Determination of lysine acetylation susceptibility of DksA and its effects on global metabolism in *Borellia Burgdorferi*

Armond J. Isaak[1], William K. Boyle[1], Hannah S. Sorensen[1], Amanda K. Zalud, and Travis J. Bourret[1]

[1]Department of Medical Microbiology and Immunology, Creighton University, Omaha, NE, United States., [2]Department of Biomedical Sciences, Creighton University, Omaha, NE, United States.

Travis J. Bourret
Creighton University
2500 California Plaza
Omaha, NE 68178
Phone: 402-280-3750
Email:
TravisBourret@creighton.edu

Abstract

For successful completion of its infectious cycle, the Lyme disease spirochete *Borrelia burgdorferi* must overcome a wide range of environmental stresses. *B. burgdorferi* harbors limited repertoire of gene regulatory proteins and the molecular mechanisms underlying their ability to direct transcriptomic responses in response to changes in environment remain enigmatic. Recently, we described the global role for the DnaK suppressor protein (DksA) in the regulation of *B. burgdorferi* gene expression in response to nutrient limitation. In a previously published study, a multitude of *B. burgdorferi* proteins, were identified as targets of lysine acetylation by endogenously produced acetyl-phosphate (Ac-PO_4) during *in vitro* growth. Lysine acetylation is a reversible post-translational modification (PTM) that contributes to the regulation of virulence gene expression in a variety of bacterial pathogens. *In silico* analysis of *B. burgdorferi* DksA suggests that five lysines (L118, L119, L121, L122, and L124) located in the C-terminus of DksA are likely targets for acetylation. In this study, I determined the susceptibility of recombinant DksA to lysine acetylation and will characterize the impact of this PTM on the gene regulatory activity of DksA required for *B. burgdorferi* to complete its infectious cycle in ticks and mice. Additionally, our previous work suggested DksA may contribute to lysine acetylation through its regulation of *ackA*, a gene that produces Ac-PO_4. Currently, I am comparing the profiles of acetylated proteins in wild-type and *dksA*-deficient *B. burgdorferi* strains to the contribution of DksA to lysine acetylation.

Introduction

Borrelia burgdorferi is the causative biological agent of Lyme disease, the most common vector-borne illness in North America and Europe. Currently, there is no Lyme disease vaccine on the market, and in effort to combat the disease, we look to *B. burgdorferi*. This agent experiences shifts in environmental stresses such pH, temperature, osmolarity, oxygen concentrations, nutrient availability as well as, reactive oxygen and nitrogen species during transmission (1). Our long-term goal is to determine mechanisms underlying the ability of *B. burgdorferi* to sense and respond to these environmental stresses during infection of its hosts, in particular *Ixodes spp*. ticks. One way *B. burgdorferi* responds to this shift in environment is by expressing genes controlled by the RNA polymerase (RNAP). Recently, the DnaK Suppressor Protein (DksA) was noted as a transcriptional regulator essential for energy conservation in response to the environmental stresses. In a previous study, various B. burgdorferi proteins were identified as targets for lysine acetylation, a reversible post-translational modification. The objective here is to determine if DksA is susceptible to lysine acetylation and characterize the impacts on its gene regulatory activity.

Methods

Acetylation. Three different proteins of DksA, bovine serum albumin (BSA) and RNA polymerase were chosen to be acetylated. DksA and RNA polymerase were not diluted and remained at their given concentrations of 0.111mM and 0.001mM, respectively. BSA was diluted to 0.100mM. Acetyl-phosphate (AcP) was added in a dilution scheme of 100mM, 10mM, 1mM, 0.1mM and 0mM to each protein sample. Due to a low amount of RNA polymerase, there was not enough of the protein solution to prepare samples for 0.1mM and 0mM. For each sample 45µL of the protein solution and 5 µL of the AcP concentrated solution was added and incubated at 37ºC for three hours.

