0273-2289/90/2425—0425$02.00

Regeneration of NADH and Ketone Hydrogenation by Hydrogen with the Combination of Hydrogenase and Alcohol Dehydrogenase

Scientific Note

ICHIRO OKURA,* KEIICHI OTSUKA, NOBUHARU NAKADA,
AND FUMIHIKO HASUMI

*Department of Bioengineering, Tokyo Institute of Technology,
Ookayama, Meguro-ku, Tokyo 152, Japan*

ABSTRACT

The regeneration of nicotinamide-adenine dinucleotide (reduced form, NADH) by the reaction of NAD with hydrogen gas was carried out in the presence of the hydrogenase from *Alcaligenes eutrophus*. And the formations of alcohol, CO_2, and 6-phospho-gluconate were observed by a combination of the above system and corresponding dehydrogenases. NADH was regenerated by hydrogen gas with the hydrogenase and recycled in these reactions.

Index Entries: Hydrogenase; alcohol dehydrogenase; formate dehydrogenase; 6-phospho-gluconate dehydrogenase; NAD; hydrogenation.

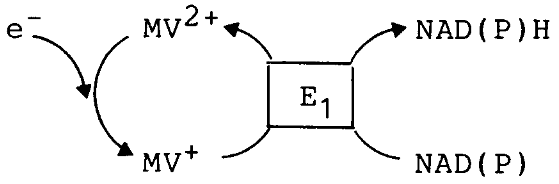
INTRODUCTION

Some photochemical (*1–3*), electrochemical (*4–7*), and enzymatic (*7–9*) systems for nicotinamide-adenine dinucleotide (phosphate) (reduced form, NAD(P)H) regeneration have been reported, as shown in Scheme 1.

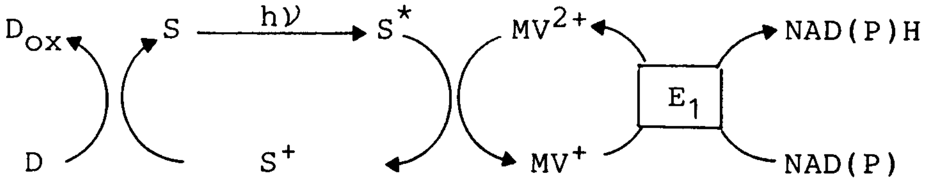
---

*Author to whom all correspondence and reprint requests should be addressed.

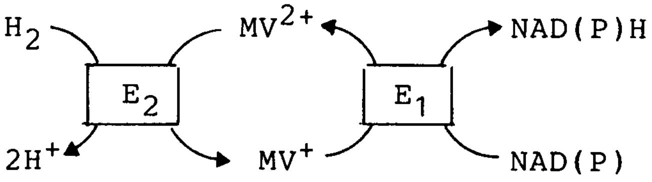
1. Electrochemical Method



2. Photochemical Method

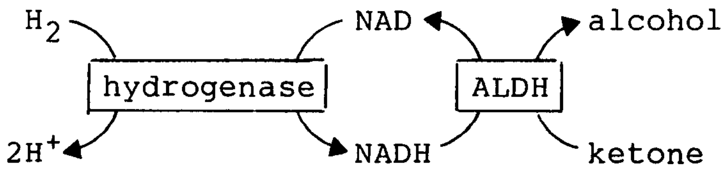


3. Enzymatic Method



E_1: Ferredoxin NADP reductase; E_2: Hydrogenase from *D. vulgaris.*

Among these systems, enzymatic systems are of great advantage to produce compounds with a high optical purity and the simplicity of the systems. When the hydrogenase from *Alcaligenes eutrophus* was used, NAD was directly reduced by hydrogen gas and no mediator, such as methyl viologen, was needed. 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 alcohol dehydrogenase (ALDH), as shown in the following scheme.



MATERIALS AND PROCEDURES

The hydrogenase from *A. eutrophus* was partly purified according to the literature (*10*). The activity (1 U) of hydrogenase used was to reduce 1 μmol 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* and other enzymes were obtained from Sigma Co, St. Louis, MO.

