Biochemical and Biophysical Research Communications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Acs is essential for propionate utilization in *Escherichia coli*

Fengying Liu [a], Jing Gu [a], Xude Wang [a], Xian-En Zhang [b,*], Jiaoyu Deng [b,*]

[a] Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
[b] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

ARTICLE INFO

Article history:
Received 29 April 2014
Available online xxxx

Keywords:
Acetylation/deacetylation
Propionyl-CoA synthetase
Acetyl-CoA synthetase
CobB
Propionate

ABSTRACT

Bacteria like *Escherichia coli* can use propionate as sole carbon and energy source. All pathways for degradation of propionate start with propionyl-CoA. However, pathways of propionyl-CoA synthesis from propionate and their regulation mechanisms have not been carefully examined in *E. coli*. In this study, roles of the acetyl-CoA synthetase encoding gene *acs* and the NAD^+-dependent protein deacetylase encoding gene *cobB* on propionate utilization in *E. coli* were investigated. Results from biochemical analysis showed that, reversible acetylation also modulates the propionyl-CoA synthetase activity of Acs. Subsequent genetic analysis revealed that, deletion of *acs* in *E. coli* results in blockage of propionate utilization, suggesting that *acs* is essential for propionate utilization in *E. coli*. Besides, deletion of *cobB* in *E. coli* also results in growth defect, but only under lower concentrations of propionate (5 mM and 10 mM propionate), suggesting the existence of other propionyl-CoA synthesis pathways. In combination with previous observations, our data implies that, for propionate utilization in *E. coli*, a primary amount of propionyl-CoA seems to be required, which is synthesized by Acs.

1. Introduction

It is well known that short chain fatty acids, such as acetate, propionate and butyrate, can be utilized as sole carbon and energy source for bacteria. Among all these fatty acids mentioned above, oxidation of propionate is the most complicated and several pathways are involved in different bacteria [1]. However, all established and putative pathways for degradation of propionate start with propionyl-CoA.

Generally, in bacteria, propionyl-CoA can be synthesized from propionate through three different routs The first route is the propionyl-CoA synthetase (PrpE), which was firstly characterized in *Salmonella typhimurium* by Horswill and Escalante-Semerena in 1999 [2]. Subsequently, PrpE was also identified in other bacterial species, suggesting that it is a common route for propionyl-CoA synthesis in bacteria [3–5]. However, from the first beginning, Horswill and Escalante-Semerena found that, though PrpE was characterized to be a propionyl-CoA synthetase, *prpE* deletion mutant could still use propionate as carbon and energy source, since the acetyl-CoA synthetase (Acs) could compensate the lack of PrpE [2]. In fact, early studies already showed that, Acs could also use propionate as substrate [6–8], so it is not surprising to find out that *acs* serves as the second route for propionyl-CoA synthesis. The third route, a rather unusual route for propionyl-CoA synthesis, is consisted of two enzymes, the propionate kinase (PduW) and the phosphotransacetylase (Pta) [9]. So far, the third route has only been identified in *S. typhimurium*, and it is induced by 1,2-propanediol [10,11].

Regulation of propionyl-CoA synthesis has also been characterized. In 1996, Tsang and Escalante-Semerena found that, *cobB* mutant of *S. typhimurium* could not use propionate as carbon and energy source [11]. In 2002, CobB was characterized to be a NAD^+-dependent protein deacetylase responsible for deacetylation of Acs, which activates the acetyl-CoA synthetase activity of the protein [12]. Later on, PrpE was found to undergo propionylation *in vivo* which inactivate its activity, and CobB was found to be responsible for its de-propionylation [13]. Except for CobB, another known regulator for propionyl-CoA synthesis has been identified to be PrpR, a transcriptional activator for *prpBCDE* operon [14–16]. Very interestingly, in *S. typhimurium*, the function of PrpR needs 2-methylcitrate as co-activator [17,18].

Though progresses have been made on bacterial propionyl-CoA synthesis pathways, little is known about propionyl-CoA synthesis pathways and their regulations in *Escherichia coli*. To better understand how reversible acetylation regulates the propionyl-CoA synthetase activity of Acs, the protein was *in vitro* overexpressed and the effect of reversible acetylation on its propionyl-CoA

* Corresponding authors. Fax: +86 10 64888148 (X.-E. Zhang). Fax: +86 27 87199492 (J. Deng).
E-mail addresses: zhangxe@sun5.ibp.ac.cn (X.-E. Zhang), dengjy@wh.iov.cn (J. Deng).

http://dx.doi.org/10.1016/j.bbrc.2014.05.015
0006-291X/© 2014 Published by Elsevier Inc.

synthetase activity was tested. To elucidate the role of Acs on propionyl-CoA synthesis in *E. coli*, the *acs* gene was deleted and the effect of *acs* deletion on bacterial growth on different concentrations of propionate was carefully examined. Furthermore, the effect of *cobB* deletion on propionate utilization of *E. coli* was also tested. The results are reported herein.

2. Material and methods

2.1. Bacterial strains, growth conditions, and plasmids

E. coli strain w3110 used in this work were derived from standard *E. coli* K-12 strain. Bacterial strains and plasmids used were listed in Table 1. Luria–Bertani broth (LB) was the rich medium used to grow *E. coli* strains. Ampicillin, kanamycin and chloramphenicol were added in medium if needed at 100, 50, and 25 μg/ml. For growth curve experiments, strains were grown to mid-log phase in LB medium, then collected by centrifugation at 8000*g* for 6 min and washed 3 times with E minimal medium (0.2 g $MgSO_4{\cdot}7H_2O$, 13.1 g $K_2HPO_4{\cdot}3H_2O$, 3.5 g $NaNH_4HPO_4{\cdot}4H_2O$, 2 g citric acid$\cdot H_2O$) [19], resuspended in E minimal medium and adjusted to OD_{600} = 0.8, 100 μl suspension culture were inoculated to 10 ml E minimal medium supplemented with varied concentrations of sodium propionate. Growth of bacteria at 37 °C was measured by taking OD_{600} every 2 days by using SyergyH1 Hybrid reader (BioTek, USA). Experiments were performed in triplicates.