SDS-PAGE. 20 µL of each protein-AcP sample was mixed with 25 µL of 2X Laemmli Sample Buffer and 5 µL ethanol to prepare for SDS-PAGE. 10 µL of the resulting solution and the ladder (Spectra™ Multicolor Broad Range Protein Ladder) were each added to a lane. Two gels were used to accommodate the number of samples prepared. Gels were run for 20 minutes at 200V with 1X TGS running buffer in BIO-RAD set-up.

Immunoblotting. PVDF membranes were prepared by soaking in 200 proof methanol and 1X transfer buffer before adding to Trans-Blot system under each SDS-PAGE gel. Trans-Blot system was run in BIO-RAD Trans-Blot Turbo Transfer System for 30 min. The resulting membranes were then incubated with blocking solution of dehydrated milk solution for an hour and rinsed with 1X TBST buffer. Next, transferred to 1:10,000 anti-acetyllysine antibody solution (20mL 1X TBST buffer with 20µL antibody) for six hours on rocker and later rinsed with 1X TBST buffer. Resulting image produced on BIO-RAD Gel Doc EZ Imager with ImageLab software.

Pull down assay. Protein A agarose beads were washed three times with B-PER lysis buffer and mixture was centrifuged at 3,000 g for 2 minutes. Anti-acetyllysine antibody was diluted 5X in Protein A/B-PER buffer and centrifuged. Once cells were grown to desired density, they were pelleted, washed with B-PER buffer, and centrifuged at 3,000 g for 10 minutes. 500µL of B-PER buffer was added before centrifugation and the supernatant was divided among four prepared agarose bead tubes and left shaking in cold room overnight. The next day, the tubes were centrifuged, and supernatant was discarded. Tubes were then washed three times each with 700µL B-PER buffer and centrifuged with supernatant collected for analysis. After third wash, 50µL of SDS buffer was added and the tubes were heated at 50ºC for ten minutes. Tubes were centrifuged and supernatant was collected. 50 µL of SDS buffer was added again and tubes were heated at 95ºC for 5 minutes, centrifuged, and supernatants collected for analysis.

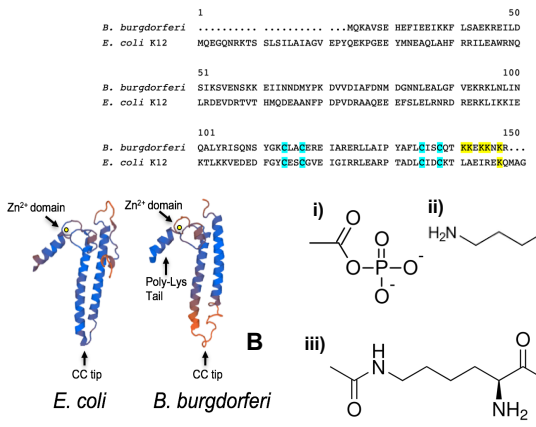

Figure 1. Protein structure of *B. burgdorferi* DksA. (A) Amino acid sequence alignment of *B. burgdorferi* and *E. coli* DksA proteins. The boxes indicate regions where *B. burgdorferi* DksA likely contains conserved coiled-coil α-helices and a zinc finger based on HHPred homology modeling. The asterisks indicate key conserved aspartic acid and cysteine residues. **(B)** SWISS-model of *E. coli* (left) and *B. burgdorferi* (right) DksA proteins illustrate predicted structural similarities based on a high-resolution crystal structure 1TJL. Color scale from blue (high) to orange (low) encodes Qmean score estimating model quality. Peptide N- and C-termini are indicated for each model. **(C)** The chemical structures of **i)** acetyl phosphate, **ii)** lysine, and **iii)** acetylated lysine.

