Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supporting Information

Modulating the catalytic activity and substrate specificity of alcohol dehydrogenases by cyclic ethers

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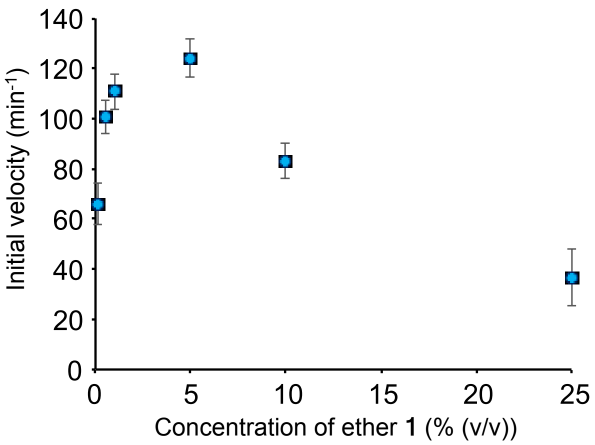
The effect of ether 1 concentration on the 2-butanol oxidation by *Tb*ADH

Oxidation of 2-butanol by TbADH in the presence of various concentrations of ether **1** was monitored. The activity was significantly increased even in the presence of 0.5% (v/v) of ether **1,** whereas the activity was decreased when the concentration is higher than 25% (v/v). The highest activity 124 min^{-1} was observed in the presence of 5% (v/v). The value difference from the main article (101 and 124 min^{-1}) is probably due to the difference of the enzyme lot.



Materials and Methods

Materials: The alcohols, THF, THP, 1,3-dioxolane, 1,3-dioxane, 1,4-dioxane, and 4-methyl-1,3-dioxane were purchased from Wako Pure Chemical Industries. The ADHs and $NAD(P)^+$ were obtained from Sigma-Aldrich. 2-Methyl-1,3-dioxolane and 4-methyl-1,3-dioxolane were purchased from Tokyo Chemical Industry.

Monitoring alcohol oxidation: We prepared 20 mM Tris-HCl containing 5% (v/v) cyclic ethers or acetonitrile, 10 mM alcohols, and 0.5–2 μM ADH. The pH was adjusted to 8.8, 8.4, and 7.8 for *Sc*ADH, HLADH, and *Tb*ADH, respectively. The reaction was initiated by the addition of 100 μM $NAD(P)^+$. NAD^+ was used for the reaction of *Sc*ADH and HLADH, and $NADP^+$ was used for the reaction of *Tb*ADH. The reaction was monitored at the 340 nm absorption maximum of NAD(P)H produced by the alcohol oxidation reaction, for the first 1 min. The background from the oxidation of contaminants in cyclic ethers was subtracted from each set of data. The amount of NAD(P)H was calculated by using the molar extinction coefficient of 6220 M^{-1} cm^{-1}.

Stability in cyclic ethers: The stability of HLADH and *Tb*ADH was confirmed by monitoring the catalytic activity after 10 min incubation with the cyclic ethers. The reaction conditions were same as those used for monitoring alcohol oxidation.

Dilution experiments: 5% (v/v) Cyclic ethers **1** or **6** were added to concentrated *Tb*ADH (50μM). *Tb*ADH (5 μL) was added to 20 mM Tris-HCL buffer (495 μL, pH 7.8) containing 100 μM $NADP^+$ and 10 mM 2-propanol at 0, 15, 30, and 60 min incubation. The catalytic activity of diluted 0.5 μM TbADH containing 0.05% cyclic ether was also measured by NADPH production.

Kinetic analysis: The catalytic activity of *Tb*ADH was measured in various substrate concentrations (0.1 to 5 mM of 2-propanol and 2-butanol) in the presence or absence of 5% (v/v) cyclic ether. The initial 1 min activities were directly fitted to the Michaelis-Menten equation and used to determine the K_m and k_{cat} values.