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Dynamic responses of the intracellular metabolite concentrations of the wild type and *pyk*A mutant *Escherichia coli* against pulse addition of glucose or NH_3 under those limiting continuous cultures

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Abstract

The dynamics of the intracellular metabolite concentrations were investigated for the wild type and *pyk*A gene knockout mutant *Escherichia coli* in responses to the glucose pulse addition during glucose-limited continuous culture and in responses to the ammonia pulse addition during ammonia-limited continuous culture. For this, we developed a new automated rapid sampling device, which enables us to take samples rapidly within a second. The intracellular concentrations of G6P, F6P, 2PG, PEP, OAA, 6PG, Ribu5P, E4P and NADPH were higher for *pyk*A mutant as compared with the wild type under both limited continuous cultures, and the concentrations of PYR, ATP and acetate were much lower for *pyk*A mutant than those of the wild type. These phenomena reflected the fact that the accumulation of PEP caused the increased flux from PEP to OAA and that the accumulated PEP inhibited pfk, which caused the accumulation of G6P and F6P, which in turn increased the flux toward pentose phosphate (PP) pathway and increased the PP pathway metabolite concentrations. Oxygen uptake rate (OUR) was a little higher for *pyk*A mutant as compared with that of its wild type, while CO_2 production rate (CER) shows the reverse trend. OUR and CER were much less for NH_3-limited condition than that of NH_3-rich condition. The intracellular concentrations of PEP, ATP and PYR decreased rapidly within several seconds, whereas the concentrations of G6P, F6P FBP, 6PG, ADP, NADH and NADPH increased after glucose pulse addition during glucose-limited condition for both wild type and *pyk*A knockout mutant. Initial decrease in PEP concentration was considered to be due to PTS system. The intracellular concentration of NADPH decreased after NH_3 pulse addition under NH_3-limited condition for both strains, which is due to the fact that NADPH is utilized through glutamate production under NH_3 addition.
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Keywords: Rapid sampling; Intracellular metabolite concentrations; Continuous culture; Transient behavior; *pyk*A gene-knockout; Pulse addition

1. Introduction

The continuous culture under carbon limitation has often been employed to investigate the cell metabolism since the culture condition can be controlled constant [1,4,7,23,24]. Other nutrient-limited chemostat cultures have also been considered including nitrogen limitation, phosphate-limitation or sulfur-limitation etc. [14,17–20,24]. The growth characteristics and the metabolism under carbon-limited and nitrogen-limited conditions have been investigated for *Saccharomyces cerevisiae, Bacillus subtilis* and other microorganisms [9,12,16,21]. Recently, the effect of nutrient limitation on the metabolism has been investigated for *Escherichia coli* based on isotope experiments [15,25]. Changes in cellular physiology such as redirection of intermediary metabolism in response to the specific gene knockout and/or the changes in culture conditions affect the overall metabolism [25,27,30].

Assimilation of nitrogen requires the synthesis of two central intermediates, glutamate and glutamine [19,24], from which other compounds are derived. It has been estimated that about 88% of the cellular nitrogen in *E. coli* is derived from

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Nomenclature

Enzymes

6PGDH	6-phosphogluconate dehydrogenase
Eno	enolase
Fba	fructose-1, 6-bisphosphate aldolase
G6PDH	glucose-6-phosphate dehydrogenase
GAPDH	glyceraldehydes-3-phosphate dehydrogenase
GDH	glutamate dehydrogenase
Hxk	hexokinase
ICDH	isocytrate dehydrogenase
LDH	lactate dehydrogenase
MDH	malate dehydrogenase
Mk	myokinase
Pgi	phosphoglucose isomerase
Ppc	phosphoenolpyruvate carboxylase
Pta	phosphotransacetylase
PTS	phosphotransferase system
Pyk	pyruvate kinase
Rpe	ribose-phosphate epimerase
Rpi	ribose-phosphate isomerase
Tkt	transketolase
Tpi	Triosephosphate isomerase

Metabolites

2PG	2-phosphoglycerate
6PG	6-phosphogluconate
AcCoA	acetyle-coenzyme A
ADP	adenosinmonophosphate
AKG	α-ketoglutarate
AMP	adenosinmonophosphate
ATP	adenosintriphosphate
DHAP	dihydroxyacetonephosphate
E4P	erythrose-4-phosphate
F6P	fructose-6-phosphate
FBP	fructose-1,6-bisphosphate
G6P	glucose-6-phosphate
GAP	glyceraldehydes-3-phosphate
ICT	isocitrate
NAD	diphosphopyridindinucleotide, oxidized
NADH	diphosphopyridindinucleotide, reduced
NADP	diphosphopyridindinucleotide-phosphate, oxidized
NADPH	diphosphopyridindinucleotide-phosphate, reduced
OAA	oxaloacetate
PEP	phosphoenolpyruvate
PYR	pyruvate
Rib5P	ribose-5-phosphate
Ribu5p	ribulose-5-phosphate
SUC	succinate

glutamate and the remaining 12% is derived from glutamine [19]. Thus, glutamate and glutamine are the key intermediates in cellular nitrogen metabolism. Ammonium is the preferred nitrogen source for the enteric bacteria. In the presence of ammonium, there is a strong repression of many systems that allow the cells to use alternative nitrogen sources such as amino acids, inorganic compounds and urea [19,24].

Intracellular metabolite concentrations play important regulatory roles in the cellular metabolic network of microorganisms. Recently, the so-called "metablomics" has been paid much attention for the quantification of intracellular and extracellular metabolite with the help of advanced analytical techniques. Metablomics are influenced by the type of fermentation experiment, sampling method and the mathematical interpretation of the data [5]. Measurement of intracellular metabolite concentrations in the time window of seconds requires rapid sampling, inactivation of metabolic enzymes and extraction of metabolites taking into account the high metabolic turnover rate of the compounds of interest. In many metabolic reactions, especially in the catabolic reactions and the reactions, which are involved in the energy metabolism, the turnover rates are in the range of 1.5–2.0 s^{-1} [10,21,23,29]. The application of sampling techniques to measure reliable intracellular metabolite concentrations can only be successful if (i) the sample can be taken from a controlled reactor without disturbing the reaction, (ii) the quenching and extraction of metabolites are rapid and reliable, (iii) the sampling, inactivation and extraction procedure do not affect the stability of metabolites, (iv) the dilution by inactivation and/or extraction is controlled, reproducible and minimal and (v) sampling rate is high enough to study rapid dynamics [23]. These are the reasons why the manual operation is not valid and not reliable to study the dynamics of the intracellular metabolite concentrations.

Dynamic responses against pulse addition of glucose during glucose-limited continuous culture have been investigated for the wild type *E. coli*, *S. cerevisiae*, *B. subtilis* and other microorganisms [4–7,21,28]. It is quite important to investigate the metabolism at transient state to make clear the metabolic regulation mechanism. In the present study, we first developed a new rapid sampling device that enables us to take the sample from the reactor every 1 s or less.

Using this device, we investigated the metabolic responses against pulse addition of glucose during glucose-limited continuous culture and the responses against NH_3 pulse addition during NH_3-limited continuous culture by measuring the intracellular metabolite concentrations. Moreover, in the present research, we investigated the metabolism of a single gene knockout mutant such as *pyk*A mutant. Previously, we investigated the metabolism of the *pyk*F mutant *E. coli* [11,13,25]. Although many researchers have reported the effect of *pyk*F knockout on the metabolism, little research is done on *pyk*A mutant. Although the impact of *pyk*A gene knockout is less than that of *pyk*F gene knockout, this type of mutation often occur in various organisms and it is worth investigating the metabolic changes.

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