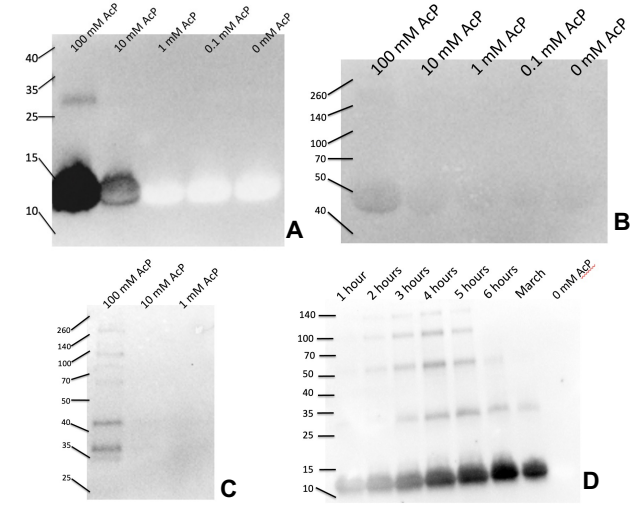

Figure 3. Western Blotting with anti-acetyllysine antibody. After SDS-PAGE was completed, the two gels were subsequently prepared for and ran under Trans-Turbo trans-blot system. The gels were them treated with 1:10,000 dilution of pan anti-acetyllysine antibody and images developed. 10µL of Spectra™ Multicolor Broad Range Protein Ladder (260 kDa) was in the first lane for each gel. **(A)** Membrane of western blot of DksA protein under dilution scheme of acetyl-phosphate (AcP) with the fifth lane containing no AcP as a negative control. **(B)** Membrane of positive control bovine serum albumin (BSA) under dilution scheme of acetyl-phosphate. The negative control of 0mM AcP is also included in the fifth lane. **(C)** Membrane of western blot of RNA polymerase with same acetyl-phosphate dilution scheme as Figure 1A and B. **(D))** Membrane of western blot of incubation time study of DksA protein with 100 mM AcP. The same 100 mM protein from Figure 2A and 0 mM AcP were used as the positive and negative controls, respectively.

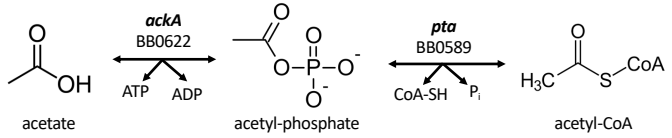

Figure 3. Borrelia burgdorferi metabolism. The metabolism of acetate to acetyl-CoA is shown with this two-step figure. The first step is mediated by the *ackA* enzyme which may be regulated by DksA and adds a phosphate to acetate. The second step, mediated by the *pta* enzyme, replaces the phosphate with Co-enzyme A so acetyl-CoA can be used for further downstream metabolism in *B. burgdorferi*.

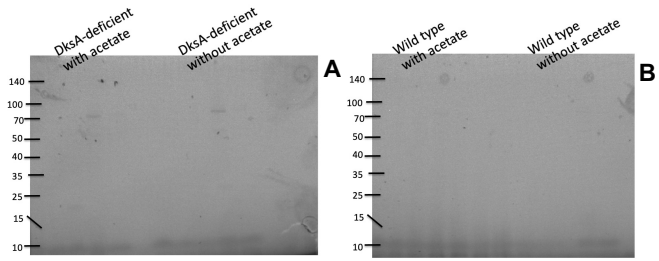

Figure 4. SDS-PAGE gel of acetylated proteins. DksA-deficient and wild type cells were grown to desired concentration of 2.0E7 cells/mL and then passaged with 30 mM acetate. Pull down assay was performed with pan anti-acetyllysine antibody and B-PER buffer to extract desired proteins. Three washes of B-PER buffer was performed for each protein and the supernatant was collected for analysis. Then, SDS buffer was added twice with each eluent collected for analysis. SDS-PAGE was then run for each protein's (DksA and wild type with and with out acetate) pull down assay supernatant and eluents (five lanes for each protein) at 200 V for 30 minutes. **(A)** Gel of DksA-deficient protein B-PER supernatants and SDS buffer eluents with and without acetate. **(B)** Gel of wild type protein B-PER supernatants and SDS buffer eluents with and without acetate.

Summary

- **DksA has been shown to contain lysines in its C-terminus that could possibly be acetylated**
- **DksA is susceptible to lysine acetylation by acetyl-phosphate via during *in vitro* growth**
- **DksA is also susceptible to lysine acetylation during *in vivo* growth when treated with acetate**

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