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Original Research Article

Lysine acetylation decreases enzyme activity and protein level of *Escherichia coli* lactate dehydrogenase

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ABSTRACT

Lactate is an important bulk chemical with widespread applications and a major byproduct of other chemicals bioprocess in microbial fermentation. Lactate dehydrogenase A (LdhA) catalyzes the synthesis of lactate from pyruvate. Lysine acetylation is an evolutionarily conserved post-translational modification; however, the mechanisms underlying the regulation of LdhA function by lysine acetylation in *Escherichia coli* remain poorly understood. Herein, we demonstrate acetylation of *E. coli* LdhA occurs via enzymatic and non-enzymatic mechanisms. Further, we show carbon source type and concentration affect the lysine acetylation status of LdhA via a non-enzymatic mechanism. Lysine acetylation significantly inhibits the enzymatic activity and protein level of LdhA. The results of the present study demonstrate lysine acetylation of *E. coli* LdhA is irreversible. Understanding of the effects of lysine acetylation on LdhA function may provide a new perspective for regulating lactate production in microbial synthesis.

1. Introduction

Cellular physiological processes are regulated by a range of complex mechanisms allowing cells to respond and adapt to changing environments. Post-translational modification (PTM) plays an important role in regulating protein structure, stability, and function [1]. PTM includes the methylation, phosphorylation, acetylation, glycosylation, and ubiquitinylation of proteins [2,3]. Lysine acetylation involves the transfer of an acetyl group to the epsilon amine of lysine. Acetylation modification abolishes the positive charge, increases the side chain size of lysine, and affects the interaction with acidic amino acids [4]. Lysine acetylation was first identified in f2a1 histone from calf thymus [5]. Lysine acetylation is highly conserved from bacteria to higher animals, involving non-enzymatic and enzymatic mechanisms [2,3]. In enzymatic acetylation, a lysine acetyltransferase (KAT) catalyzes the transfer of an acetyl group from acetyl coenzyme A to the ϵ-amino group of target lysine residues [6]. In the non-enzymatic mechanism, acetyl phosphate (AcP) can directly transfer its acetyl group to the lysine ϵ-amino group [7,8]. AcP-mediated non-enzymatic acetylation is the primary mechanism of lysine acetylation in the majority of bacterial species [9–11].

Over the past 50 years, protein acetylation has been extensively studied due to its role in histone modification in eukaryotes [12]. Following the development of high-affinity immune separation and nano-HPLC/MS/MS, the bacterial acetylome was first characterized in 2008 and increasing numbers of acetylated proteins have been identified by subsequent studies [7,13-15]. However, the function and regulation of lysine acetylation remains poorly understood in bacteria. The regulation of cellular processes by lysine acetylation has yet to be fully elucidated. NAD-dependent lactate dehydrogenase A (LdhA) catalyzes the synthesis of lactate from pyruvate and is found in almost all species. In human pancreatic cancer, lysine acetylation reportedly regulates LdhA function with effects on cell proliferation and migration [16].

In microbial fermentation, LdhA catalyzes the conversion of accumulating pyruvate to lactate [17,18]. Lactate is an important byproduct during the bioproduction of numerous chemicals, affecting yields, and conversion efficiency. In addition, lactate is an important bulk chemical with a wide range of applications in the fields of cosmetics, herbicides, and pharmaceuticals [19,20]. Lactate is also used as a monomer in the production of polylactate, a biodegradable plastic [21]. Therefore, increased understanding of lysine acetylation of LdhA may provide new

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Table 1
Primers used in this study.

Name	Sequence
pETDuet1-*ldhA*-5′	CCGGGATCCGATGAAACTCGCCGTTTATAGC
pETDuet1-*ldhA*-3′	CCGGAGCTCTTAAACCAGTTCGTTCGGGC
pACYCDuet1-*patZ*-5′	CGCGGATCCGATGAGTCAGCGAGGACTGGAAGC
pACYCDuet1-*patZ*-3′	CCAAGCTTTCATGATTCCTCGCGCTGGGCAA
pACYCDuet1-*cobB*-5′	CGCGGATCCGATGCTGTCGCGTCGGGGTCATC
pACYCDuet1-*cobB*-3′	CCAAGCTTTCAGGCAATGCTTCCCGCTTTT
pET28a-*patZ*-5′	CGCGAATTCATGAGTCAGCGAGGACTGGA
pET28a-*patZ*-3′	CCCAAGCTTTCATGATTCCTCGCGCTGGG
ID-*pat*-5′	CCAATATTGTACTGCCGAGG
ID-*cobB*-5′	CTGACCTGGTCGTCATTGGT
ID-*pta*-5′	AAGACGCGAGCCGCCTGACTGCCTG
ID-*ackA*-5′	CATAAAACGGATCGCATAACGC
kan -In –3′	GGTGAGATGACAGGAGATCC

