0273-2289/95/5501—0001$04.80

Accelerated Article

Synthesis of Glutamate by Reductive Amination of 2-Oxoglutarate with the Combination of Hydrogenase and Glutamate Dehydrogenase

Scientific Note

FUMIHIKO HASUMI,[1] YASUMITSU MIYAMOTO,[2] AND ICHIRO OKURA*,[2]

[1]Department of Chemistry and Biochemistry, Numazu College of Technology, Shizuoka, Japan; and [2]Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 226, Japan

Received April 17, 1995; Accepted May 17, 1995

ABSTRACT

Glutamate synthesis by reductive amination of 2-oxoglutarate was performed by the combination of NADH regeneration system and glutamate dehydrogenase (GluDH). The conversion of 2-oxoglutamate to glutamate was 98% after 3 h, and the turnover number of NAD^+ was 17.

Index Entries: Hydrogenase; glutamate dehydrogenase; NAD^+; hydrogenation.

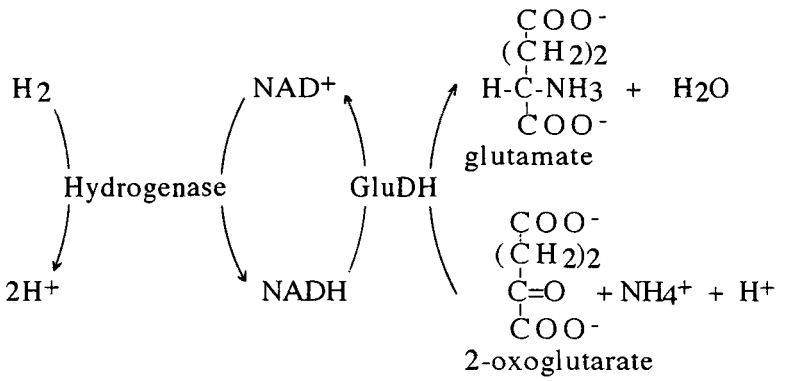
INTRODUCTION

An enzymatic NADH regeneration system has been established using hydrogenase from *Alcaligenes eutrophus* with hydrogen gas as a reducing agent (*1–3*). The enzymatic systems offer a great advantage in the production of compounds with high optical purity.

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

As previously reported, 2-oxoglutarate can be produced from n-paraffin with *Corynebacterium* sp. and *Arthrobacter* sp. (*4*). If the NADH regeneration system with hydrogenase is combined with GluDH, glutamate is produced from 2-oxoglutarate and a useful material, glutamate, is produced from n-paraffin in a total enzymatic system. In this study, glutamate synthesis by a combination of the above regeneration system and glutamate dehydrogenase was attempted. This is depicted in the following scheme.

Glutamate formation by the combination of hydrogenase and glutamate dehydrogenase.



MATERIALS AND METHODS

The hydrogenase from *A. eutrophus* was partly purified according to the literature (*5*). One unit of hydrogenase activity (1 U) reduced 1 μmol of NAD^+ per 1 min. Glutamate dehydrogenase from beef liver was obtained from Oriental Yeast Co., Ltd.

The reaction of glutamate formation was carried out as follows. The sample solution, consisting of hydrogenase (3.3 U), NAD^+ (3.8×10^{-5} mol dm^{-3}), GluDH (100 U) 2-oxoglutarate (8.0×10^{-4} mol dm^{-3}), and ammonia (8.0×10^{-4} mol dm^{-3}) in 5×10^{-2} mol dm^{-3} phosphate buffer (pH 8.0), was deaerated by repeated freeze-pump-thraw cycles. The reaction was carried out at 30°C by the introduction of hydrogen gas into the above system. Glutamate was analyzed by HPLC with Nucleosil 5C18 (Chemco Scientific Co., Ltd.) column using 5.0×10^{-2} mol dm^{-3} phosphate buffer (pH 7.5) as eluent. The concentration of NADH was determined from the absorbance at 340 nm. The sample solution was deproteinized with sodium tungstate solution, and the remaining ammonia was removed in advance by vacuum evaporation at 50°C.

