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Regeneration of NADH and Hydrogenation of Ketones to Alcohols with the Combination of Hydrogenase and Alcohol Dehydrogenase

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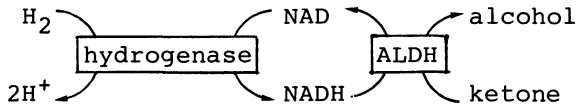
The regeneration of nicotinamide-adenine dinucleotide (reduced form, NADH) by the reduction of NAD with hydrogen gas was carried out in the presence of the hydrogenase from Alcaligenes eutrophus, and ketone hydrogenation was tried by the combination of the above system and alcohol dehydrogenase (ALDH). The ketones such as 2-butanone, 2-pentanone, and 2-hexanone were easily hydrogenated by hydrogen gas. After 65 h, the turnover number of NAD was 64 for 2-butanone showing a recurrence of NAD by hydrogen gas.

Some photochemical, electrochemical, and enzymatic systems for NAD(P)H regeneration have been reported.[1-3] Among these systems, enzymatic systems are of great advantage to produce compounds with a high optical purity, and to the simplicity of the systems. In this study the hydrogenase from A. eutrophus was used for the regeneration of NADH from NAD with hydrogen gas as a reducing agent; ketone hydrogenation was tried by a combination of the system with ALDH as shown in the scheme.



The hydrogenase from A. eutrophus was purified according to the literature.[4] The activity (1 unit) of hydrogenase used was to reduce 1 μmol of NAD for 1 min in the system containing hydrogenase and NAD (2.01×10^{-4} mol dm^{-3}) in 4.0 ml of a 5.0×10^{-3} mol dm^{-3} Tris-HCl buffer.(pH 8.0) under 300 Torr hydrogen pressure at 30 °C. ALDH from Thermoanaerobium brockii was obtained from SIGMA Co.

The sample solution, which consisted of hydrogenase, NAD, ALDH, and ketone (if included) in a Tris-HCl buffer (pH 8.0), was deaerated by repeated freeze-pump-thaw cycles. The reaction was carried out at 30 °C by the introduction of hydrogen gas into the above system. Ketones and alcohols were analyzed by GLC with a 3-m long column of PEG 6000 at 80 °C by using nitrogen as a carrier gas. The electronic spectra were measured using a Shimadzu MPS 5000 spectrometer, and the concentration of NADH was determined from the absorbance at 340 nm.

When hydrogen gas was introduced to a system containing hydrogenase and NAD, the spectrum of the solution changed with reaction time, and the characteristic absorption band of the reduced form (NADP) of NAD was observed at 340 nm. The NADH concentration increased linearly with the reaction time at low NAD conversion; then, the NADH formation rate decreased. The rate decrease was caused by a decrease in the concentration of the starting material, NAD. When 4.04 x 10^{-4} mol dm^{-3} NAD was used, the conversion of NAD after 20 min was 49.5%. Since NAD is reduced by hydrogen with the hydrogenase, the hydrogenation of ketones to alcohols could be accomplished by adding ALDH to the above system. The hydrogenation of some ketones were tried. As ketones, 2-butanone, 2-pentanone, and 2-hexanone were used. All the ketones were hydrogenated and the corresponding alcohols were formed and no byproducts were observed. As an example, time dependence of 2-butanol formation is shown in Fig. 1. 2-Butanol formed almost linearly with reaction time and then the formation rate decreased. When another hydrogenase was added after 27 h reaction, the 2-butanol formation rate again increased remarkably. It is apparent that the rate decrease strongly depends on the hydrogenase activity. The depression of hydrogenase deactivation is important to get the stationary formation of 2-butanol in this system. The turnover number of NAD against 2-butanol after an 65-h reaction was 64. Similar turnover numbers were obtained in the cases of 2-pentanone and 2-hexanone. The results show that NAD recycled catalytically in this system, and that ketone hydrogenation by hydrogen gas was accomplished.

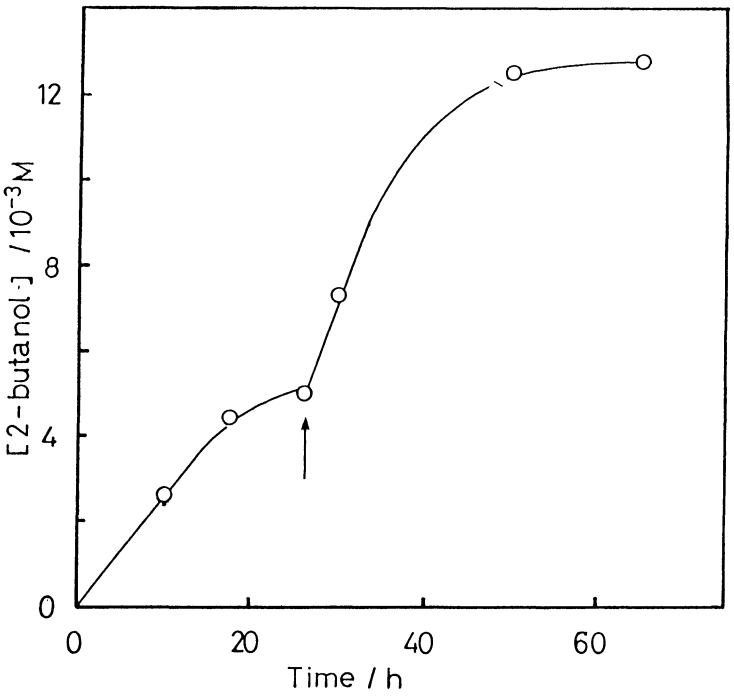


Fig. 1. Time dependence of 2-butanol formation. The sample solution (7.0 ml) contains hydrogenase (1.0 ml), NAD (7.0 x 10^{-4} mol dm^{-3}), ALDH (5 U), and 2-butanol (1.47 x 10^{-2} mol dm^{-3}). The reaction was carried out under hydrogen atmosphere (340 Torr) at 30 °C. The arrow indicates another hydrogenase (1.0 ml) added.

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