perspectives on the regulation of lactate synthesis. *E. coli* is one of the most widely used species for lactate production in microbial fermentation. However, the role of lysine acetylation in regulating the function in *E. coli* LdhA still remains unclear. In the present study, we demonstrate acetylation of *E. coli* LdhA by acetyltransferase -mediated enzymatic and AcP-dependent non-enzymatic mechanisms and the irreversibility of LdhA acetylation. Further, lysine acetylation decreases the enzyme activity and protein level of *E. coli* LdhA.

2. Materials and methods

2.1. Plasmids and strains construction

All primers used in this study were listed in Table 1, and all plasmids and strains used in this study were listed in Table 2. *E. coli* DH5α was used for the construction of recombinant plasmids. Protein expression was performed in *E. coli* BL21(DE3). Plasmid construction was performed using the polymerase chain reaction and restriction enzyme digestion following the general principles of molecular manipulation. Knockout of chromosomal genes in *E. coli* BL21(DE3) was performed via P_1 vir-mediated transduction as previously described [22]. Donor strains were purchased from the Keio collection [23].

2.2. Protein expression, purification, and stability experiments

Bacterial strains were cultivated in LB medium with appropriate antibiotics. After overnight growth at 37 ˚C, cultures were transferred into fresh LB medium with 2% glucose by 1:50 dilution and further incubated under the same conditions. When the culture absorbance had reached an OD_{600} of approximately 0.6, 100 μM isopropyl-β-d-thiogalactopyranoside (IPTG) was added for T_7 promoter induction and cells were cultivated at 30 ˚C for 18 h. Cultures were collected by centrifugation and resuspended in phosphate-buffered saline (PBS; pH 7.5). High pressure was used for cell disruption. Cell lysates were centrifuged and purified using a Ni-NTA His·Bind Column (Novagen) according to the manufacturer's instructions. Purified proteins were used for acetylation and enzyme activity experiments.

For protein stability experiments, Q3685, Q3673, and Q3776 strains were cultured in LB medium containing 2% glucose. After IPTG induction for 1.5 h, strains were treated with 200 μg/mL chloromycetin for varying durations. Treated samples were calibrated according to absorbance at OD_{600}. Cells were collected by centrifugation and resuspended in PBS. Protein loading buffer was added to resuspended samples before incubation at 100 ˚C for 15 min. Extracted proteins were stored until use in SDS-PAGE and Western blot analyses.

2.3. Western blotting

LdhA protein was fractionated by electrophoresis on 12% SDS-PAGE gels. Protein samples were then transferred to polyvinylidene difluoride (PVDF) membranes for 1.5 h at 15 V. Membranes were blocked for 1 h at room temperature using quick block Western reagent (Beyotime, China). Membranes were incubated overnight at 4 ˚C with anti-acetyllysine mouse monoclonal antibody (EASYBIO, China, 1:2000) as primary antibody. Membranes were then incubated with anti-mouse antibody combined with horseradish peroxidase (EASYBIO, China, 1:10,000) as a secondary antibody for 1 h. After washing three times with PBS with Tween20, signals were detected using the enhanced chemiluminescence (ECL) system according to the manufacturer's instructions.

2.4. LdhA activity assays

LdhA activity assays were performed in a 100 mM phosphate buffer (pH 7.5), 1 mM NADH, 1 mM pyruvate, and 40 nM purified protein. Reaction rates were determined at 340 nm absorbance using a multimode microplate reader (Spark, Tecan).

2.5. In vitro acetylation and deacetylation

Acetyltransferase Pat-mediated acetylation was performed *in vitro* according to a previously described method with modifications [24]. The reaction mixture contained 50 mM Tris–HCl (pH 7.8), 0.1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 10 mM sodium butyrate, 0.3 mM acetyl-CoA, 80 μg acetyltransferase, and 40 μg LdhA in a 200 μL volume. Reaction mixtures were incubated at 37 ˚C for 1 h. AcP-mediated acetylation was assessed *in vitro* by mixing 50 μg LdhA with varying concentrations of AcP in 100 μL volumes and incubation at 37 ˚C for varying durations.

Deacetylase CobB-mediated deacetylation *in vitro* was performed according to a previously described methods with modifications [24]. Reaction mixtures contained 40 mM HEPES (pH 7.0), 6 mM $MgCl_2$, 1 mM NAD^+, 1 mM dithiothreitol, 10% glycerol, 80 μg CobB, and 40 μg LdhA in 200 μL volumes. Reaction mixtures were incubated at 37 ˚C for 1 h. Following enzymatic reactions, samples were used for Western blot analysis and enzyme assays.

