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Stereospecificity of Hydrogen Transfer by the NAD+-linked Alcohol Dehydrogenase from the Antarctic Psychrophile *Moraxella* sp. TAE123.

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Abstract

Investigation of the stereochemistry of the hydride transfer in reactions catalyzed by the recently isolated NAD+-linked alcohol dehydrogenase from the Antarctic psychrophile *Moraxella* sp. TAE123 was accomplished by using ^{1}H NMR spectroscopy of the deuterated coenzyme. It was found that this new psychrophilic enzyme is a type A dehydrogenase. *Moraxella* sp. ADH reduces stereospecifically 2-butanone to produce (S)-2-butanol.

Keywords: stereochemistry; stereospecificity; enzymes and enzyme reactions; coenzymes

The prospect of expanding the limits of biocatalysis and, in turn, the range of associated applications, has led recently numerous scientists to investigate enzymes isolated from organisms inhabiting unconventional ecosystems.[1, 2]

It has been shown that enzymes from ectothermic organisms have considerably greater activity, accompanied with shifted thermal stability, than the homologous enzymes produced by homeotherms.[3, 4] Although no crystallographic structures are available, many structure-stability studies have been accomplished by molecular modelling in order to elucidate the thermodynamic properties of these extremophiles.[5] The psychrophilic enzymes possess a more flexible structure which is necessary to accommodate slower catalytic rates at reduced temperatures.[4] In the last few years, the potential applications and the molecular characteristics of psychrophiles have been extensively studied.[6]

In an effort to investigate the potential applications of psychrophilic enzymes in asymmetric synthesis, we have recently isolated and purified to homogeneity a new alcohol dehydrogenase from the antarctic psychrophile *Moraxella* sp. TAE123.[7] From initial screening studies, this new enzyme showed an attractive substrate specificity.[7] This enzyme displayed mainly oxidizing activity towards primary and secondary aliphatic alcohols, as shown below.[7] Interestingly, it can oxidize benzhydrol[7], while HLADH

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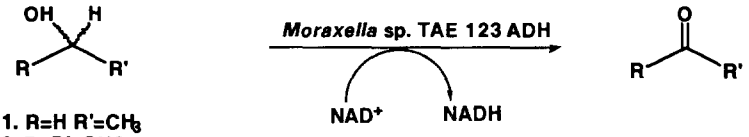
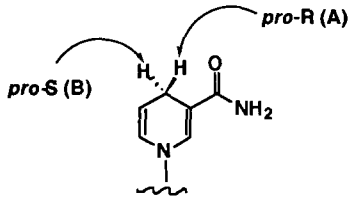
(mesophilic) and TBADH (thermophilic) do not.



1. R=H R'=CH_3
2. R=R'=CH3
3. R=CH_3 R'=CH_2CH_3
4. R=CH_2CH_3 R'=$(CH_3)_2C$
5. R=R'=Ph
6. R=H R'=$PhCH_2$

In order to determine whether the alcohol dehydrogenase from the psychrophilic bacteria *Moraxella* sp. TAE123 is a type A or type B enzyme, we studied the coenzyme face stereospecificity in the hydride equivalent transfer from the alcohol to the NAD^+. The stereospecificity of this transfer was established by comparing the ^1H NMR spectrum of NADD, obtained from the oxidation of ethanol-d_6 with *Moraxella* sp. TAE123 ADH, with the corresponding spectra of the unlabelled NADH and of that of (4R)-[4D]-NADH that was prepared from the reduction of NAD^+ with HLADH - a type A specific enzyme-using ethanol -d_6 as a substrate.



The NMR clearly shows that the NADD generated in the reaction with the psychrophilic ADH contains only one hydrogen at C4 of the pyridine ring of this reduced nicotinamide cofactor, and that the resonance of this hydrogen is identical with that of the 4-hydrogen in (4R)-[4D]-NADH generated by HLADH. Thus, we conclude that this enzyme catalyzes the hydrogen transfer to the re-face of NAD^+, i.e., it is a type-A dehydrogenase.

To establish the face stereospecificity of the hydride transfer from NADH to the carbonyl substrate, we investigated the reduction of 2-butanone with NADH. The reaction mixture contained 10mM potassium phosphate buffer (pH 6.0), 1.5mM NADH, 20mM 2-butanone and 0.1 units *Moraxella* sp. TAE 123. The reaction was held at 0° C and its progress was followed by GC analysis. The products were analyzed with chiral gas chromatography by using a HP Chiral column (20% permethylated B-Cyclodextrin, 30m x 0.25mm). The identification of the two alcohol signals was accomplished by comparison of the corresponding S- and R-2-butanol signals produced in the reduction of 2-butanone with HLADH[8]. This analysis showed that up to 16% conversion the only product observed was (S)-2-butanol (>99%ee).

