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Scientific Note

Synthesis of Alanine and Leucine by Reductive Amination of 2-Oxoic Acid with Combination of Hydrogenase and Dehydrogenase

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ABSTRACT

Alanine synthesis by reductive amination of pyruvate was performed by the combination of NADH regeneration system and alanine dehydrogenase (AlaDH). The conversion of pyruvate to alanine was 99% after 1 h. Leucine synthesis was also carried out by the combination of NADH regeneration system and leucine dehydrogenase (LeuDH). The conversion of 4-methyl-2-oxovalerate to leucine was 60% after 1.5 h.

Index Entries: Hydrogenase; alanine dehydrogenase; leucine dehydrogenase; NAD^+; hydrogenation.

INTRODUCTION

Enzymatic NADH regeneration system has been established with the hydrogenase from *Alcaligenes eutrophus* by hydrogen gas as a reducing agent (*1–3*). The enzymatic systems are of great advantage to produce

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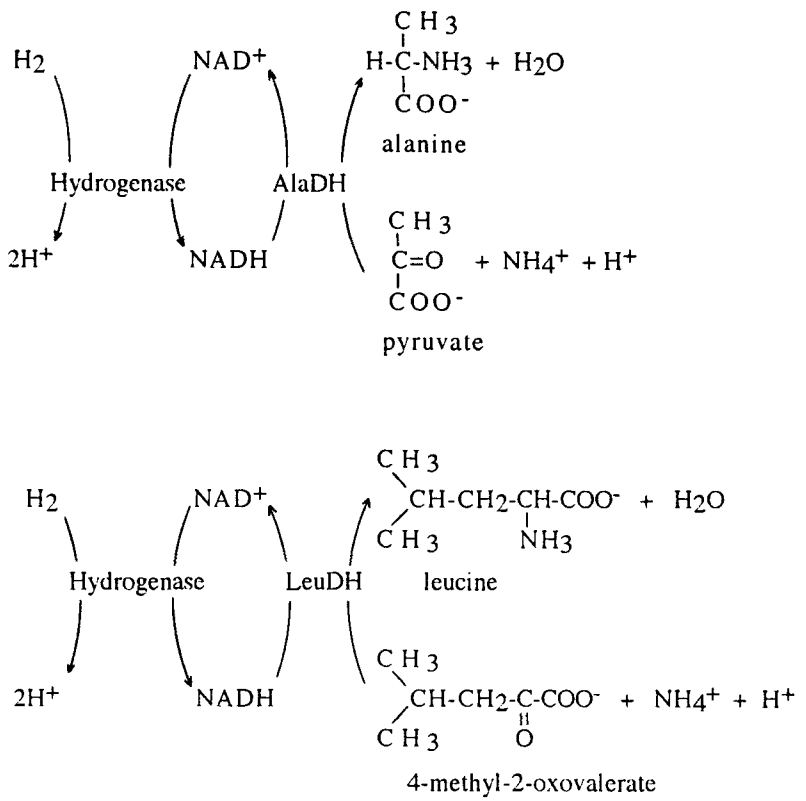

Scheme 1. Alanine and Leucine formation by the combination of hydrogenase and dehydrogenase.

compounds with a high optical purity. In this study, synthesis of alanine and leucine by a combination of the above regeneration system and the corresponding dehydrogenase is shown in the following scheme.

MATERIALS AND PROCEDURES

The hydrogenase from *A. eutrophus* was partly purified according to the literature (4). The activity (1 U) of hydrogenase used was to reduce 1 μmol of NAD^+ for 1 min. AlaDH from *Bacillus subtilis* and LeuDH from *Bacillus sphaerious* were obtained from Sigma Co.

Alanine formation reaction was carried out as follows. The sample solution, which consisted of hydrogenase, NAD^+, AlaDH, pyruvate, and ammonia in phosphate buffer (pH 9.0), 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. The leucine formation was attempted as follows. The sample solution contained hydrogenase, NAD^+, LeuDH, 4-methyl-2-oxovalerate, and ammonia in a phosphate buffer (pH 9.0). The reaction was carried out at 30°C by the introduction of hydrogen