3. Results and discussion

3.1. Non-enzymatic acetylation of LdhA

Lysine acetylation of *E. coli* LdhA was first determined by Western blotting using an anti-acetyllysine monoclonal antibody. Lysine acetylation can occur via non-enzymatic or enzymatic mechanisms. In the non-enzymatic mechanism, the donor of the acetyl group is AcP, an intermediate in the phosphotransacetylase-acetate kinase (Pta-AckA) pathway (Fig. 1A), with the intracellular concentration of AcP previously found to be higher in an *ackA* mutant and lower in a *pta-ackA* double mutant than in the wild-type strain [25,26]. To study whether AcP concentration affects LdhA acetylation *in vivo, pta* and *ackA* knockout strains were constructed by P1 vir-mediated transduction. LdhA acetylation levels were compared in the wild-type strain and individual mutant strains. As expected, LdhA acetylation was decreased by 0.13-fold in the *pta* mutant and increased by 1.89-fold in the *ackA* mutant (Fig. 1B). These results demonstrate that modulation of the Pta-AckA pathway can alter AcP levels and further affect LdhA acetylation status.

In order to study whether AcP can directly acetylate LdhA in *vitro*, purified LdhA protein was incubated with varying concentrations of AcP at over varying durations.

The acetylation level of LdhA increased with incubation time, and higher AcP concentrations increased LdhA acetylation over the same treatment duration (Fig. 1C). LdhA acetylation increased in an AcP dose- and time-dependent manner. These findings demonstrate that LdhA can be acetylated by an AcP-mediated non-enzymatic mechanism (Fig. 1A).

AcP regulates the expression of hundreds of genes involved in many bacterial cellular processes including protein degradation, chemotaxis,

Table 2
Plasmids and strains used in this study.

Plasmids and Strains	Description	Source
Plasmids		
pACYCDuet1	*Cm*^{r} *oriP*$_{15A}$ *lacI*^{q} P$_{T7}$	Novagen
pETDuet1	Amp^r oriP$_{BR322}$ lacIq P$_{T7}$	Novagen
pET28a	Kan^r oriP$_{BR322}$ lacIq P$_{T7}$	Novagen
pCP20	reppSC101ts Ap^R Cm^R cI857 λP$_R$ FLP	CGSC[a]
pETDuet1-*ldhA*	Amp^r oriP$_{BR322}$ lacIq P$_{T7}$ ldhA	This study
pACYCDuet1-*patZ*	*Cm*^{r} *oriP*$_{15A}$ *lacI*^{q} *P*$_{T7}$ *patZ*	This study
pET28a -*patZ*	Kan^r oriP$_{BR322}$ lacIq P$_{T7}$ *patZ*	This study
pACYCDuet1-*cobB*	*Cm*^{r} *oriP*$_{15A}$ *lacI*^{q} *P*$_{T7}$ *cobB*	This study
Strains		
E. coli DH5α	F$^-$ *supE*44 Δ*lacU*169 (ϕ80 *lacZ* Δ*M15*) *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	Invitrogen
E. coli BL21(DE3)	F$^-$ *ompT gal dcm lon hsdSB* (rB$^-$ mB$^-$) λ(DE3)	Invitrogen
JW2568	BW25113 Δ*pat::Km*^{R}	Keio collection
JW1106	BW25113 Δ*cobB::Km*^{R}	Keio collection
JW2294	BW25113 Δ*pta::Km*^{R}	Keio collection
JW2293	BW25113 Δ*ackA::Km*^{R}	Keio collection
Q3509	*E. coli* BL21(DE3)/ pACYCDuet1-*patZ*	This study
Q3510	*E. coli* BL21(DE3)/ pACYCDuet1-*cobB*	This study
Q3620	*E. coli* BL21(DE3) Δ*patZ*	This study
Q3621	*E. coli* BL21(DE3) Δ*cobB*	This study
Q1437	*E. coli* BL21(DE3) Δ*pta*	This study
Q1951	*E. coli* BL21(DE3) Δ*ackA*	This study
Q3685	*E. coli* BL21(DE3)/pETDuet1-*ldhA*	This study
Q3673	*E. coli* BL21(DE3) Δ*patZ*/ pETDuet1-*ldhA*	This study
Q3674	*E. coli* BL21(DE3) Δ*cobB*/ pETDuet1-*ldhA*	This study
Q3675	*E. coli* BL21(DE3) Δ*pta*/ pETDuet1-*ldhA*	This study
Q3676	*E. coli* BL21(DE3) Δ*ackA*/ pETDuet1-*ldhA*	This study
Q3776	*E. coli* BL21(DE3)/pETDuet1-*ldhA*/pET28a-*patZ*	This study

[a] The Coli Genetic Stock Center at Yale University.

