Microbial Pathogenesis 49 (2010) 323–329

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Screening of nitrosative stress resistance genes in *Coxiella burnetii*: Involvement of nucleotide excision repair

Sung-Hyun Park, Hyun-Wook Lee, Weiguo Cao*

Department of Genetics and Biochemistry, South Carolina Experiment Station, Clemson University, Room 219 Biosystems Research Complex, 51 New Cherry Street, Clemson, SC 29634, USA

ARTICLE INFO

Article history:
Received 19 May 2010
Received in revised form
28 July 2010
Accepted 2 August 2010
Available online 10 August 2010

Keywords:
Nitric oxide
Reactive nitrogen intermediates
Nitrosative stress
uvrB
Nucleotide excision repair
DNA repair

ABSTRACT

Coxiella burnetii, an obligate intracellular Gram-negative bacterium, is the etiological agent of Q fever. This work takes advantage of a hypersensitive *Escherichia coli* genetic system to identify genes involved in resistance to nitrosative stress imposed by reactive nitrogen intermediates. Among the ten candidate genes identified, the transposase, UvrB and DNA topoisomerase IV are involved in DNA transaction; the sigma-32 factor and the putative DNA-binding protein may be involved in transcriptional regulation; IF-2 is involved in protein translation; malate dehydrogenase and carbamoyl-phosphate synthase are metabolic enzymes; and the ABC transporter is a membrane-bound protein. In addition, a hypothetical protein was identified. The role of the DNA repair gene *uvrB* in resistance to RNI was further confirmed by investigating the sensitivity of *uvrB* deletion mutant and complementation by *C. burnetii uvrB*. Deletion of two other components of the UvrABC nuclease, *uvrA* and *uvrC* also renders the cell sensitive to RNI. The relationship between UvrABC and nitrosative stress is discussed.

1. Introduction

Coxiella burnetii, an obligate intracellular Gram-negative bacterium, is the etiological agent of Q fever [1–5]. The typical means of transmission is through inhalation of contaminated aerosols, enabling its spread to large population. Once inhaled, *C. burnetii* targets alveolar macrophages, is internalized by phagocytosis and resides in acidic phagolysosomes [6,7]. Upon infection, macrophages are well known to trigger "respiratory burst" to release large amount of reactive radicals known as reactive oxygen intermediate (ROI) which imposes oxidative stress and reactive nitrogen intermediate (RNI) which imposes nitrosative stress to kill or contain invading pathogens [8,9]. Despite of such a powerful host defense system, some macrophage-residing pathogens such as the facultative intracellular *Mycobacterium tuberculosis* and the obligate intracellular *C. burnetii* remain viable after the assault by these reactive radicals that inflict a variety of damage to essential macromolecules such as DNA and proteins. Evidently, these organisms have developed an unusual ability to resist the killing effects caused by ROI and RNI. It is of great interest to understand the underlying resistance mechanisms that confer pathogen's ability to survive in the host, for study of pathogen biology, prevention, and therapeutic interaction.

How bacteria cope with oxidative stress imposed by ROI has been a subject of intense investigation for many years [10,11]. Resistance of nitrosative stress imposed by RNI was not recognized until the role of nitric oxide synthase (NOS) in host defense emerged (for review see Ref. [12]). Since then, studies in *Mycobacterium*, *Salmonella* and other bacteria started to unravel bacterial defense mechanisms against RNI [13,14]. Yet, compared with ROI, our understanding of resistance to RNI appears still in a very early stage. Use of *M. tuberculosis* as a model system to study biochemical basis of resistance to RNI is facilitated by the ability to cultivate it outside the host cells and availability of tools for genetic manipulation. Given its unusual ability to survive in host macrophage cells, *C. burnetii* serves as a unique model system to understand its resistance mechanism to RNI. However, the obligate host requirement for growth and lack of a manipulatable genetic system present a challenge [15]. Because many genetic screening and selection methodologies are not applicable due to these limitations, alternative strategies have to be explored.

To investigate the resistance mechanisms to RNI adopted in *C. burnetii*, we developed an *Escherichia coli*-based genetic system to allow for screening of genes involved in resistance to RNI. This system takes advantage of the hypersensitivity of an *E. coli* triple mutant strain to nitrosative stress imposed by RNI. In this study, we

* Corresponding author. Tel.: +1 864 656 4176; fax: +1 864 656 0393.
E-mail address: wgc@clemson.edu (W. Cao).

0882-4010/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2010.08.001

screened a *C. burnetii* genomic library for candidate genes involved in resistance to RNI. Among the genes positively identified, *uvrB* encodes a helicase as part of the UvrABC nuclease involved in nucleotide excision repair. Based on the finding of *uvrB* gene, we examined the sensitivity of *uvrA* and *uvrC* to RNI-mediated stress. Both *uvrA* and *uvrC* deletion mutants were sensitive to nitrosative stress. To our knowledge, this is the first report examining the relationship between all three components of UvrABC nuclease (*uvrA*, *uvrB* and *uvrC*) and nitrosative stress.

2. Results

2.1. E. coli-based genetic system

To investigate the resistance mechanisms to RNI adopted by bacteria pathogens, we sought to develop a genetic system to allow for screening of genes involved in resistance to RNI. Since endonuclease V (*nfi*), alkyladenine DNA glycosylase (*alkA*) and endonuclease VIII (*nei*) are involved in repair of DNA damage caused by RNI, the lack of these repair genes may render the cells sensitive to nitrosative stress. To test the sensitivity of this triple mutant strain to nitrosative stress, we treated it with different concentrations of acidified sodium nitrite. The triple mutant strain BW1739 that lacks *nfi alkA* and *nei* was significantly more sensitive than the corresponding wild type (wt) strain BW1466 (Fig. 1). After treating with more than 20 mM of acidified nitrite, BW1739 completely lost viability, indicating that the triple mutant strain BW1739 becomes hypersensitive under nitrosative stress (Fig. 1). These results were consistent with previous observations of reduced survival of *Pseudomonas aeruginosa,* and *Mycobacterium smegmatis, and Salmonella typhimurium* in glycosylase-deficient mutants under nitrosative stress conditions [16,17].

