CHEMISTRY LETTERS, pp. 951-954, 1992.

Asymmetric Reduction of Butyl Pyruvate Catalyzed by Immobilized Glycerol Dehydrogenase in Organic-Aqueous Biphasic Media[†]

Kaoru NAKAMURA,* Satoshi TAKANO,[††] Kumi TERADA,[†††] and Atsuyoshi OHNO
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611

The rate of asymmetric reduction of butyl pyruvate catalyzed by glycerol dehydrogenase is largely enhanced when the enzyme system is immobilized by a water-adsorbent polymer and the reaction is run in an organic solvent with cooperation of cyclopentanol as a reducing reagent.

Enzymes are known to be versatile and used widely as catalysts in asymmetric syntheses.1) However, the utility of an enzyme is restricted to an aqueous solution except for lipase-catalyzed reactions. Since a majority of substrates employed for organic reactions are unstable and insoluble in water, the use of organic solvent may be advantageous to prevent the substrate from decomposition and to dissolve them. For example, Shimizu *et al.* reported2) that the reduction of 4-chloro-3-oxobutanoate is promoted by using an organic-aqueous biphasic system.

Recently, we reported3) an asymmetric reduction of ketones by glycerol dehydrogenase from *Geotrichum candidum* (GGDH). Several keto esters are reduced enantioselectively to the corresponding D-hydroxy esters by the GGDH-catalyzed reaction using 2-propanol as a reducing reagent. Two serious problems remained in the reaction, however. The first one is that the reaction rate is limited because the catalytic reduction of NAD^+ to NADH is slow disturbing smooth recycling of the coenzyme. The second is that the chemical yield is unsatisfactory because the

---

[†] Dedicated to celebrate 80th birthday of Professor Emeritus Osamu Simamura of The University of Tokyo.

[††] Present address : Department of Applied Chemistry, Osaka Institute of Technology, Asahi-ku, Osaka 535.

[†††] Present address : Department of Applied Chemistry, Doshisha University, Kamigyou-ku, Kyoto 602.

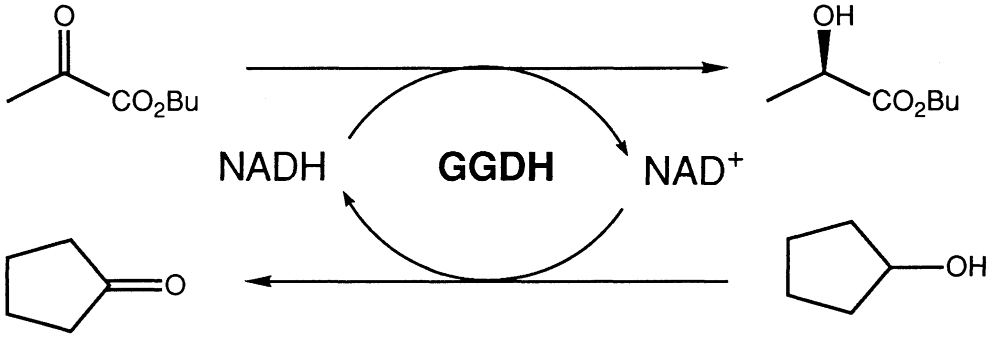
substrates in an aqueous solution are unstable. To overcome these difficulties, we have looked for a new reduction system and have found an excellent one where the enzyme system (an enzyme and its coenzyme) is immobilized by a water-adsorbent polymer and the reaction is run in an organic solvent with cyclopentanol as a reducing reagent. The results being reported in this letter. This system enhances the rate of enzymatic reaction appreciably compared with those in other systems such as non-immobilized and mono-phasic (aqueous) reduction.

The water-adsorbent polymer (BL 100,4) 60 mg) was added to an aqueous solution (1 ml, Tris, pH 8.0) containing GGDH (10.4 unit) and NAD^+ (1.5×10^{-6} mol) to prepare a biocatalyst. A hexane solution (4 ml) of butyl pyruvate (0.2 mmol) and cyclopentanol (0.22 mmol) was added to the biocatalyst and the system was shaken (140 rev/min) at 35 °C. The reaction was followed by GC-analysis of the organic layer.5) The reaction without the polymer was also conducted as a reference. The result shown in Fig. 1 indicates that the use of water-adsorbent polymer enhances largely the reactivity of the enzymatic reaction. Butyl pyruvate was reduced to butyl lactate quantitatively within 30 min by immobilized biocatalyst in hexane. The acceleration in reaction rate (about 3 to 10 times depending on the solvent used) was observed when the enzymatic system is immobilized. Although turn over number (TN) of the coenzyme is about 133 in the system exemplified in Fig. 1, it was confirmed that TN can be increased to 2500 by using less amount (0.08×10^{-6} mol) of NAD^+, where the reaction completed within 100 min and again proceeded quantitatively.6)

The reduction gave the (*R*)-lactate enantioselectively as anticipated from the previously reported reduction of ethyl pyruvate.3) GC analysis of the MTPA derivative7) indicates that butyl (*R*)-lactate was obtained in >99% enantiomeric excess from the all reactions mentioned above.