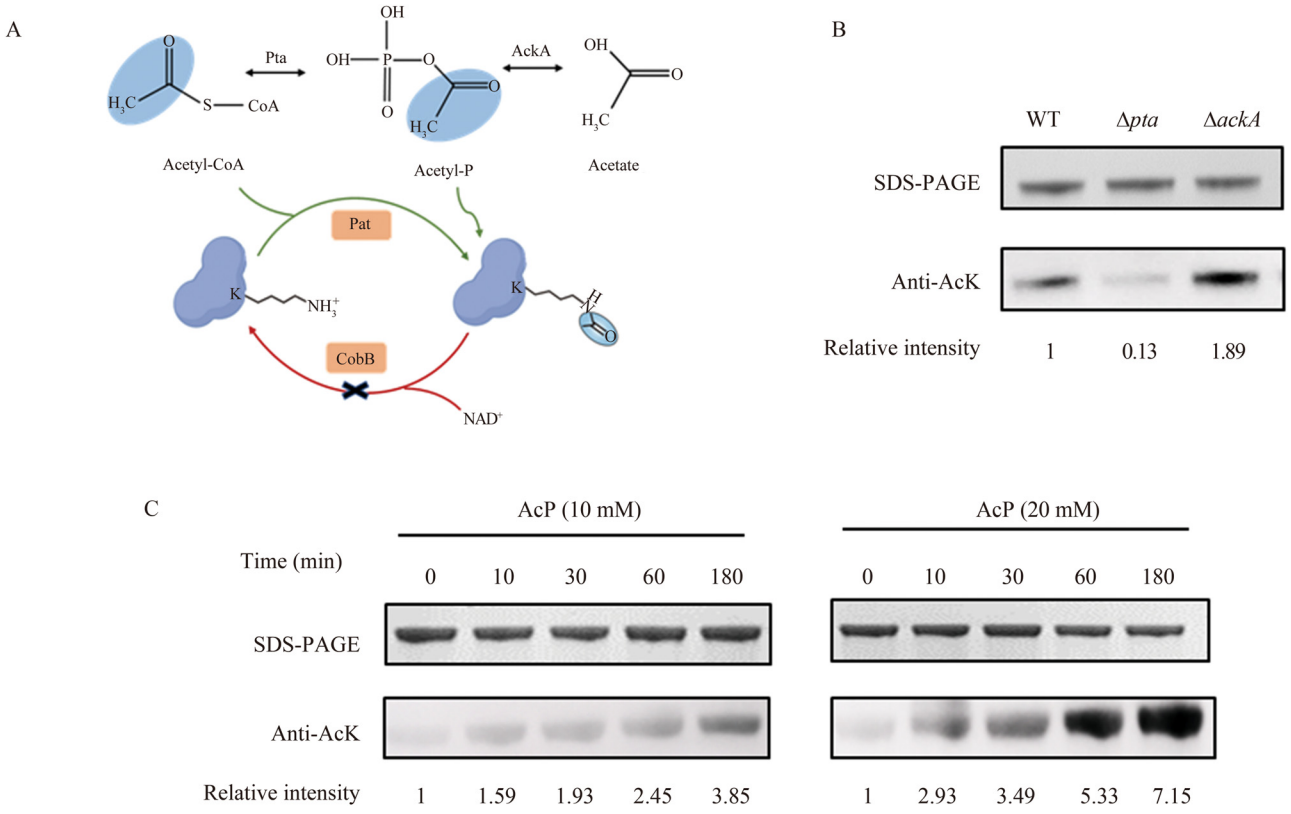

Fig. 1. AcP-mediated non-enzymatic acetylation of *E. coli* LdhA protein. (A) Mechanisms of *E. coli* LdhA acetylation. (B) LdhA acetylation in Pta and AckA strains. (C) LdhA acetylation following *in vitro* AcP treatment for varying durations.