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 gas chromatography 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.

RESULTS AND DISCUSSION

Reduction of NAD by Hydrogen

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 of NAD was observed at 340 nm, as shown in Fig. 1. The NADH concentration increased linearly with the reaction time at low NAD conversion. When 4.04×10^{-4} mol dm^{-3} NAD was used, the conversion of NAD after 20 min was 49.5%. The initial reduction rate of NAD by hydrogen strongly depended on NAD concentration and hydrogen pressure. Especially at low pressure of hydrogen, the rate was proportional to hydrogen pressure.

Reduction of Ketone by Hydrogen

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, 2-hexanone, and cyclohexanone were used. All the ketones were hydrogenated, the corresponding alcohols were formed, and no byproducts were observed. Table 1 shows time dependence of alcohol formation. Alcohol formed almost linearly with reaction time, and then the formation rate decreased. Table 1 also shows the turnover number of NAD against alcohol after 12 h reaction. The results show that NAD recycled catalytically in the system, and ketones were easily hydrogenated by hydrogen gas with the combination of hydrogenase and alcohol dehydrogenase.

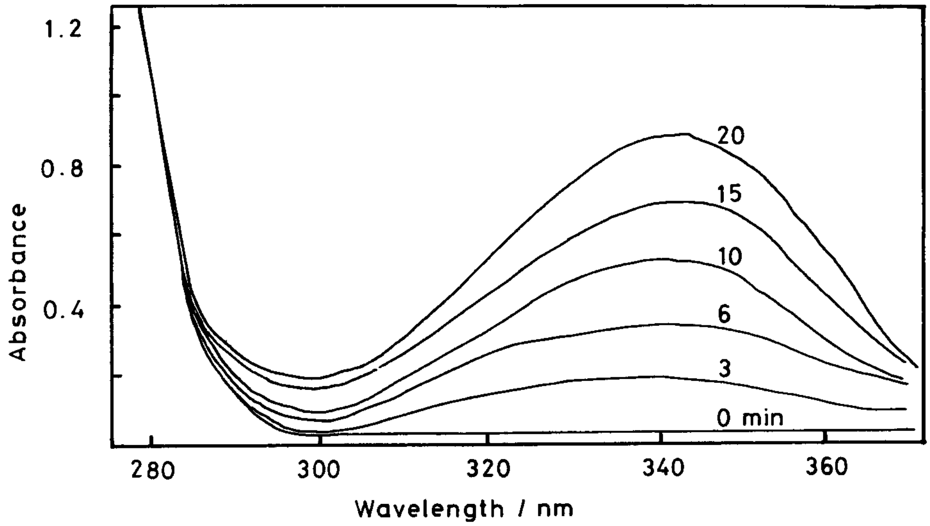


Fig. 1. Time dependence of the spectrum change of the solution containing NAD (2.0×10^{-4} mol dm^{-3}) and hydrogenase (4 U) in the presence of hydrogen (320 torr).

Table 1
Time Dependence of Alcohol Formation[a]

Reactant	Product, 10^{-3} mol dm^{-3}	Reaction time/h 2	4	8	12	Turnover number
2-Butanone	2-Butanol	3.10	6.78	9.83	10.0	10
2-Pentanone	2-Pentanol	0.89	1.56	3.94	4.78	8
2-Hexanone	2-Hexanol	1.39	3.44	6.00	7.72	8
Cyclohexanone	Cyclohexonal	1.83	3.72	6.67	8.44	5

[a] The sample solution (7.0 mL) contains hydrogenase (18 U), NAD (1.0×10^{-3} mol dm^{-3}), ALDH (5 U), and ketone (1.25×10^{-2} mol dm^{-3}). The reaction was carried out under a hydrogen atmosphere (340 torr) at 30°C.