The characteristics of the present system is the large enhancement in reaction rate and the easiness of operation. Since this method does not require any severe conditions that usually deactivate enzymes, other enzymes may be immobilized similarly.

The system described herein has two further advantages. Since the product is transferred to the organic phase and its concentration at the vicinity of the enzyme is kept low compared with a mono-aqueous phasic system, product inhibition is diminished or avoided. Product inhibition has been the most serious limitation in using enzymes as catalysts in organic synthesis as was seen in the oxidation of diols by glycerol dehydrogenase.8) The second advantage is that the system does not require any other enzymes to recycle the coenzyme because NAD^+ is reduced smoothly by GGDH and cyclopentanol. We believe that the present system is applicable in general and strongly recommend the use of water-adsorbent polymer in an organic solvent for enzymatic reactions of organic compounds which are susceptible to aqueous solutions.



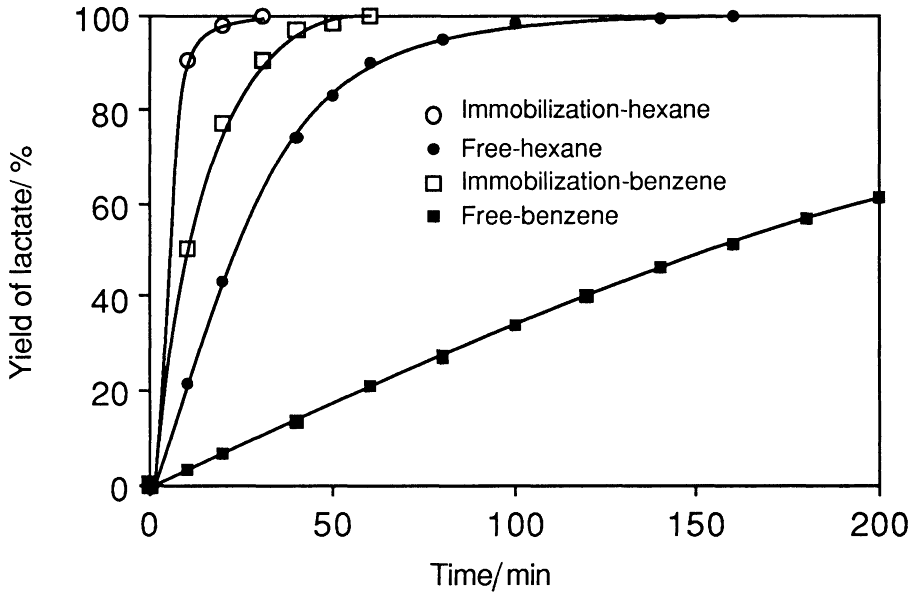


Fig. 1. Effect of immobilization on GGDH-catalyzed reduction of butyl pyruvate in organic solvents.

The authors specially thank Osaka Yuki Kagaku Kogyo Co., Ltd. and Kao Corp. for kind supply of the water-adsorbent polymers. We also thank Amano Pharmaceutical Co., Ltd. for providing GGDH.

References

1) a) G. M. Whitesides and C-H. Wong, *Angew. Chem.,Int. Ed. Engl*., **24**, 617 (1985); b) J. B. Jones, *Tetrahedron*, **42**, 3351 (1986); c) S. Servi, *Synthesis*, **1990**, 1; d) R. Csuk, and B. I. Glänzer, *Chem. Rev*., **91**, 49 (1991).

2) S. Shimizu, M. Kataoka, M. Katoh, T. Morikawa, T. Miyoshi, and H. Yamada, *Appl. Environ. Microbiol*., **56**, 2374 (1990).

3) K. Nakamura, T. Yoneda, T. Miyai, K. Ushio, A. Ohno, and S. Oka, *Tetrahedron Lett*., **29**, 2453

(1988).

4) Water-adsorbent polymer was supplied by Osaka Yuki Kagaku Kogyo Co., Ltd. One gram of BL-100 (sodium polyacrylate as the base component) can adsorb more than 100 g of water. Other water-adsorbent polymers of different types are also usable. We used "Wander gel" from Kao Corp. and obtained a similar result.

5) GC conditions; PEG 20M, 0.25 mm x 25 m, 90 °C. Partition of the materials between organic and aqueous phases was taken into account.

6) We confirmed that the reaction with excellent efficiency in TN can be run in a column packed by the biocatalyst.

7) The authentic sample of butyl (*R*)-lactate was prepared from methyl (*R*)-lactate by transesterification. Absolute configuration of the enzymatic product was determined by comparing GC-spectrum of the MTPA derivative with that of the authentic sample. GC conditions; PEG-20M, 0.25 mm x 25 m, 170 °C.

8) L. G. Lee and G. M. Whitesides, *J. Org. Chem.*, **51**, 25 (1986).

(Received March 6, 1992)