehyde-1-d_1 in the reductive step of the dismutation (Fig. 4a). Therefore, in this step, a mixture of ethanol-1,1-d_2 and ethanol-1d_1 is formed, while the oxidized form of NAD^+ released will be only partially labeled. As mentioned in Section 1, the psychrophilic *Moraxella* sp. TAE 123 catalyzes the dismutation of aldehydes in rates comparable to the oxidation of the produced alcohols. It is therefore expected that the mixture of ethanol-1,1d_2 and ethanol-1d_1 will in turn be oxidized to acetaldehyde and release all forms of the reduced coenzyme (Fig. 4b).

3. Conclusion

In conclusion, both YADH and the thermophilic TBADH exhibit the same stereospecificity as that seen in the oxidative step of the dismutation of acetaldehyde as in the oxidation of alcohols, i.e. they catalyze the pro-*R* hydride transfer from C(4) of the nicotinamide ring of NADH. Surprisingly, the psychrophilic *Moraxella* sp. TAE 123 ADH does not exhibit any specificity in the oxidation of acetaldehyde to acetic acid.

4. Experimental

4.1. Materials and methods

YADH and TBADH were purchased from Sigma Chemical Co. The psychrophilic *Moraxella* sp. TAE123 ADH was purified according to the method reported in the literature. All other chemicals, including the oxidized and reduced forms of the coenzymes 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 reported in Hz.

4.2. 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 acetaldehyde-1-d_1, 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) by using solvent suppression with solvent presaturation.

The course of the reaction was followed by integrating the methyl proton absorptions of all the organic species present in the reaction mixture.

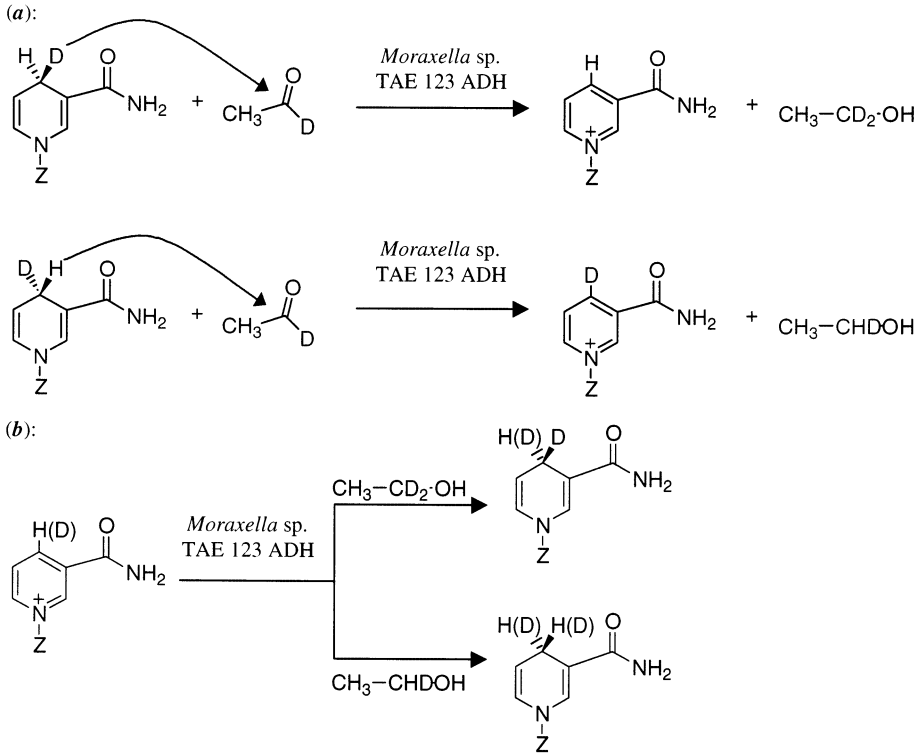

Figure 4. (a) Type A ADH stereospecific hydride equivalent transfer, during the reductive step of the *Moraxella* sp. TAE 123-catalyzed dismutation of acetaldehyde-1-d_1. (b) Stereospecificity during the concomitant oxidation of the mixture of ethanol-d_1 and ethanol-d_2 produced during the oxidative step of dismutation.

Acetaldehyde-1d_1: δH 2.20 (3H, d, J 2.9, CH_3); ***gem*-diol**: δH 1.30 (3H, d, J 5.2, C H_3); **acetate**: δH 1.88 (3H, s, C H_3); **ethanol**: δH 1.14 (3H, t, J 7.1, C H_3).

The conversion was calculated from the sum of the integrals of the methyl hydrogen absorptions of acetate and ethanol, versus the sum of the integrals of the methyl hydrogen absorptions of reagents and products, i.e. acetaldehyde, *gem*-diol, acetate and ethanol.

4.3. Dismutation of acetaldehyde-1-d_1 catalyzed by YADH

The dismutation of acetaldehyde-1-d_1 catalyzed by YADH was performed under standard assay conditions for dismutation reactions, by using 0.1 U mL^{-1} of *S. cerevisiae* ADH at pH 8.8. The reaction was performed at 30°C. ^{1}H NMR spectra were acquired every 2.5 min until the observation of NADD release and every hour until the completion of the reaction.

4.4. Dismutation of acetaldehyde-1-d_1 catalyzed by *Moraxella* sp. TAE123 ADH

The dismutation of acetaldehyde-1-d_1 catalyzed by *Moraxella* sp. TAE123 ADH was performed under standard assay conditions for dismutation reactions, by using 0.01 U mL^{-1} of *Moraxella* sp. TAE123 ADH at pH 8.8. The reactions were performed at 30 and 0°C and were followed by ^{1}H NMR spectroscopy (500 MHz). Spectra were acquired every 2 min until 50% conversion, and then every 30 min until completion of the reaction (6–8 h).

4.5. Dismutation of acetaldehyde catalyzed by TBADH

The dismutation of acetaldehyde-1-d_1 catalyzed by TBADH was performed under the standard assay conditions for dismutation reactions, by using 0.1 U mL^{-1} of *T. 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.

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