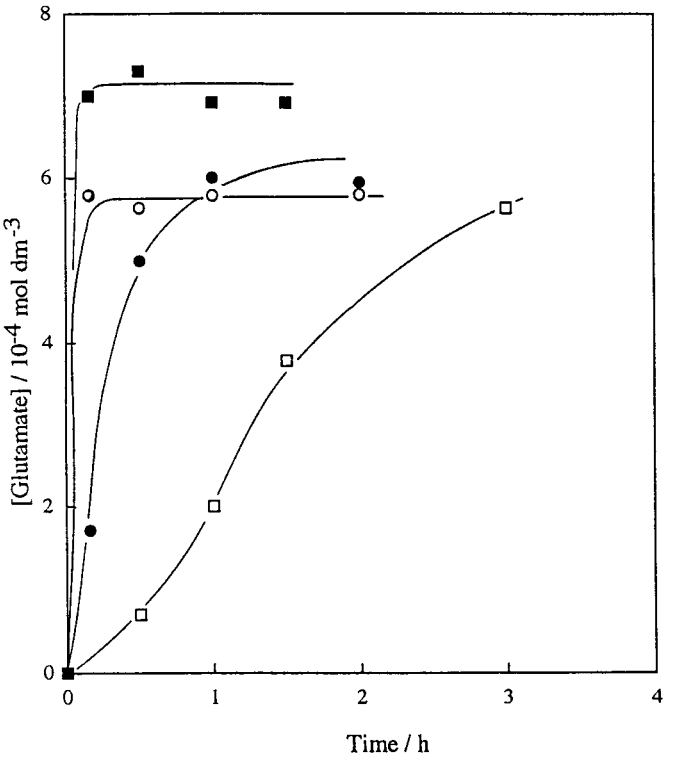


Fig. 1. Time dependence of glutamate formation. The sample solution (10.0 mL) contains NAD^+ (3.8×10^{-5} mol dm^{-3}), ammonia (8.0×10^{-4} mol dm^{-3}), 2-oxoglutarate (8.0×10^{-4} mol dm^{-3}), GluDH (100 U), and hydrogenase. The reaction was carried out under hydrogen atmosphere (555 torr) at 30°C. Amount of hydrogenase: □, 0.66 U; ●, 1.65 U; ○, 3.30 U; ■, 6.60 U.

RESULTS AND DISCUSSION

As we reported previously, the hydrogenase from *A. eutrophus* can reduce NAD^+ in the presence of hydrogen gas (*1–3*). When hydrogen gas was introduced to the system containing hydrogenase and NAD^+, the NADH concentration increased with reaction time. The conversion of NAD^+ to NADH was 85% after 40 min. By the introduction of GluDH and ammonia into the system, the reduced NADH was rapidly oxidized within 5 min, showing that NAD^+ in this system is recycled.

When hydrogen gas was introduced into the system containing hydrogenase, NAD^+, 2-oxoglutarate, GluDH, and ammonia, reductive amination of 2-oxoglutarate to glutamate proceeded as shown in Fig. 1. The new substance glutamate formation rate was proportional to the quantity of hydrogenase indicating that NAD^+ reduction with hydrogenase is a

rate determining step in this glutamate formation reaction. When the reaction was carried out at 30°C with the sample solution containing excess hydrogenase (6.6 U), GluDH (100 U), 2-oxoglutarate (8.0×10^{-4} mol dm^{-3}), ammonia (8.0×10^{-4} mol dm^{-3}), and NAD^+ (3.8×10^{-5} mol dm^{-3}) under hydrogen atmosphere (555 torr), the conversion of 2-oxoglutarate to glutamate was 86% after 10 min and the turnover number of NAD^+ was 17.

When the reaction was carried out under high pressure of hydrogen gas (2400) torr at 30°C with the sample solution containing GluDH (100 U), 2-oxoglutarate (8.0×10^{-4} mol dm^{-3}), ammonia (8.0×10^{-4} mol dm^{-3}), NAD^+ (3.8×10^{-4} mol dm^{-3}), and hydrogenase (0.7 U), the conversion of 2-oxoglutarate to glutamate was 98%.

From the above results, glutamate synthesis by reductive amination of 2-oxoglutarate with the combination of hydrogenase and glutamate dehydrogenase was accomplished.

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