Other Hydrogenation Reactions

When formate dehydrogenase or 6-phospho-gluconate dehydrogenase (6-PGDH) was used instead of ALDH, the corresponding hydrogenation proceeded by hydrogen gas.

$$H_2 + CO_2 \rightarrow HCOOH$$

$$H_2 + CO_2 + \text{Ribulose 5-Phosphate} \rightarrow \text{6-phospho-gluconate}$$

Figure 2 shows the time dependence of formic acid formation with the combination of hydrogenase and formate dehydrogenase. In this reaction, mixed gases of H_2 and CO_2 were introduced. Figure 3 shows the time

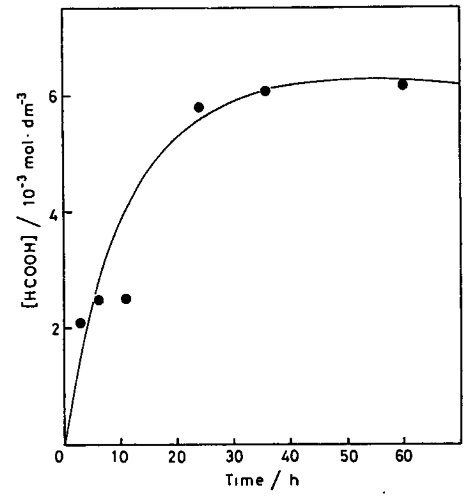


Fig. 2. Time dependence of formic acid formation. The sample solution (7.0 mL) contains hydrogenase (6.8 U), NAD (1.0×10^{-3} mol dm^{-3}), and formate dehydrogenase (3.0 U). The reaction was carried out under a hydrogen (314 torr) and CO_2 (215 torr) atmosphere at 30°C.

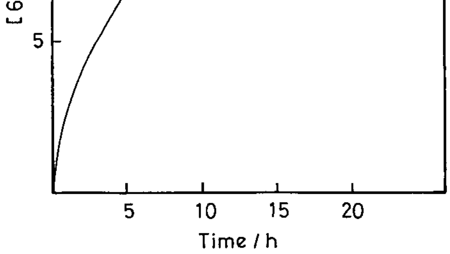


Fig. 3. Time dependence of 6-phospho-gluconate formation. The sample solution (7.0 mL) contains hydrogenase (18 U), NAD (4.09×10^{-3} mol dm^{-3}), 6-PGDH (10 U), and ribulose 5-phosphate (1.0×10^{-2} mol dm^{-3}). The reaction was carried out under a hydrogen (340 torr) and CO_2 (215 torr) atmosphere at 30°C.

dependence of 6-phospho-gluconate formation with the combination of hydrogenase and 6-PGDH. In this case, mixed gases of H_2 and CO_2 also were used. From the above results, hydrogenations by hydrogen gas with the combination of hydrogenase and corresponding dehydrogenase were accomplished.

ACKNOWLEDGMENT

This work was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas No. 63612507 "Dynamic Interactions and Electronic Processes of Macromolecular Complexes" from the Ministry of Education, Science and Culture.

REFERENCES

1. Mandler, D. and Willner, I. (1984), *J. Am. Chem. Soc.* **106,** 5352.
2. Cuendet, P. and Gratzel, M. (1984), *Photochem. Photobiol.* **39,** 609.
3. Wienkamp, R. and Steckhan, E. (1983), *Angew, Chem.* (Int. ed., England) **22,** 497.
4. Shaked, Z., Barber, J. J., and Whitesides, G. M. (1981), *J. Org. Chem.* **46,** 4101.
5. Dicosimo, R., Wong, C.-H., Daniels, L., and Whitesides, G. M. (1981), *J. Org. Chem.* **46,** 4623.
6. Wienkamp, R. and Steckhan, E. (1982), *Angew. Chem.* (Int. ed., England) **21,** 782.
7. Simon, H., Bader, J., Gunther, H. Neumann, S., and Thanos, J. (1985), *Angew. Chem.* (Int. ed., England) **24,** 539.
8. Wong, C.-H. and Whitesides, G. H. (1983), *J. Am. Chem. Soc.* **105,** 5012.
9. Shaked, Z. and Whitesides, G. M. (1980), *J. Am. Chem. Soc.* **102,** 7104.
10. Schneider, K. and Schlegel, H. G. (1976), *Biochem. Biophys. Acta* **452,** 66.