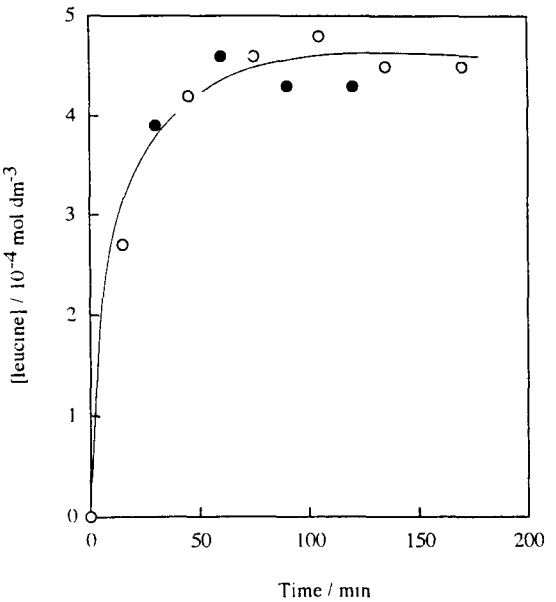

Fig. 1. Time dependence of leucine formation. The sample solution (10 mL) contains hydrogenase (3.3 μ), ammonia (8.0×10^{-4} mol dm^{-3}), 4-methyl-2-oxovalate (8.0×10^{-4} mol dm^{-3}), LeuDH (10 μ), and NAD^+; ●: 3.8×10^{-5} mol dm^{-3}; ○: 3.8×10^{-4} mol dm^{-3}. The reaction was carried out under hydrogen atmosphere (500 torr) at 30°C.

gas into the above system. Alanine and leucine were analyzed by HPLC with the Nucleocil 5C18 (Chemco Scientific) column using 50% methanol and 50% 50 m*M* phosphate buffer (pH 7.5) mixed solution as eluate. The sample solution was deproteinized with sodium tungstate solution, and the remaining ammonia was removed by vacuum evaporator at 50% in advance.

RESULTS AND DISCUSSION

Leucine Formation

When hydrogen gas was introduced into the system containing hydrogenase, NAD^+, 4-methyl-2-oxovalate, ammonia, and LeuDH, reductive amination of 4-methyl-2-oxovariate to leucine proceeded as shown in Fig. 1. Leucine formation rate or conversion of 4-methyl-2-oxovariate to leucine was independent of NAD^+ concentration, showing that rate determining step for leucine formation is the reductive amination of 4-methyl-2-oxovariate by LeuDH. When the reaction was carried out at 30°C with the sample solution containing hydrogenase (3.3 μ), LeuDH (10.0 μ), 4-methyl-2-oxovariate (7.9×10^{-4} mol dm^{-3}), ammonia (8.0×10^{-4} mol dm^{-3}), and NAD^+ (3.8×10^{-4} mol dm^{-3}) under hydrogen atmosphere (500 torr), and

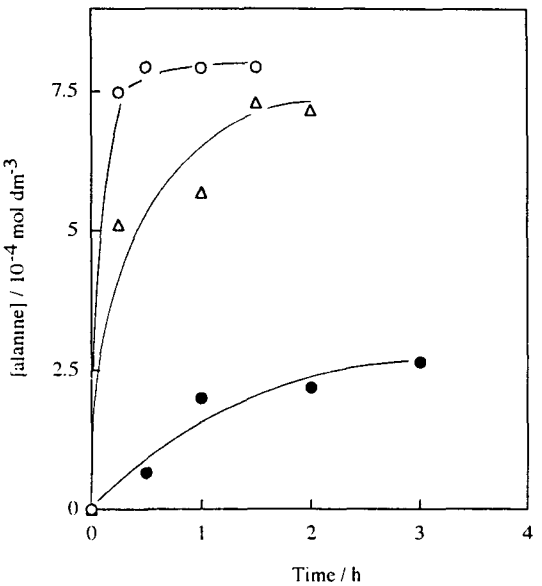

Fig. 2. Time dependence of alanine formation. The sample solution (10 mL) contains hydrogenase (3.3 μ), NAD^+ (3.8×10^{-4} mol dm^{-3}), ammonia (1.6×10^{-3} mol dm^{-3}), pyruvate (8.0×10^{-4} mol dm^{-3}), AlaDH (20 μ) and oxamate; ●: 0 mol dm^{-3}; △: 8.0×10^{-5} mol dm^{-3}; ○: 8.0×10^{-4} mol dm^{-3}. The reaction was carried out under hydrogen atmosphere (500 torr) at 30°C.

conversion of 4-methyl-2-oxovariate to leucine was 60% after 1.5 h and the turnover number of NAD^+ was 11.

Alanine Formation

Partly purified hydrogenase contains lactate dehydrogenase, which serves as a catalyst for pyruvate reaction (3). To prevent lactate dehydrogenase activity, oxamate was introduced to the system. When hydrogen gas was introduced into a system containing hydrogenase, NAD^+, pyruvate, ammonia, and AlaDH, reductive amination of pyruvate to alanine proceeded as shown in Fig. 2. No by-products were observed. Alanine formation rate increased with NAD^+ concentration and reached a constant value. When 8.0×10^{-4} mol dm^{-3} NAD^+ was used, the conversion of pyruvate was 99% after 1 h and the turnover of NAD^+ was 10.

From the above results, leucine and alanine synthesis by reductive amination of appropriate 2-oxoic acids with the combination of hydrogenase and corresponding dehydrogenase were accomplished.

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