Note

Escherichia coli Transformant Expressing the Glucose Dehydrogenase Gene from Bacillus megaterium as a Cofactor Regenerator in a Chiral Alcohol Production System

Michihiko KATAOKA,† Luh Poni Sri ROHANI,†† Masaru WADA, Keiko KITA,* Hideshi YANASE,* Itaru URABE,** and Sakayu SHIMIZU

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan
*Department of Biotechnology, Faculty of Engineering, Tottori University, 4-101 Koyama, Tottori 680, Japan
**Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565, Japan

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Escherichia coli JM109 (pGDA2) overexpressing the glucose dehydrogenase (GDH) gene from Bacillus megaterium IWG3 was examined for use as a cofactor regenerator. In the asymmetric reduction of ethyl 4-chloro-3-oxobutanoate by E. coli JM109 (pKAR) which is an aldehyde reductase-overproducing transformant, E. coli JM109 (pGDA2) can act as an NADPH regenerator with NADP⁺ and glucose, similarly to commercially available GDH.

Key words: glucose dehydrogenase; Bacillus megaterium; NADPH regeneration; aldehyde reductase; asymmetric reduction

The asymmetric reduction of various carbonyl compounds using stereoselective oxidoreductases is quite a significant method for the production of useful optically active alcohols.¹⁻⁵ Most of such oxidoreductases are NAD(P)H-dependent enzymes,¹⁻⁹ and consequently require an NAD(P)H regeneration system for sufficient reduction of carbonyl compounds.⁴⁻⁵,¹⁰,¹¹ In the asymmetric reduction of ketopantoyl lactone by washed cells of Candida parapsilosis IFO 0708,¹² which produced NADPH-dependent ketopantoyl lactone reductase, the addition of sugars such as glucose and fructose to the reaction mixtures increased the molar yields of the products. In this case, intact cells of C. parapsilosis have their own NADPH regeneration system, and it was found that the enzymes responsible for the formation of NADPH were mainly di-glucose 6-phosphate dehydrogenase and 6-phospho-d-gluconate dehydrogenase in the hexose monophosphate pathway.¹³ Recently, we developed a method for asymmetric reduction of ethyl 4-chloro-3-oxobutanoate (COBE) to ethyl (R)-4-chloro-3-hydroxybutanoate (CHBE) involving Escherichia coli JM109 (pKAR) cells expressing the aldehyde reductase gene from Sporobolomyces salmonicolor AKU 4429¹⁴,¹⁵ as a catalyst.¹⁶ Since E. coli cells do not have a sufficient cofactor regeneration system, commercially available glucose dehydrogenase (GDH, from Bacillus sp.; Amano Pharmaceutical Co., Japan) has to be added to the reaction mixture together with glucose and NADP⁺. Makino et al.¹⁷ cloned and sequenced the gene encoding GDH from Bacillus megaterium IWG3, and then the GDH gene was expressed in E. coli cells. In this paper, we show the possibility of the application of an E. coli transformant expressing the GDH gene as a cofactor regenerator in the asymmetric reduction of COBE with E. coli JM109 (pKAR).

E. coli JM109 (pGDA2) cells expressing the GDH gene from B. megaterium¹⁷ were cultivated in Luria-Bertani (LB) medium, comprising 1% Bacto-tryptone (Difco Lab., USA), 0.5% yeast extract (Oriental Yeast Co., Japan), and 1% NaCl, pH 7.0, supplemented with 50 µg/ml ampicillin at 37°C, with reciprocal shaking (120 strokes/min). Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM when the turbidity of the culture at 600 nm reached 0.6. After further incubation for 5 h, the cells were harvested by centrifugation. A cell-free extract of E. coli JM109 (pGDA2) was prepared as described previously,¹⁵ and then total activity of GDH in the cell-free extract prepared from 1 g (wet weight) of the cells was found to be 261 units. The aldehyde reductase-overproducing strain, E. coli JM109 (pKAR), was cultivated as described previously.¹⁶

