Biochemical Engineering Journal 54 (2011) 151–157

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Metabolic pathway analysis of 1,3-propanediol production with a genetically modified *Klebsiella pneumoniae* by overexpressing an endogenous NADPH-dependent alcohol dehydrogenase

Zhen Chen [a], Hongjuan Liu [b,*], Dehua Liu [a,*]

[a] *Institute of Applied Chemistry, Department of Chemical Engineering, Tsinghua University, Beijing 100084, PR China*
[b] *Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing 100084, PR China*

ARTICLE INFO

Article history:
Received 30 June 2010
Accepted 4 February 2011
Available online 22 February 2011

Keywords:
Metabolic pathway analysis
Elementary mode analysis
HOR
3-Hydroxypropionaldehyde
1,3-Propanediol
Overxpression

ABSTRACT

Coenzyme limitation is one of the most important issues for 1,3-propanediol (PDO) production. Elementary mode analysis indicated that pentose phosphate pathway and TCA cycle were the most efficient pathways for generating reducing equivalent NADPH and NADH. Under the optimal condition for PDO production, 0.542 mol NADPH/(mol glycerol), accounting for 61.7% of the total reducing equivalent would be produced, which requires the fast conversion of NADPH for PDO synthesis. Based on the above analysis, an endogenous NADPH-dependent alcohol dehydrogenase (HOR) was cloned and overexpressed for NADPH usage in *Klebsiella pneumoniae* ACCC10082. The activities of HOR and total 1,3-propanediol dehydrogenase (PDOR) increased 5.8-fold and 1.1-fold than that of the wild type strain. In the fed-batch fermentation, the PDO concentration and yield of the constructed strain increased 10.4% and 9.4% while the highest 3-hydroxypropionaldehyde accumulation reduced 35.1% compared with that of the wild type strain. Metabolic flux analysis suggested that the increase of PDO yield was due to the enhanced carbon flux flowed to pentose phosphate pathway which provided coenzyme for HOR utilization. This work is helpful for the further understanding of PDO metabolism in *K. pneumoniae* and also useful for the strain improvement of PDO production.

1. Introduction

The biological conversion of glycerol to 1, 3-propanediol (PDO), a promising chemical as the monomer of the novel polymer polytrimethylene terephathalate (PTT), has been refocused in recent years as a result of the surplus of glycerol that is being produced as a major by-product in biodiesel industry [1,2]. The mechanism for the glycerol conversion by *Klebsiella pneumoniae* has been extensively studied [3,4]. The metabolic pathway can be divided into two branches: the reductive branch and the oxidative branch (Fig. 1). In the reductive branch, glycerol is first converted to 3-hydroxypropionaldehyde (3-HPA) by a coenzyme B12-dependent glycerol dehydratase (GDHt) and the latter is further reduced to PDO by a NADH-dependent 1,3-propanediol oxidoreductase (PDOR) [5]. On the other hand, glycerol is oxidized to dihydroxyacetone (DHA) by a NAD^+-dependent glycerol dehydrogenase (GDH) or phosphorylated into glycerol-3-phosphate (G3P) by glycerol kinase and both DHA and G3P can be further transferred into DHA phosphate (DHAP) which will enter glycolysis [6].

It has been generally recognized that the conversion of 3-HPA to PDO is mainly catalyzed by PDOR with the association of coenzyme NADH. Recent years, an NADPH-dependent alcohol dehydrogenase was found more effective than NADH-dependent 1,3-propanediol dehydrogenase for PDO production. A substitution of dhaT gene to yqhD gene encoding a NADPH-dependent alcohol dehydrogenase in recombinant *Escherichia coli* resulted in high titers of approximately 130 g/L PDO production, which have never been obtained in the identical strain utilizing dhaT gene [7]. The proteomic analysis of *K. pneumoniae* from glycerol fermentation identified an endogenous NADPH-dependent oxidoreductase (HOR) in *K. pneumoniae* [8]. HOR was supposed to play important role in the conversion of 3-HPA to PDO and showed high activity at the late phase of the fermentation [8]. In the latest report, Seo et al. confirmed that HOR was the isoenzyme of PDOR and was responsible for the biotransformation of PDO production [9]. However, the effect of HOR on the metabolism of *K. pneumoniae* was still not clear.

Elementary mode analysis is one of the most powerful tools for metabolic pathway analysis which calculate the solution space that contains all possible steady-state flux distributions of a network by considering the stoichiometry of carbon and cofactor [10]. The stoichiometry of the metabolic network, including carbon as well as cofactor requirements, is fully considered in elementary mode analysis. On the other hand, elementary mode analysis also allows

* Corresponding authors. Tel.: +86 10 62794742; fax: +86 10 62794742.
E-mail addresses: dhliu@tsinghua.edu.cn (D. Liu), liuhongjuan@tsinghua.edu.cn (H. Liu).