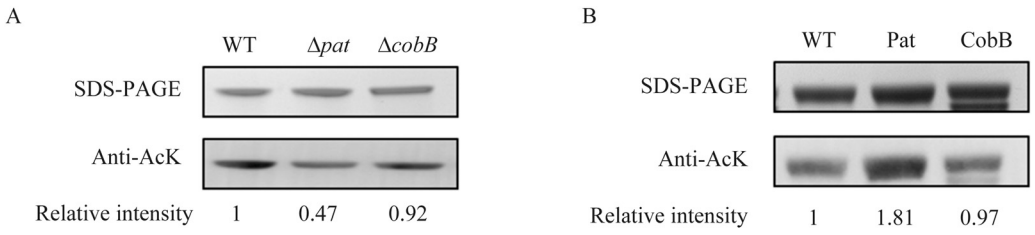

Fig. 2. Enzymatic acetylation of *E. coli* LdhA protein. (A) LdhA acetylation in *pat* and *cobB* strains. (B) LdhA acetylation following Pat and CobB treatment *in vitro*.

cell metabolism, and flagella biosynthesis [27]. AcP-dependent acetylation is the predominant mechanism for protein acetylation in *E. coli*. Modulation of AcP metabolism may have effects on protein acetylation and the regulation of multiple cellular physiological and metabolic processes. The Pta-AckA pathway is an important target for regulating AcP synthesis. In addition, Schilling et al. reported knockout of a number of carbon regulators, including *arcA, cra, csrA, rcsB, crp*, and *cyaA*, altered cellular acetylation patterns [10]. Thus, the methods described above may have utility in modulating LdhA acetylation level, thereby allowing modulation of LdhA activity and lactate production.

3.2. Enzymatic acetylation of LdhA

In *E. coli*, Pat and CobB are the predominate acetyltransferase and deacetylases, respectively, in enzymatic protein acetylation [1] We therefore assessed the roles of Pat and CobB in LdhA modification both *in vivo* and *in vitro*. Firstly, *pat* and *cobB* knockout mutants were constructed and LdhA acetylation levels were compared between the two mutant and the wild-type strain. As shown in Fig. 2A, LdhA acetylation levels decreased by 0.47-fold following the deletion of *pat* and were unchanged in the *cobB* knockout strain (Fig. 2A). Then, Pat and CobB proteins were purified and individually co-incubated with LdhA *in vitro*. Pat treatment increased LdhA acetylation by 1.81-fold, whereas CobB treatment had no effect on LdhA acetylation, corroborating the *in vivo* findings (Fig. 2B). These results demonstrate that *E. coli* LdhA can be acetylated by Pat. Further, CobB was unable to deacetylate LdhA. Studies in human pancreatic cancer have reported knockout and overexpression of the deacetylase, SIRT2, resulted in significantly increased and reduced levels of LdhA lysine acetylation, respectively [16].

The above results demonstrate that both Pat-mediated enzymatic and AcP-dependent non-enzymatic mechanisms can acetylate LdhA; however, acetylated LdhA cannot be deacetylated by CobB (Fig. 1A). Consistent with these findings, AbouElfetouh et al. stated not all acetylated proteins have evidence of reversal in *E. coli* [28]. Even so, efforts to discover new deacetylases have continued and several proteins, such as a serine hydrolase YcgC in *E. coli* [29] and MSMEG4620 in *Mycobacterium smegmatis* [30], have been reported as putative deacetylases. However, further studies have suggested YcgC is unlikely to be a deacetylase as the deacetylation of RutR protein by YcgC could not be replicated by other researchers [31,32]. Accordingly, there remain a number of challenges and opportunities in the study of bacterial deacetylases.

3.3. Effect of carbon sources on LdhA acetylation

The type and concentration of carbon sources are the determinants of *E. coli* acetate metabolism which affects the intracellular concentration of the acetyl group donor, AcP [1]. Previous studies have reported that carbon sources affect the overall bacterial acetylome [24,33]. Thus, carbon sources have been posited to influence LdhA acetylation status. To test this hypothesis, LdhA protein was purified from *E. coli* cells grown in LB medium supplemented with glucose or glycerol and subjected to Western blotting to measure acetylation status. As shown in Fig. 3A, supplementation with either glucose or glycerol markedly improved the acetylation level of LdhA protein, with more acetylated LdhA observed in cells cultivated with glucose than with the same concentration of glycerol. However, the acetylation level of LdhA protein was similar between cells cultivated in 1% or 2% glucose. A glucose concentration of 1% may be sufficient for maximal acetylation of LdhA. To determine whether the acetylation level of LdhA is dependent on glucose concentration, several additional concentrations were evaluated. Glucose improved the acetylation level of LdhA protein in a dose-dependent manner for glucose concentration ranging between 0% and 1%, with no changes in the acetylation level of LdhA observed for glucose concentration between 1% and 2% (Fig. 3B).