For preliminary examination to measure the ability of E. coli JM109 (pGDA2) as an NADPH regenerator, cells of E. coli JM109 (pKAR) were incubated in the reaction mixture with various amounts of cells of E. coli JM109 (pGDA2) instead of GDH. The results after 3 h of incubation are summarized in the Table. These results showed that GDH could be replaced by E. coli JM109 (pGDA2) cells as an NADPH regenerator. E. coli JM109 (pGDA2) cells at half the amount of E. coli JM109 (pKAR) cells were required for effective reduction reactions. The amount of GDH (13.1 units) in the cells required for sufficient reaction (50 mg wet weight)

† To whom correspondence should be addressed.
†† Present address: Department of Biology, Bandung Institute of Technology, Jalan Ganesha 10 Bandung 40132, Indonesia.

Abbreviations: GDH, glucose dehydrogenase; COBE, ethyl 4-chloro-3-oxobutanoate; CHBE, ethyl 4-chloro-3-hydroxybutanoate; ee, enantionic excess.
was one-fourth the amount of commercially available GDH (51.1 units). The addition of *E. coli* JM109 (pGD2A) cells to the reaction mixtures had no effect on the optical purity of (R)-CHBE formed (92% ee). When either NADP⁺ or glucose was omitted from the reaction mixture, the formation of CHBE was not observed.

The introduction of a water-solvent two-phase system to the COBE reduction reaction improved the molar conversion yield and the product amount of (R)-CHBE. Cells of *E. coli* JM109 (pGD2A) were used for the reduction of COBE in a two-phase system reaction, with an initial concentration of 608 mM (100 mg/mL). The reaction conditions and results are shown in the Figure. COBE, NADP⁺, and glucose were added to the reaction mixture periodically. After 58 h of incubation, the concentrations of CHBE formed in the organic and aqueous phases were 1570 (261) and 99.3 mM (16.5 mg/mL), respectively. The molar conversion yield (molar ratio of total CHBE formed to total COBE consumed), molar reaction yield (molar ratio of total CHBE formed to total COBE added), and optical purity (for (R)-CHBE formed) were 94.1, 91.4%, and 92% ee, respectively. The calculated turnover of NADP⁺ based on the amounts of NADP⁺ added and CHBE formed was 4380 mol/mol. These results are almost equal to those of the reactions involving *E. coli* JM109 (pKAR) and commercially available GDH as catalysts under the optimal conditions (1530 mM (255 mg/mL) of (R)-CHBE was formed in the organic phase, with a molar conversion yield of 91.1%, an optical purity of 91% ee, and an NADP⁺ turnover of 5130 mol/mol).

NADP⁺ and NADPH might be not able to easily permeate the cell membrane of *E. coli* cells. However, in these reaction systems described above, substrate and organic solvent added to the reaction mixture might partly damage the cell membrane, consequently NADP⁺ and NADPH (including COBE and CHBE) could pass through the cell membrane. On the other hand, GDH and aldehyde reductase, which are comparatively large molecules, seemed to be held in the *E. coli* cells. Based on the amount of CHBE formed at 3 h reaction under the monophase (see Table) and two-phase reaction systems (see Fig.), apparent CHBE-producing specific activities were calculated to be 0.982 and 3.44 mmol of CHBE/g of cells (wt) per h, respectively. One of the cause of this improvement in CHBE-producing velocity under two-phase reaction system might be an increase of permeability of cell membrane by addition of organic solvent described above.

These results showed the possibility of the application of *E. coli* (pGD2A) cells expressing the GDH gene as a cofactor regenerator to the enzymatic production of chiral compounds. Since GDH can also reduce NAD⁺ to NADH, *E. coli* JM109 (pGD2A) cells might be applicable to enzymatic reduction processes requiring NADH as a cofactor.

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