Furthermore, we studied the oxidation of racemic 2-butanol with *Moraxella* sp. ADH and determined the enantiomeric purity of the remaining alcohol. The reaction mixture contained 20mM tris-HCl buffer (pH 7.4), 10mM $CaCl_2$, 1.5mM NAD^+, 30mM 2-butanol and 0.1 units *Moraxella* sp. TAE 123. It was held at 0°C and monitored by G.C. Chiral gas chromatographic analysis showed that the remaining alcohol

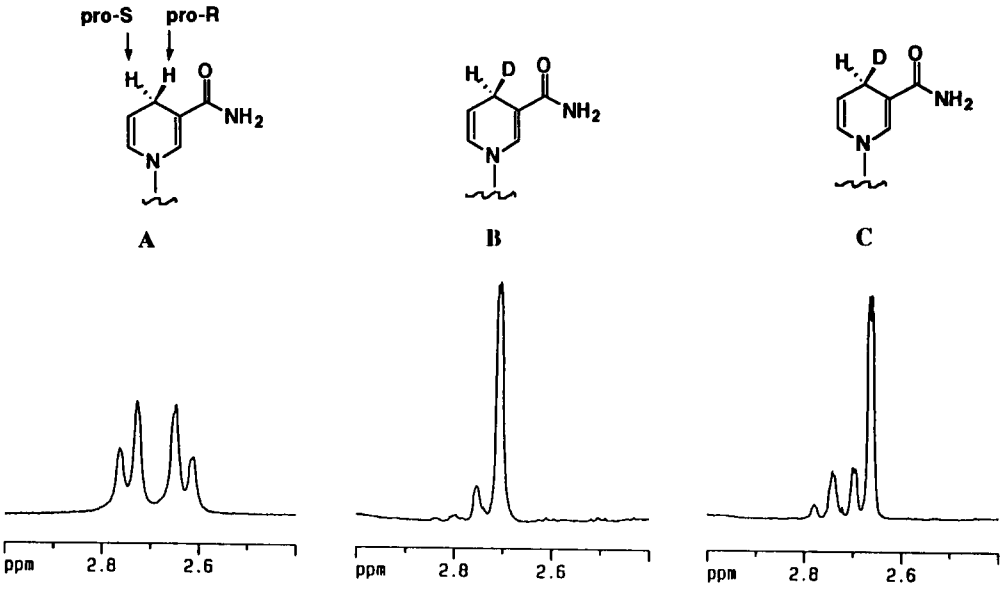
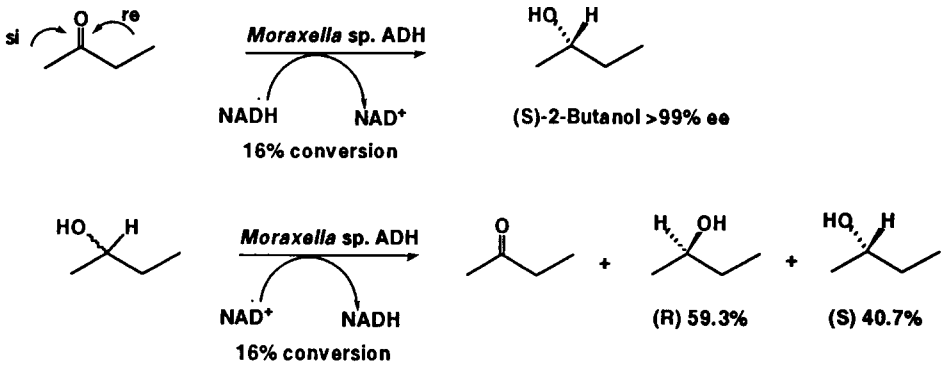


FIGURE 1. ^{1}H NMR (500MHz) spectra of the hydrogen at C4 of NADH in D_2O, with solvent supression. (A) commercially available NADH. (B) NADD generated from the reduction of NAD^+ with HLADH, using EtOH-d_6. (C) NADD obtained from the reduction of NAD^+ with *Moraxella* sp. TAE 123 ADH, using EtOH-d_6. The commercially available NAD^+ used in cases B and C contained 4% EtOH.

was enriched in the R enantiomer, with a 56.5/43.5 ratio of (R)/(S) enantiomers at 13.4% conversion, and 59.3/40.7 ratio at 16% conversion.



These results clearly indicate the high enantiopreference (>99%ee) of *Moraxella* sp. TAE ADH for the S-2-butanol, which is in agreement with the face enantiospecificity of this new alcohol dehydrogenase.

It is interesting to point out that the above observed enantiospecificity of psychrophilic *Moraxella* sp. ADH is much higher than the corresponding enantiospecificities of mesophilic HLADH (72.8%ee)[8] and YADH (67%ee)[9] and that of the thermophilic TBADH (48%ee).[10]

In conclusion, we have shown that the psychrophilic NAD^+ dependent enzyme *Moraxella* sp. TAE123 is a type-A enzyme and catalyzes stereospecifically the transfer of the pro-*R* hydrogen from the pyridine 4-position of the reduced coenzyme to the re face of the carbonyl substrate, to produce the (S)-alcohol with 99%ee.

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