Of note, lysine acetylation showed no obvious differences when *E. coli* cells grown on the same concentration of xylose and glucose [33]. The mechanisms underlying the regulation of lysine acetylation by carbon sources have yet to be elucidated. Schilling et al. posited that concentrations of downstream metabolites are more important than concentrations of carbon sources themselves [33]. Thus, we measured the concentrations of AcP and acetate in wild-type and *pat* knockout strains cultured with different carbon sources. We observed similar results in these two strains with very low AcP concentrations observed following culture with three different carbon sources. No significant differences in AcP concentration were observed between the three conditions. Acetate levels were positively correlated with LdhA acetylation level (Fig. 3C–D).

Taken together, these results indicate that the acetyltransferase, Pat, may not play a major role in the regulation of LdhA acetylation in response to changes in carbon source. The effects of carbon source on lysine acetylation predominantly depend on carbon flux through glycolysis and the accumulations of downstream metabolites, processes which are regulated by non-enzymatic mechanisms. Previous studies have reported that acetylation levels in a *pta* mutant do not increase in response to glucose supplementation [7,34]. In addition, lysine acetylation reportedly changes dynamically during the cell growth phase and increase when cells enter the stationary phase, correlating with acetate excretion due to overflow metabolism [10]. As AcP is a precursor for acetate synthesis, LdhA acetylation levels correlate positively with acetate concentration during the stationary phase.

Both carbon source type and concentration are optimized in microbial fermentation to alleviate overflow metabolism, overcome the accumulation of byproducts, and increase the production of target chemicals [35,36]. Lysine acetylation represents a cellular response to carbon flux and is closely related to overflow metabolism. Therefore, lysine acetylation of LdhA plays an important role in regulating lactate production and carbon flux distribution in response to different carbon source types and concentrations.

3.4. Lysine acetylation decreases LdhA activity

To test the effect of lysine acetylation on LdhA activity, the enzymatic activity of LdhA was measured after treatment with Pat, CobB, or AcP. Increased LdhA acetylation in response to Pat treatment decreased LdhA activity by 20%, while LdhA activity following treatment with CobB was similar to control without any treatment (Fig. 4A), as CobB was unable to deacetylate LdhA (Fig. 2B). Moreover, AcP treatment