2.2. Unmarking of acs and cobB deletion mutants

The antibiotic resistance gene of the *acs* and *cobB* deletion mutants were eliminated with plasmid pCP20 as previously described [20]. Elimination of resistance gene was verified by antibiotic-resistance. Unmarked mutants were verified by PCR with primers designed up/downstream of target genes. Following primers were used: cobB-s, 5′-atctcttacctgtagctcgtgttccg-3′ (sense), cobB-a, 5′-aaaagtgggcgtgtattattccg-3′ (antisense); acs-s, 5′-ccccta tgtgtaacaaataacca-3′ (sense), acs-a, 5′-tatcaggcctacaaaccgttac-3′ (antisense).

2.3. Overexpression and purification of proteins

Bacteria strains were first grown overnight in 5 ml LB medium, and then cultures were transferred into 500 ml fresh LB medium at 37 °C in shaking flasks. IPTG was added to a final concentration of 0.2 mM when OD_{600} reached to 0.6–0.8. Then cells were grown for an additional 3 h at 37 °C for *E. coli* BL21 (DE3)/pET32a-*cobB* and 12 h at 25 °C for *E. coli* DH5α/pTrcHis2C-*acs*, harvested by centrifugation and washed once with ice-cold PBS buffer, resuspended in 30 ml Binding Buffer (20 mM Tris–HCl pH 7.9, 500 mM NaCl and 10 mM imidazole), sonicated on ice at the intensity of 3 s burst at 200 W with a 5 s cooling period between each burst with an Ultrasonic Cell Disruptor (VCX750, SONICS, US) until cell suspension becomes clear. The lysate was centrifuged at 10,000*g* for 30 min at 4 °C and the supernatant was harvested. Proteins were purified as previously described [21]. Protein concentrations were measured by the Bradford Protein Assay Kit (Beyotime Institute of Biotechnology, China) with BSA as standard, according to manufacturer's instructions.

2.4. In vitro enzymatic assay

Enzymatic activity of Acs was determined as previously described [2,22,23] with modifications. Briefly, 1 ml reaction mixtures contains 50 mM Tris–HCl (pH 7.5), 500 mM hydroxylamine (2.0 M solution of hydroxylamine was prepared before use by mixing equal volume of 4.0 M $NH_2OH{\cdot}HCl$ and 4.0 M KOH), 10 mM $MgCl_2$ and 2 mM DTT, 5 mM acyl substrate, 5 mM CoA and 5 mM ATP. The reaction mixture was preincubated at 37 °C for 5 min before the addition of Acs (5 μM). After addition of Acs, the reaction mixture was incubated at 37 °C for 10 min and terminated with the addition of equal volume of stop solution [2% (w/v) $FeCl_3$ in 2 M HCl, 5% (w/v) trichloroacetic acid (TCA)]. Reaction tubes were centrifuged at 10,000*g* for one minute to remove turbidity, and the color generated was measured at 540 nm. Samples without CoA were used as blank. The reaction product of acetylhydroxamate by acetyl-phosphate (sigma) served as the standard. The kinetic parameters and their standard errors were determined by non-linear regression to fit the data to the Michaelis–Menten equation.

2.5. Acetylation and CobB mediated deacetylation of Acs

Purified Acs was incubated with acetyl-phosphate (freshly prepared) in the buffer consisted of 300 mM NaCl and 50 mM Tris–HCl (pH 8.0) to obtain acetylated Acs (Ac-Acs). Ac-Acs was separated from the reaction mixture by dialysis, then deacetylated by CobB as previously described [24]. The acetylation level of Acs was analyzed by Western blot and enzyme activities of Acs, Ac-Acs and de-Acs (deacetylated by CobB) were determined as mentioned above.

2.6. Western blot analysis

Proteins were separated by 10% SDS–PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore). The PVDF membrane was blocked at 37 °C for 2 h in TBST [25 m M Tris–HCl (pH 8.0), 150 mM NaCl, 0.1% Tween-20] containing 5% non-fat dry milk (NFDM). Primary rabbit anti-acetyl lysine

Table 1
Strains and plasmids used in this study.

Strain and plasmid	Relevant properties	Reference or source
E. coli strains		
W3110	Wild type strain, F-λ-IN(rrnD-rrnE)1 rph-1	Laboratory stock
RL001	W3110 Δ*cobB*::Km^r	Laboratory stock
RL002	W3110 Δ*acs*::Km^r	Laboratory stock
W3110 Δ*cobB*	W3110 derivative *cobB* unmark deleted	This study
W3110 Δ*acs*	W3110 derivative *acs* unmark deleted	This study
DH5α	Host for plasmid propagation	Laboratory stock
BL21(DE3)	Host for CobB protein expression	Laboratory stock
Plasmids		
pCP20	FLP helper plasmid, Apr, Cm^r	Yale CGSC
pTrcHis2C-*acs*	Acs-His expression plasmids, Ap^r	Laboratory stock
pET32a-*cobB*	CobB expression plasmids, Ap^r	Laboratory stock

Please cite this article in press as: F. Liu et al., Acs is essential for propionate utilization in *Escherichia coli*, Biochem. Biophys. Res. Commun. (2014), http://dx.doi.org/10.1016/j.bbrc.2014.05.015