Microbiological Research 166 (2011) 521—530

Available online at www.sciencedirect.com

ScienceDirect

Microbiological Research

www.elsevier.de/micres

^{13}C-metabolic enrichment of glutamate in glutamate dehydrogenase mutants of *Saccharomyces cerevisiae*

Yijin Tang, Alex Sieg, Pamela J. Trotter*

Guehler Biochemistry Laboratory, Department of Chemistry, Augustana College, 639-38th Street, Rock Island, IL 61201, United States

Received 8 July 2010 ; received in revised form 14 October 2010; accepted 24 October 2010

KEYWORDS
Yeast;
Saccharomyces cerevisiae;
Metabolism;
Glutamate;
NMR;
Citric acid cycle

Summary

Glutamate dehydrogenases (GDH) interconvert α-ketoglutarate and glutamate. In yeast, NADP-dependent enzymes, encoded by *GDH1* and *GDH3*, are reported to synthesize glutamate from α-ketoglutarate, while an NAD-dependent enzyme, encoded by *GDH2*, catalyzes the reverse. Cells were grown in acetate/raffinose (YNAceRaf) to examine the role(s) of these enzymes during aerobic metabolism. In YNAceRaf the doubling time of wild type, *gdh2Δ*, and *gdh3Δ* cells was comparable at ∼4 h. NADP-dependent GDH activity (Gdh1p + Gdh3p) in wild type, *gdh2Δ*, and *gdh3Δ* was decreased ∼80% and NAD-dependent activity (Gdh2p) in wild type and *gdh3Δ* was increased ∼20-fold in YNAceRaf as compared to glucose. Cells carrying the *gdh1Δ* allele did not divide in YNAceRaf, yet both the NADP-dependent (Gdh3p) and NAD-dependent (Gdh2p) GDH activity was ∼3-fold higher than in glucose. Metabolism of [1,2-^{13}C]-acetate and analysis of carbon NMR spectra were used to examine glutamate metabolism. Incorporation of ^{13}C into glutamate was nearly undetectable in *gdh1Δ* cells, reflecting a GDH activity at <15% of wild type. Analysis of ^{13}C-enrichment of glutamate carbons indicates a decreased rate of glutamate biosynthesis from acetate in *gdh2Δ* and *gdh3Δ* strains as compared to wild type. Further, the relative complexity of ^{13}C-isotopomers at early time points was noticeably greater in *gdh3Δ* as compared to wild type and *gdh2Δ* cells. These *in vivo* data show that Gdh1p is the primary GDH enzyme and Gdh2p and Gdh3p play evident roles during aerobic glutamate metabolism.

*Corresponding author. Tel.: +1 309 794 3469.
E-mail address: pamtrotter@augustana.edu (P.J. Trotter).

0944-5013/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.micres.2010.10.004

Table 1. Strains and doubling times on experimental media.

Strain[a]	Genotype	Doubling time (min)[b]	
		YNDex	YNAceRaf
Wild Type (BY4742)	*MATα his3 leu2 lys2 ura3*	127 ± 13	254 ± 43
gdh1Δ (*YOR375c*)	*MATα his3 leu2 lys2 ura3 gdh1Δ::G418*	132 ± 11	ND[c]
gdh2Δ (*YDL215c*)	*MATα his3 leu2 lys2 ura3 gdh2Δ::G418*	132 ± 6	232 ± 43
gdh3Δ (*YAL062w*)	*MATα his3 leu2 lys2 ura3 gdh3Δ::G418*	133 ± 6	272 ± 33

[a] Wild type is followed in parentheses by the strain name; for mutants the strain name used in these studies is followed in parentheses by the designation for the disrupted open reading frame.
[b] Doubling times during logarithmic growth in glucose (YNDex) or acetate plus raffinose media (YNAceRaf); Average of 3—5 separate determinations ± standard deviation. ND, not detectable.
[c] Although the *gdh1Δ* strain does not divide, no loss of viability is observed after 17—19 h in this medium.

1. Introduction

The yeast *Saccharomyces cerevisiae* thrives with ammonia as its only source of nitrogen, as it is capable of assimilating ammonia into glutamate, which accounts for about 80% of cellular nitrogen (Magasanik 2003). Glutamate may be produced in two ways. An ATP-dependent glutamine synthetase (encoded by *GLN1*) may catalyze the transfer of ammonia onto glutamate, producing glutamine. This is followed by the action of a NAD-dependent glutamate synthase (encoded by *GLT1*), which reduces and transfers the amide group onto α-ketoglutarate (2-oxoglutarate) to produce two molecules of glutamate. Alternatively, in the favored route at lower metabolic cost, two NADP-dependent glutamate dehydrogenases (NADP-GDH; encoded by *GDH1* and *GDH3*) produce glutamate by catalyzing amination and reduction of α-ketoglutarate (Magasanik 2003; DeLuna et al. 2001). Degradation of glutamate to α-ketoglutarate and free ammonia is mediated by a related NAD-dependent glutamate dehydrogenase (NAD-GDH; encoded by *GDH2*) (Miller and Magasanik 1990).

Studies by DeLuna et al. (2001) on the expression and activity levels of the three glutamate dehydrogenase enzymes indicate that Gdh1p is the sole NADP-GDH showing activity when cells grow on glucose. Gdh3p is induced during the diauxic shift or by growth in ethanol, indicating a role during respiration, whereas Gdh1p activity is the same or decreased under these conditions. The Gdh2p enzyme is also subject to regulation (Coschigano et al. 1991), being repressed by glucose and elevated during fermentation (low glucose, ethanol), respiration (acetate) or growth with amino acids as nitrogen source. Such control is consistent with an anaplerotic role for Gdh2p during respiration (Coschigano et al. 1991).

The regulation of the GDH enzymes indicates different physiological roles in the cell. In these studies we aimed to examine the biosynthesis of glutamate *in vivo* using ^{13}C-metabolic enrichment followed by nuclear magnetic resonance (NMR) spectral analysis. Glutamate formation was examined during aerobic metabolism in normal, wild type cells as compared to single disruption mutants for each of the GDH enzymes (*gdh1Δ*, *gdh2Δ* and *gdh3Δ*). These data allow us to make some conclusions about the relative importance of each of the three enzymes in regulating the level of glutamate formation during aerobic metabolism.

2. Materials and methods

2.1. Yeast strains, media and growth

The yeast strains used in these studies and their genotypes are listed in Table 1. Genotypes were confirmed by growth phenotype for nutritional markers or PCR analysis of *GDH* alleles (data not shown). Synthetic medium (YN) contained 0.6% yeast nitrogen base, 0.5% yeast extract, and the necessary amino acid supplements (see Table 1) (Sherman 1991). The carbon sources were either 2% glucose (YNDex) or 1% each of acetate and raffinose (YNAceRaf). Minimal medium for ^{13}C-metabolic enrichment contained 0.67% yeast nitrogen base without amino acids. All media reagents were from Difco or Sigma Chemical.

Doubling times were determined for cells during logarithmic growth. YNDex-grown cells were diluted to an optical density of 0.05—0.2 at 600 nm (OD_{600}) and OD_{600} readings were taken for 8 h (YNDex) to 24 h (YNAceRaf) at 30 °C with shaking at 200 rpm. Growth after the transfer of cells from YNDex to YNAceRaf proceeded only after a significant lag (∼10 h; data not shown). To examine the viability of *gdh1Δ* cells after culture in YNAceRaf medium, cells pre-grown overnight in YNDex were pelleted by centrifugation, resuspended in