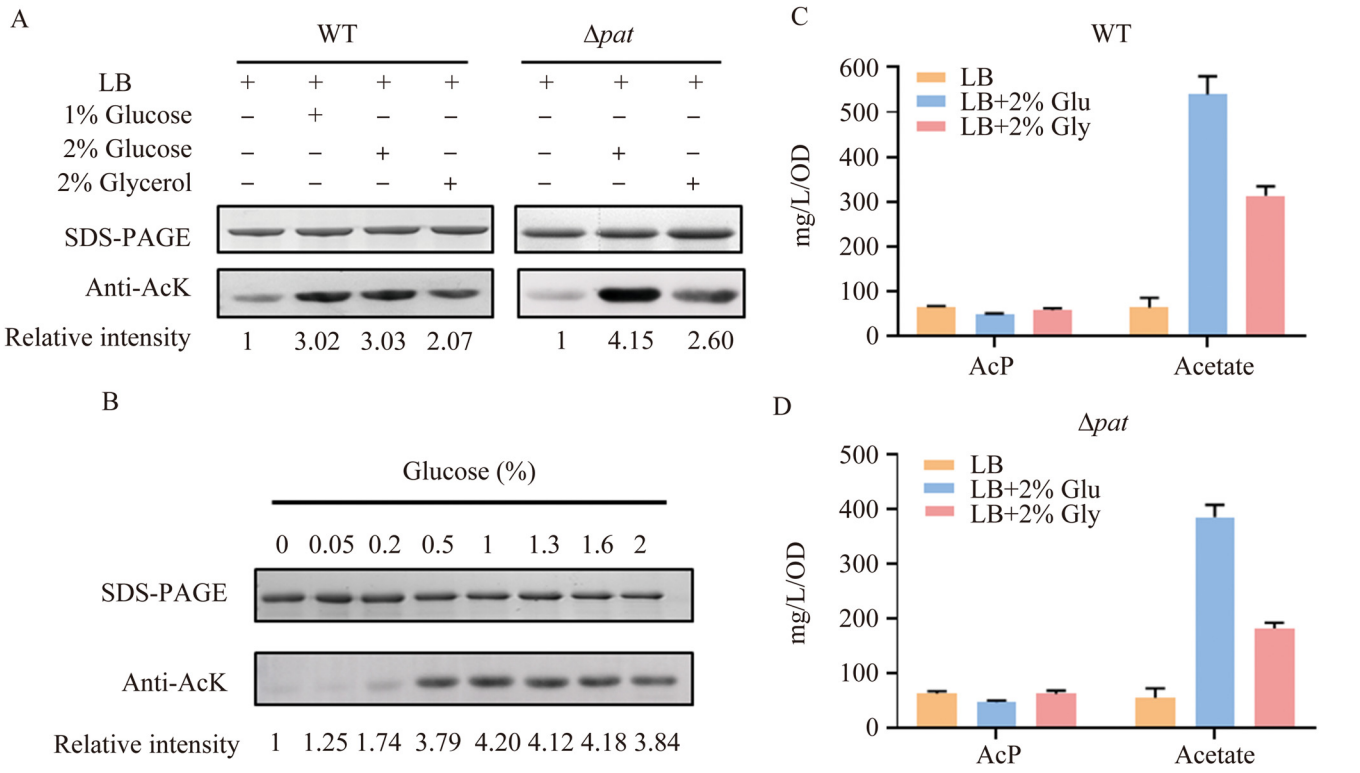

Fig. 3. Carbon sources affect LdhA acetylation. (A) The effects of different carbon sources on LdhA acetylation in *E. coli* wild-type and *pat* knockout strains. (B) The effects of different glucose concentrations on LdhA acetylation. (C) Concentrations of AcP and acetate under different conditions in wild-type *E. coli* strain. (D) Concentrations of AcP and acetate under different conditions in *pat* knockout strain.

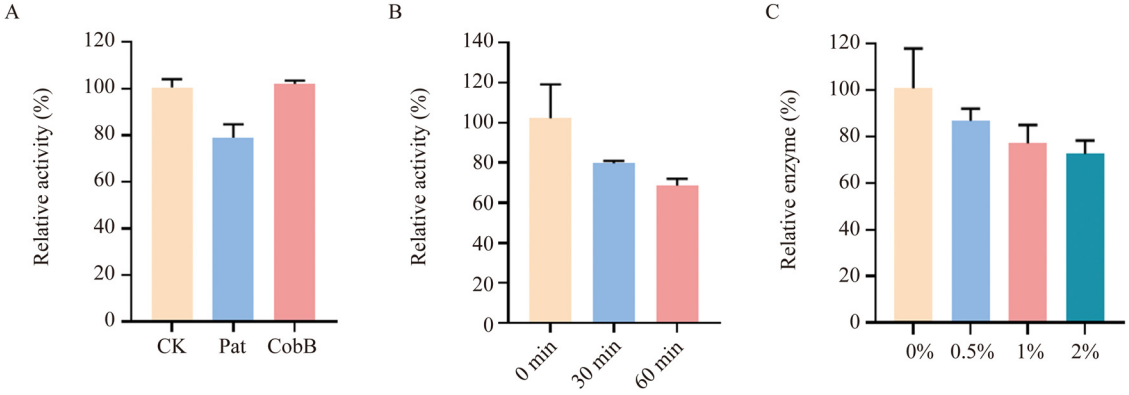

Fig. 4. Lysine acetylation inhibits the overall activity of LdhA. (A) Effect of Pat and CobB treatment on LdhA activity. (B) Effect of AcP treatment for varying durations on LdhA activity. (C) LdhA activity in cells grown in different glucose concentrations.

decreased LdhA activity, with greater reductions seen with increasing treatment duration. LdhA activity decreased to 79.7% and 68.5% following treatment with 20 mM AcP for 30 min and 60 min, respectively (Fig. 4B). Additionally, we measured LdhA activity in *E. coli* cells grown in the presence or absence of glucose. As glucose increased the acetylation level of LdhA *in vivo*, supplementation with glucose reduced LdhA activity (Fig. 4C).

LdhA catalyzes the synthesis of lactate from pyruvate, thereby competing with glycolysis for carbon flux. Lactate is an important byproduct of microbial fermentation that inhibits the production of other target chemicals. Knockout of the *ldhA* gene is commonly used for overcoming lactate accumulation in traditional methods. However, *ldhA* deletion affects cell growth and NAD replenishment. Our results demonstrate that lysine acetylation can inhibit the overall activity of LdhA. The regulation of LdhA activity by lysine acetylation may coordinate the balance between lactate accumulation and cell growth to increase metabolic fitness. In addition, proteins generally have more than one acetylated lysine site. The effects of individual acetylated lysine residues on enzyme activity differ depending on their spatial location. Accordingly, the overall effect of lysine acetylation on enzyme activity is the combination of the effects of all acetylated lysine sites. Therefore, identifying the acetylated lysine sites of LdhA and determining the contribution of each site to LdhA activity may increase the application of lysine acetylation in regulating lactate synthesis.

