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The effect of intracellular ppGpp levels on glutamate and lysine overproduction in *Escherichia coli*

Akira Imaizumi *, Hiroyuki Kojima, Kazuhiko Matsui

Laboratories of Fermentation and Biotechnology, Ajinomoto, CO., Inc., Kawasaki-shi, Kanagawa, 210-8681, Japan

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Abstract

Although the enhancement of amino-acid synthesis by guanosine-3′,5′-tetraphosphate (ppGpp) is well known, the effect of intracellular ppGpp levels on amino-acid overproduction in *Escherichia coli* has not been investigated. In this study, we demonstrate that overexpression of the *relA* gene, encoding ppGpp synthetase, increases the accumulation of amino acids, such as glutamate and lysine, in amino-acid-overproducing strains of *E. coli*. Elevation of intracellular ppGpp levels due to depletion of required amino acids also enhances glutamate overproduction. Moreover, the extent of overproduction is highly dependent on the intracellular ppGpp level. These results demonstrate that amino-acid overproduction in *E. coli* is closely connected to amino-acid auxotrophy via the accumulation of ppGpp.

Keywords: *Escherichia coli*; ppGpp; Stringent response; *relA*; L-glutamate; L-lysine

1. Introduction

Amino acids are widely used as seasonings, nutritional supplements, animal feeds, and drug intermediates and so on. Several amino acids are produced by fermentation methods. *Escherichia coli* is commonly used for amino-acid production because of the extensive knowledge of its metabolism (Herrmann and Somerville, 1983), well-established recombinant DNA technology (Swartz, 1996) and availability of the complete genome sequence (Blattner et al., 1997). Many studies of amino-acid production using *E. coli* have exploited these advantages (Aiba et al., 1980; Hashiguchi et al., 1999; Jetten and Sinskey, 1995; Kojima et al., 1995; Ogawa-Miyata et al., 2001; Tsujimoto et al., 1993; Usuda and Kurahashi, 2005), and standard breeding strategies have focused mainly on increasing the flux to target substances (Eggeling and Sahm, 1999; Hashiguchi et al., 1999; Jetten et al., 1995; Patnaik and Liao, 1994). Methods such as release from feedback inhibition and/or repression, amplifica-

Abbreviations: OD, optical density; ppGpp, guanosine-3′,5′-tetraphosphate; rRNA, ribosomal RNA; tRNA, transfer RNA

* Corresponding author at: Institute of Life Sciences, Ajinomoto CO., Inc., 1-1,Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Kanagawa, 210-8681, Japan. Tel.: +81 44 210 5928; fax: +81 44 244 4258.
E-mail address: akira_imaizumi@ajinomoto.com (A. Imaizumi).

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tion of genes encoding enzymes of the relevant pathway and disruption of genes encoding degradative enzymes, have all been reported (Eggeling and Sahm, 1999; Hashiguchi et al., 1999; Jetten et al., 1995; Kikuchi et al., 1996; Ogawa-Miyata et al., 2001; Usuda et al., 2005). The resulting overproducing strains are often auxotrophic for some other amino acid because of inactivation or weakening of the corresponding biosynthetic pathway.

Guanosine-3′,5′-tetraphosphate (ppGpp) is a nucleotide that plays an important role in the signal-transduction pathway activated in response to several kinds of starvation (Cashel et al., 1996; Magnusson et al., 2005). A well-studied ppGpp-mediated response is that evoked by amino-acid starvation, the so-called 'stringent response' (Cashel et al., 1996); when amino acids are depleted, ribosome-bound RelA protein recognizes uncharged transfer RNA and synthesizes ppGpp (Hazeltine and Block, 1973), which binds to the RNA polymerase core enzyme (Chatterji et al., 1998; Toulokhonov et al., 2001) resulting in immediate various responses, such as growth arrest, repression of the synthesis of ribosomes, degradation of ribosomal proteins, retardation of translation speed, enhancement of amino-acid biosynthesis, and so on (reviewed in Cashel et al., 1996). Furthermore, ppGpp may act an important role on transcription of the *rmf* gene (Izutsu et al., 2001), encoding ribosome modulation factor, whose knockout results the increase of lysine production of lysine overproducing strain of *E. coli* (Imaizumi et al., 2005b).

It has been reported that knockout of *relA* enhanced exogenous protein production in *E. coli* because it eliminates repression by ppGpp (Dedhia et al., 1997). By contrast, overproduction of ppGpp enhanced production of secondary metabolites, such as antibiotics, in *Streptomyces griseus* and *Bacillus subtilis* (Ochi, 1987; Ochi and Ohsawa, 1984). Although there have been several demonstrations of upregulation of amino-acid-synthesizing genes by ppGpp (Barker et al., 2001a, 2001b; Cashel et al., 1996; Paul et al., 2005), there have been no reports of amino-acid overproduction in *E. coli*.

Here, we investigated the effects of intracellular ppGpp levels on growth and amino-acid production in overproducing derivatives of *E. coli*. We used *relA* and *spoT* knockouts, and *relA*-overproducing mutants of amino-acid-overproducing strains of *E. coli*, to test whether accumulation of ppGpp is essential for amino-acid overproduction, and to determine how ppGpp accumulation influences growth and amino-acid overproduction when overproducing cells are starved of required amino acids.

2. Material and methods

2.1. Strains, plasmids and culture conditions

The strains and plasmids used in this study are listed in Table 1. MG1655S is an L-glutamate-overproducing strain obtained by in-frame disruption of *sucA* gene

Table 1
List of bacterial strains and plasmids used in this study

Strain or plasmid	Phenotype or gene	Source or reference
Strain		
MG1655	Wild type	Our laboratory stock
MG1655A	MG1655Δ*relA*	This study
MG1655S	MG1655Δ*sucA*	This study
MG1655SA	MG1655Δ*sucA* Δ*relA*	This study
MG1655ST	*MG*1655Δ*sucA* Δ*spoT*	This study
MG1655SAT	MG1655Δ*sucA* Δ*relA* Δ*spoT*	This study
WC196	Lysine producing mutant derived from W3110	Kikuchi et al. (1996, 1997)
Plasmid		
pMAN997	pSC101 derivative, ori^{ts}, Amp^r	Tanaka et al. (2001)
pM15	Plasmid vector, Amp^r	Imaizumi et al. (2005a, 2005b)
pMrelA′	pM15 carrying truncated *relA* gene	Imaizumi et al. (2005a, 2005b)
pMrelA	pM15 carrying *relA* gene	Imaizumi et al. (2005a, 2005b)
pCAB1	RSF1010 derivatives, carrying *dapA**, *dapB* and *lysC** Sm^r	Kojima et al. (1995)

Amp^r, ampicillin resistant; Sm^r, streptomycin resistant; ori^{ts}, temperature-sensitive replication origin of plasmid.