2.2. Screening of candidate genes in C. burnetii

We reasoned that genes involved in resistance to RNI may complement the hypersensitive phenotype demonstrated in the triple mutant strain. Since *C. burnetii* is known for its ability to survive under nitrosative stress condition in the host, we thought this genetic system may allow us to identify RNI resistance genes in *C. burnetii*. We thus constructed a genomic expression library using a Lambda ZAP Express Vector (Stratagene). The quality of the library was assessed by the lacZ-based blue/white color selection assay on X-gal plates. Approximately 98% of clones contained inserts, indicating a high ratio between recombinant clones and self-ligated plasmid.

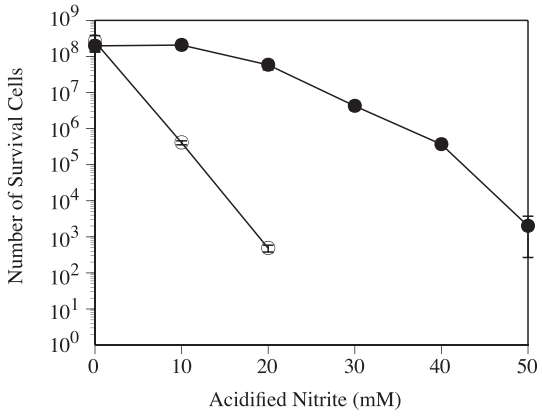

Fig. 1. Sensitivity of *E. coli* strains BW1466 and BW1739 to acidified sodium nitrite treatment.Colonies from each strain was grown to saturation in 3.5 ml of LB medium. Cell density was approximated using a spectrophotometer and divided into six aliquots of 0.5 ml (containing approximately 1×10^8 cell numbers). Each aliquot was treated with 0, 10, 20, 30, 40, and 50 mM acidified nitrite (pH 4.6) and then incubated at 37 °C for 10 min. Dilution and plating were performed as described in Materials and methods. (●) wt *E. coli* strain (BW1466); (○) DNA repair deficient *E. coli* strain (BW1739).The data are the means ± SD of at least three independent experiments.

The genomic expression library was electroporated into the hypersensitive BW1739 cells. The screening was carried out by treating the electroporated BW1739 cells with ascending concentrations of acidified sodium nitrite at pH 4.6. As shown in Fig. 2, the cells with the empty plasmid were still hypersensitive to RNI as they could not survive the treatment above 20 mM level. However, a large number of colonies were observed in the *C. burnetii* library at concentrations above 20 mM. Some cells were viable at 40 mM and even 50 mM concentration (Fig. 2B).

To verify these results, each individual surviving colonies from 40 to 50 mM concentrations were selected, grown, and treated with 0–50 mM acidified sodium nitrite at pH 4.6 again. Colonies that still maintained viability at 40 or 50 mM treatment were kept for further confirmation while those did not were discarded. To confirm that the survival is caused by the candidate genes instead of a change in the host, plasmids were isolated from the clones and retransformed back to the hypersensitive BW1739 cells. The cells that again showed complementation to the triple mutant cells were considered confirmed candidate genes. After these screening and confirmation steps, ten genes from *C. burnetii* were identified (Table 1). Among the list, the transposase, UvrB and DNA topoisomerase IV are involved in DNA transaction; the sigma-32 factor and the putative DNA-binding protein may involved in transcriptional regulation; IF-2 is involved in protein translation; malate dehydrogenase and carbamoyl-phosphate synthase are metabolic enzymes; and the ABC transporter is a membrane-bound protein. In addition, a hypothetical protein was identified.

2.3. UvrB and RNI resistance

The DNA repair gene *uvrB* gene caught our attention because of its role in nucleotide excision repair. To assess the relationship between *uvrB* gene and the RNI resistance, we measured the sensitivity of an *E. coli uvrB* deletion strain. While the isogenic wild type BW25113 strain showed resistance to RNI, the survival of Δ*uvrB* strain was subdued at high acidified nitrite concentrations (Fig. 3). The cell counts were substantially reduced by 30 mM treatment and reduced at zero by 40 and 50 mM treatments. To test whether *C. burnetii uvrB* can complement the phenotype, we cloned the gene into the pBK-CMV plasmid and transformed it into the Δ*uvrB* strain. The cell counts of the survival colonies were substantially increased in all treatments (Figs. 3 and 4). A large number of *uvrB*-containing cells survived 30 mM treatment. Even at 40 or 50 mM concentration, some of the *uvrB*-containing cells still survived (Fig. 3). These results suggest that the DNA repair gene *uvrB* is involved in resistance to RNI.

2.4. UvrA, UvrC and RNI resistance

Since UvrA and UvrC, along with UvrB, consist of the UvrABC nuclease, we were curious whether *uvrA* and *uvrC* are also involved in resistance to RNI. We, therefore, tested the sensitivity of *uvrA* and *uvrC* deletion strains to acidified nitrite treatment. The *uvrA* deletion strain became very sensitive to RNI, as indicated by complete loss of survival at 40 or 50 mM and reduction of colony count to several orders of magnitude at 30 mM concentration (Fig. 5). Similarly, the survival of the *uvrC* deletion strain was also reduced (Figs. 5 and 6), as indicated by substantial decrease of colony counts from 20 to 50 mM. However, *uvrA* appeared far more sensitive than