3.5. Lysine acetylation decreases LdhA protein levels

Lysine acetylation reportedly regulates LdhA levels by modulating protein aggregation and half-life [37–39]. In order to study the effect of lysine acetylation on LdhA levels, LdhA protein was expressed in wild-type, *pat* knockout, and *pat* overexpressing *E. coli* strains. Chloramphenicol was used to further inhibit protein translation in order to observe the

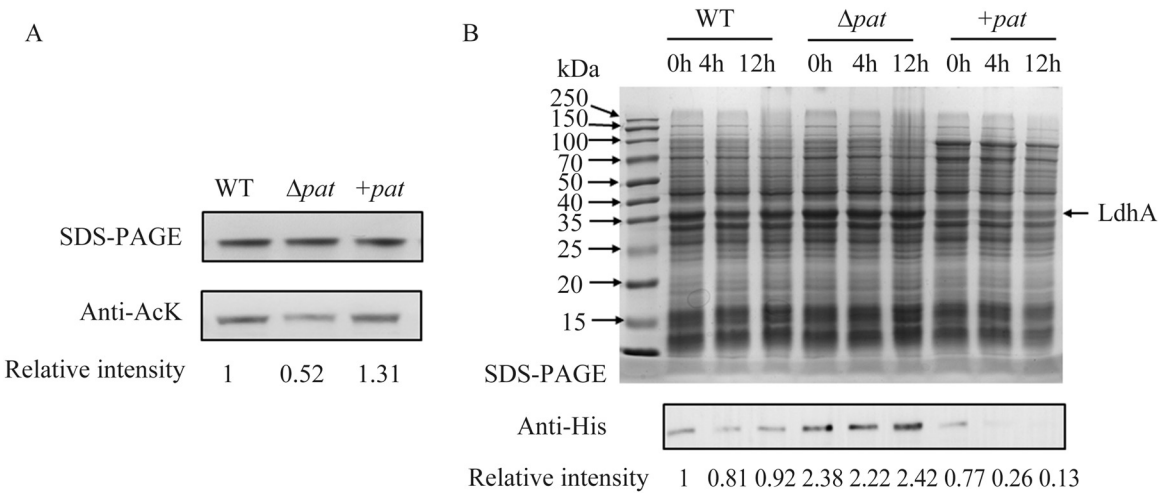

Fig. 5. Acetylation decreased LdhA protein levels. (A) Acetylation levels in wild-type, *pat* knockout, and *pat* overexpressing strains. (B) LdhA protein levels in wild-type, *pat* knockout, and *pat* overexpressing following chloramphenicol treatment.

stability of existing LdhA protein. As shown in Fig. 5A, deletion of *pat* increased LdhA acetylation levels and overexpression of *pat* decreased LdhA acetylation levels.

After treatment with chloramphenicol, LdhA protein levels remained unchanged for 12 h in wild-type and *pat* mutant strains; however, a constant decrease in LdhA protein levels over time was observed in the *pat* overexpressing strain (Fig. 5B). The LdhA acetylation level in the *pat* overexpressing strain decreased by 0.26-fold and 0.13-fold after chloramphenicol treatment for 4 h and 12 h, respectively. These results demonstrate that lysine acetylation decreases protein levels of LdhA by promoting its degradation. LdhA protein levels showed significantly different among all three strains at the timepoint of chloramphenicol addition. LdhA protein levels were substantially higher in the *pat* knockout strain and substantially lower in the *pat* overexpressing strain compared to the wild-type strain, indicating that lysine acetylation may affect LdhA protein levels through other mechanisms. As previously reported, lysine acetylation of ribosomal proteins affects ribosome assembly [40,41]. Accordingly, Pat overexpression may decrease protein translation through acetylation of ribosomal proteins resulting in lower LdhA levels and *vice versa* in the *pat* knockout strain. Further studies are required to fully elucidate the detailed mechanisms underlying the effect of acetylation on LdhA levels.

4. Conclusion

As an abundant PTM, lysine acetylation plays an important role in many cellular physiology and metabolism processes. Although a large number of bacterial acetylated proteins have been identified in acetylome, the functional role and regulation of protein acetylation remains poorly understood. The present study systematically characterized the effects of lysine acetylation on the enzymatic activity and protein stability of *E. coli* LdhA, and uncovered the regulatory effect of carbon sources on LdhA acetylation. The regulation of lactate synthesis is an enduring example of metabolic engineering, and LdhA is the key enzyme catalyzing lactate synthesis from pyruvate. Therefore, lysine acetylation of LdhA may provide a new strategy for regulating lactate synthesis in microbial fermentation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

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