1369-703X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2011.02.005

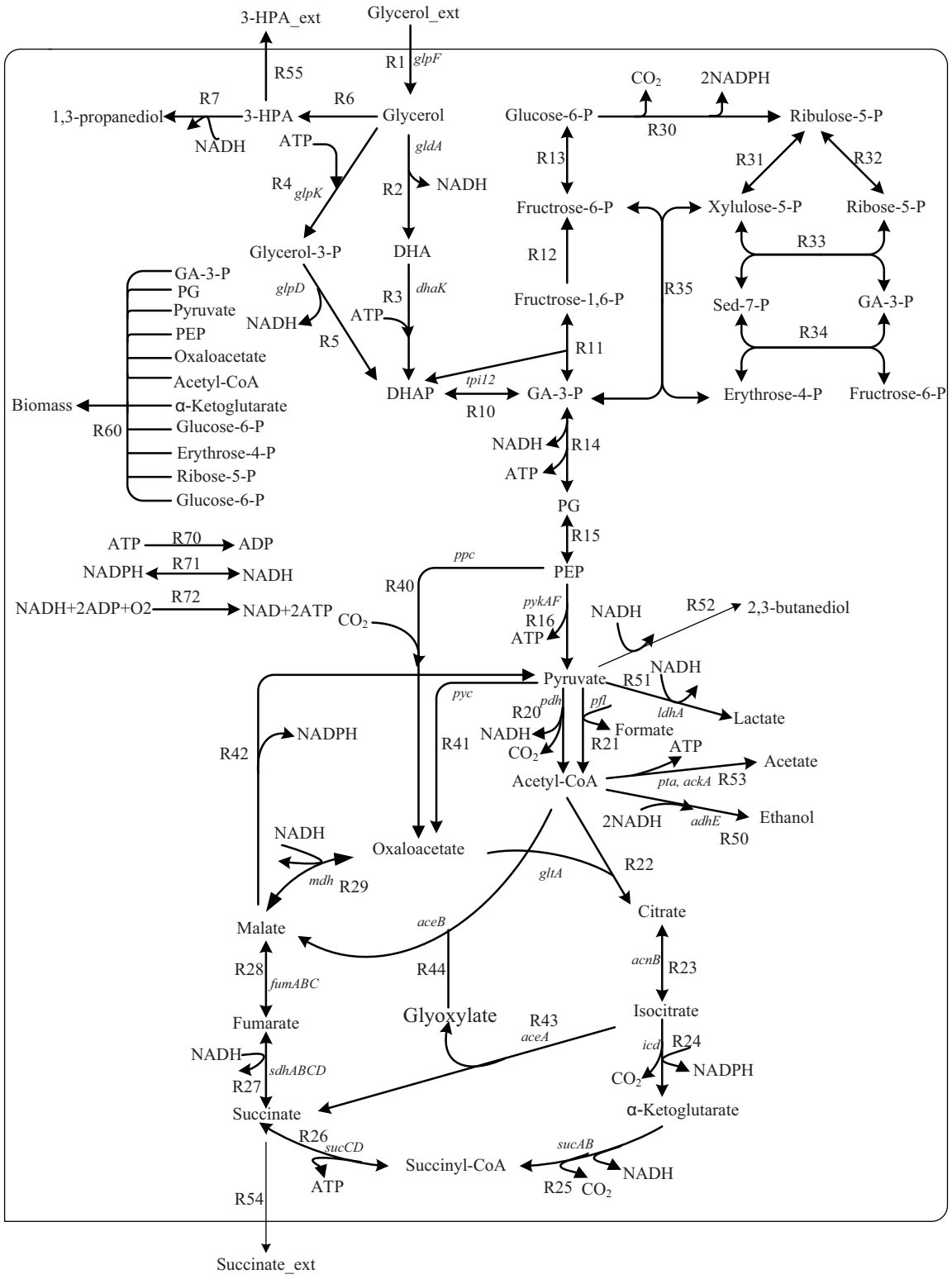

Fig. 1. The representative metabolic pathway of glycerol. Abbreviations: 3-HPA, 3-hydroxypropionaldehyde; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; GA-3-P, glyceraldehyde-3-phosphate; PG, phosphoglycerate; PEP, phosphoenolpyruvate.

determining the overall capacity, i.e., theoretical maximum yield, of a cellular system and studying the effects of any genetic modification [11]. Based on such studies, rational design can be obtained for the efficient production and genetic modification. Recently, elementary mode analysis have been used for genome scale metabolic studies dealing with, e.g., the rational design of methionine production in *E. coli* and *Corynebacterium glutamicum* [12], the production of polyhydroxybutanoate in yeast [13], the growth-related aspects in *Saccharomyces cerevisiae* [14,15] and *E. coli* [16,17].

To discuss the function of the endogenous HOR on the PDO synthesis by *K. pneumoniae*, an endogenous HOR gene was cloned and overexpressed in *K. pneumoniae* ACCC10082 in this study.